Archival Report

Biological Psychiatry

Regulation of Brain-Derived Neurotrophic Factor Exocytosis and Gamma-Aminobutyric Acidergic Interneuron Synapse by the Schizophrenia Susceptibility Gene Dysbindin-1

Qiang Yuan, Feng Yang, Yixin Xiao, Shawn Tan, Nilofer Husain, Ming Ren, Zhonghua Hu, Keri Martinowich, Julia S. Ng, Paul J. Kim, Weiping Han, Koh-ichi Nagata, Daniel R. Weinberger, and H. Shawn Je

ABSTRACT

BACKGROUND: Genetic variations in dystrobrevin binding protein 1 (*DTNBP1* or dysbindin-1) have been implicated as risk factors in the pathogenesis of schizophrenia. The encoded protein dysbindin-1 functions in the regulation of synaptic activity and synapse development. Intriguingly, a loss of function mutation in *Dtnbp1* in mice disrupted both glutamatergic and gamma-aminobutyric acidergic transmission in the cerebral cortex; pyramidal neurons displayed enhanced excitability due to reductions in inhibitory synaptic inputs. However, the mechanism by which reduced dysbindin-1 activity causes inhibitory synaptic deficits remains unknown.

METHODS: We investigated the role of dysbindin-1 in the exocytosis of brain-derived neurotrophic factor (BDNF) from cortical excitatory neurons, organotypic brain slices, and acute slices from dysbindin-1 mutant mice and determined how this change in BDNF exocytosis transsynaptically affected the number of inhibitory synapses formed on excitatory neurons via whole-cell recordings, immunohistochemistry, and live-cell imaging using total internal reflection fluorescence microscopy.

RESULTS: A decrease in dysbindin-1 reduces the exocytosis of BDNF from cortical excitatory neurons, and this reduction in BDNF exocytosis transsynaptically resulted in reduced inhibitory synapse numbers formed on excitatory neurons. Furthermore, application of exogenous BDNF rescued the inhibitory synaptic deficits caused by the reduced dysbindin-1 level in both cultured cortical neurons and slice cultures.

CONCLUSIONS: Taken together, our results demonstrate that these two genes linked to risk for schizophrenia (BDNF and dysbindin-1) function together to regulate interneuron development and cortical network activity. This evidence supports the investigation of the association between dysbindin-1 and BDNF in humans with schizophrenia.

Keywords: Brain-derived neurotrophic factor (BDNF), DTNBP1, Dysbindin-1, Exocytosis, Gamma-aminobutyric acid (GABA), Interneuron synapse, Schizophrenia

http://dx.doi.org/10.1016/j.biopsych.2015.08.019

In the mammalian central nervous system, neurons receive inhibitory synaptic inputs from gamma-aminobutyric acid (GABA)ergic interneurons and excitatory inputs from glutamatergic neurons (1,2). Converging experimental and clinical evidence suggests that the dysfunction of appropriate GABAergic inhibition and the consequent imbalance between excitation and inhibition in the cerebral cortex underlie the pathophysiology of schizophrenia, a complex psychiatric disorder with lifetime morbid risk close to 1% of the general population (3–5). For example, in the neocortex of schizophrenia patients, the activity of the rate-limiting synthetic enzyme glutamic acid decarboxylase (GAD) is reduced (6). This finding has been repeatedly confirmed and extended in subsequent studies that showed alterations in several presynaptic and postsynaptic components of the GABAergic system (7).

Dystrobrevin binding protein 1 (*DTNBP1* or dysbindin-1), which encodes dysbindin-1, has been implicated in several studies as a potential susceptibility gene for schizophrenia (8–11). In schizophrenia, dysbindin-1 expression has been reported to be reduced in cortical and limbic neurons, suggesting that reduced dysbindin-1 protein levels may be a disease-related trait (11–15). In mice, a loss-of-function mutation in *Dtnbp1* (dys-MT) disrupts both glutamatergic and GABAergic transmission in the prefrontal cortex, and this disruption is associated with working memory deficits (16–21). Most importantly, in dys-MT mice, cortical pyramidal neurons in the prefrontal cortex display enhanced excitability, presumably due to a reduction in GABAergic signaling (19–21). The mechanism underlying the loss of inhibitory activity in dys-MT mice remains unclear because dysbindin-1 expression is

SEE VIDEO CONTENT ONLINE

largely restricted to excitatory neurons rather than interneurons (13,22).

In this study, we discovered an unexpected role of dysbindin-1 in the exocytosis of brain-derived neurotrophic factor (BDNF), an important trophic factor for the differentiation and survival of inhibitory neurons from excitatory neurons (23). We further determined that this reduction in BDNF secretion reduces the number of functional GABAergic synapses that are transneuronally formed on excitatory neurons (24). Given that interneurons cannot synthesize BDNF by themselves but require BDNF for the formation and maintenance of their synapses (25–27), our results demonstrate that these two well-known schizophrenia susceptibility gene products (BDNF and dysbindin-1) function together to regulate interneuron synapse development. Furthermore, our results provide considerable insight into the cellular and molecular mechanisms that regulate the development of the neural circuitry in the brain and link these abnormalities in BDNF secretion to cognitive disease.

METHODS AND MATERIALS

A detailed description of the experimental procedures can be found in the Supplement. All animal experiments in this study were approved by the Institutional Animal Care and Use Committee of the Duke-National University of Singapore Graduate Medical School and the Lieber Institute for Brain Development.

Cell Culture

Cortical neurons were prepared from embryonic day 17 rat embryos and cultured in Neurobasal medium with B27 supplement (Invitrogen, Carlsbad, California). At day-in-vitro (DIV) 7, cultures were transfected with a calcium phosphate transfection kit (Clontech, Madison, Wisconsin). Human embryonic kidney 293 cells and PC12 cells were maintained using standard protocols and were transfected with Lipofectamine 2000 (Invitrogen).

Molecular Biology

Dysbindin-1 and scrambled short hairpin RNAs (shRNAs) were generated using pLentilox 3.7 vector. Dysbindin-1 shRNA target sequences were conserved in both mouse and rat dysbindin-1A and C isoforms. pCAG-MCS2-myc-dysbindin-1 was generated as reported previously (28), and the shRNA-resistant mutant of dysbindin-1 was generated by polymerase chain reaction-based mutagenesis.

Fluorescent Immunocytochemistry

Cultured rat neurons and mouse brain slices (postnatal day [P] 28) were stained with various primary antibodies, followed by incubation with appropriate fluorophore-conjugated secondary antibodies and imaged. Detailed antibody information can be found in the Supplement.

Imaging

Images were acquired with an LSM 710 (Zeiss, Singapore) confocal microscope using either 40× or 63× oil objectives and total internal reflection fluorescence microscopy imaging was conducted as previously reported (29).

Electrophysiology

Cortical neurons were transfected with various constructs as previously described (29). Whole-cell voltage clamp recordings were performed on DIV14 to DIV15 neurons. For acute slice recording, wild-type and dys-MT male mice at P15 to P35 were used as previously described (21).

Statistical Analysis

Unless otherwise stated, error bars represent the standard error of the mean. All statistical analyses are listed in the Supplement.

RESULTS

Increased Excitatory Synaptic Transmission in Layer 2/3 Pyramidal Neurons in the Prefrontal Cortex of dys-MT Mice

The prefrontal cortex (PFC) is a brain structure that is known to play a critical role in executive function and is prominently implicated in the pathophysiology of schizophrenia (21). In the PFC, top-down information is transmitted via pathways from layer (L) 2/3 pyramidal neurons to pyramidal neurons in L5, which is a major corticofugal output layer of the PFC network. We characterized the synaptic properties of L2/3 pyramidal neurons within the prelimbic area (PrL), homologous to the dorsolateral prefrontal cortex in primates, by measuring spontaneous excitatory postsynaptic currents (sEPSCs) using whole-cell patch-clamp recordings (Figure 1A, B) (20). Intriguingly, we found that dys-MT mice exhibited a dramatic increase in the frequency of the sEPSCs that were recorded from L2/3 pyramidal cells, without changing the amplitude (WT: 2.69 ± .62 Hz, 10.50 ± .29 pA; dys-MT: 6.65 ± 1.50 Hz, 10.99 ± 1.25 pA; $p < .05$) (Figure 1C, D). These results indicate an increase in excitatory synaptic transmission in the PFC of dys-MT mice.

Previous reports suggested that the decreased excitability of interneurons and decreased inhibitory transmission onto pyramidal neurons partially account for the increased frequency of sEPSCs in the PFC of dys-MT mice (20). To test this further, we monitored spontaneous miniature inhibitory postsynaptic currents (mIPSCs) in L2/3 pyramidal neurons within PrL. In the presence of glutamatergic antagonists and sodium channel blockers, we observed significant reduction in the frequency of mIPSCs (WT: 5.23 ± .30 Hz; dys-MT: 4.29 ± .27 Hz; $p < .05$) (Figure 1E, F). However, no change in mIPSC amplitude was detected in PrL of dys-MT mice (WT: 22.19 ± 1.98 pA; dys-MT: 18.31 ± 1.32 pA) (Figure 1E, G). Intriguingly, immunocytochemistry revealed that the density (WT: 1.52 ± .08 per 10 μm^2; dys-MT: 1.24 ± .04 per 10 μm^2; $p < .01$) and size (WT: 1.46 ± .05 μm^2; dys-MT: 1.33 ± .04 μm^2; $p < .05$) of vesicular GABA transporter (VGAT) (an inhibitory presynaptic marker)-expressing boutons, which represent GABAergic inhibitory synaptic terminals, in the L2/3 within the PrL of dys-MT mice were reduced significantly, indicating the reduced inhibitory inputs onto L2/3 pyramidal neurons (Supplemental Figure S1).

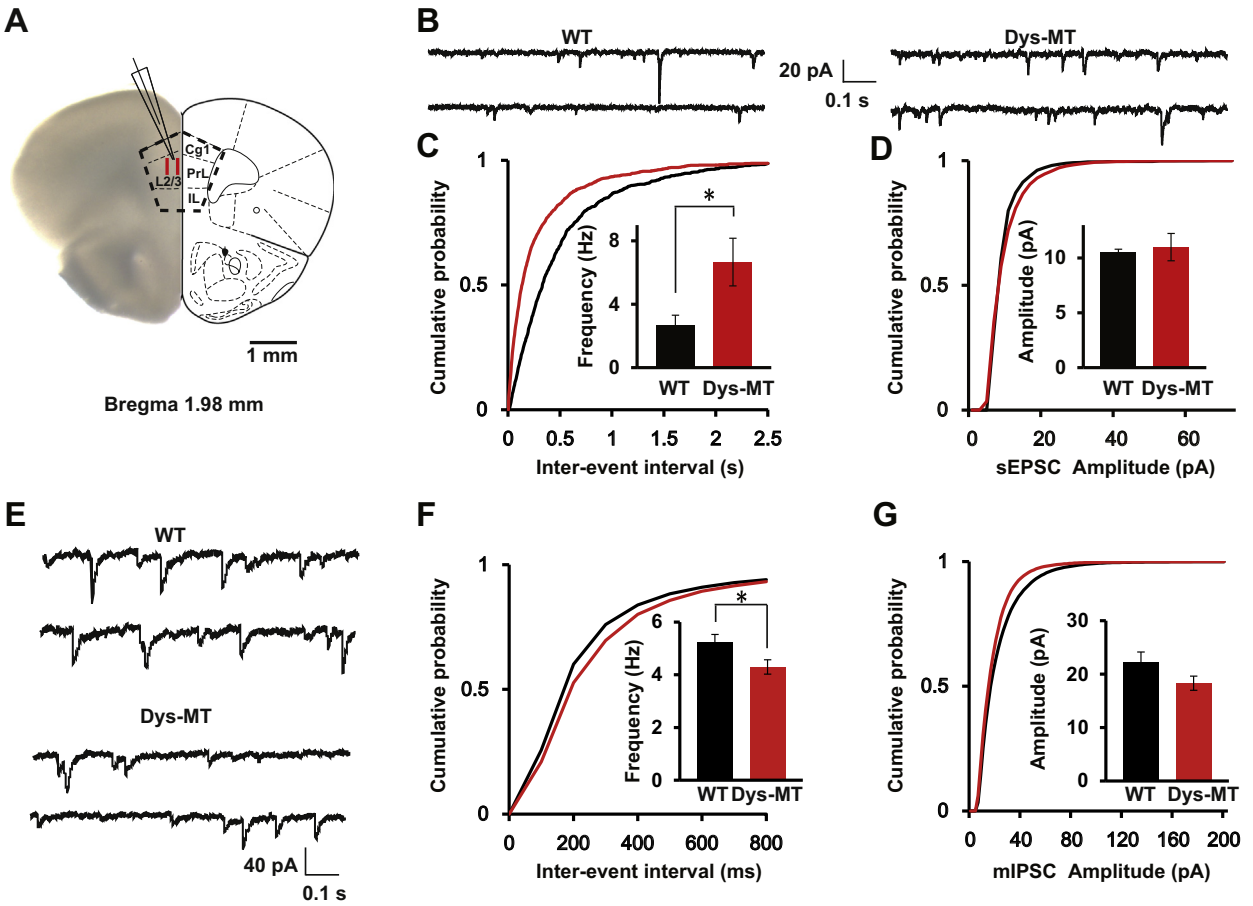

Figure 1. Enhanced excitatory basal transmission of pyramidal neurons in the medial prefrontal cortex layer 2/3 in dysbindin-1 mutant (Dys-MT) mice. **(A)** Schematic diagram of medial prefrontal cortex. **(B)** Sample traces of spontaneous excitatory postsynaptic currents (sEPSCs) recorded from the prelimbic (PrL) layer 2/3 (L2/3) pyramidal neurons in wild-type (WT) mice (left panel) and Dys-MT mice (right panel). **(C, D)** Cumulative plots and histograms of sEPSC frequency **(C)** and amplitude **(D)** recorded from the layer 2/3 pyramidal neurons in WT and Dys-MT mice (n = 9 and 11, respectively). **(E)** Sample traces of miniature inhibitory postsynaptic currents (mIPSCs) recorded from the layer 2/3 pyramidal neurons in WT and Dys-MT mice. **(F, G)** Cumulative plots and histograms of mIPSC frequency **(F)** and amplitude **(G)** recorded from the layer 2/3 pyramidal neurons in WT and Dys-MT mice (n = 26 and 19, respectively. *$p < .05$. Cg1, cingulate cortex; IL, intralimbic cortex.

Reduced Inhibitory Synapse Number in Cultured Cortical Excitatory Neurons Upon Dysbindin-1 Knockdown

Our results indicated that decreased inhibitory synaptic transmission onto pyramidal neurons in the PrL of dys-MT mice might result in increased network activity. However, it remains unclear how the loss of dysbindin-1 results in these changes. First, we investigated whether the loss of endogenous dysbindin-1 in either pyramidal neurons or inhibitory neurons affects inhibitory synaptic input. To that end, we used dissociated cultured cortical neurons and generated a series of bicistronic lentiviral constructs expressing shRNAs together with enhanced green fluorescence protein (EGFP). The expression of a specific shRNA construct targeting rat and mouse dysbindin-1 (dys-shRNA) reduced the expression of recombinant dysbindin-1 to approximately 52% of control levels in human embryonic kidney cells and reduced the expression levels of endogenous dysbindin-1 in cultured cortical neurons to 48% (Supplemental Figure S2). Cultured cortical neurons were transfected with either scrambled (scr) shRNA or dys-shRNA and were subsequently immunostained against VGAT (Figure 2A). We restricted our analysis to excitatory neurons identified based on somatic morphology as well as a calcium/calmodulin-dependent protein kinase II reporter-driven, lentiviral probe that specifically labels glutamatergic excitatory neurons (Supplemental Figure S3) (30). The knockdown of dysbindin-1 significantly reduced the inhibitory synapse density, as assessed using the number of VGAT-positive clusters compared with neurons transfected with scrambled shRNA (scr: .151 ± .018 boutons/μm; dys-shRNA: .081 ± .011 boutons/μm; $p < .01$) (Figure 2A, B). Moreover, we analyzed the number of inhibitory synapses using another inhibitory presynaptic marker, GAD65/67, and we found a similar reduction in synapses from dys-shRNA transfected neurons (scr: .145 ± .017 boutons/μm; dys-shRNA: .082 ± .016 boutons/μm; $p < .01$) (Supplemental Figure S4). To further confirm the specificity of the shRNA experiments, we performed a rescue experiment using an shRNA-resistant form of

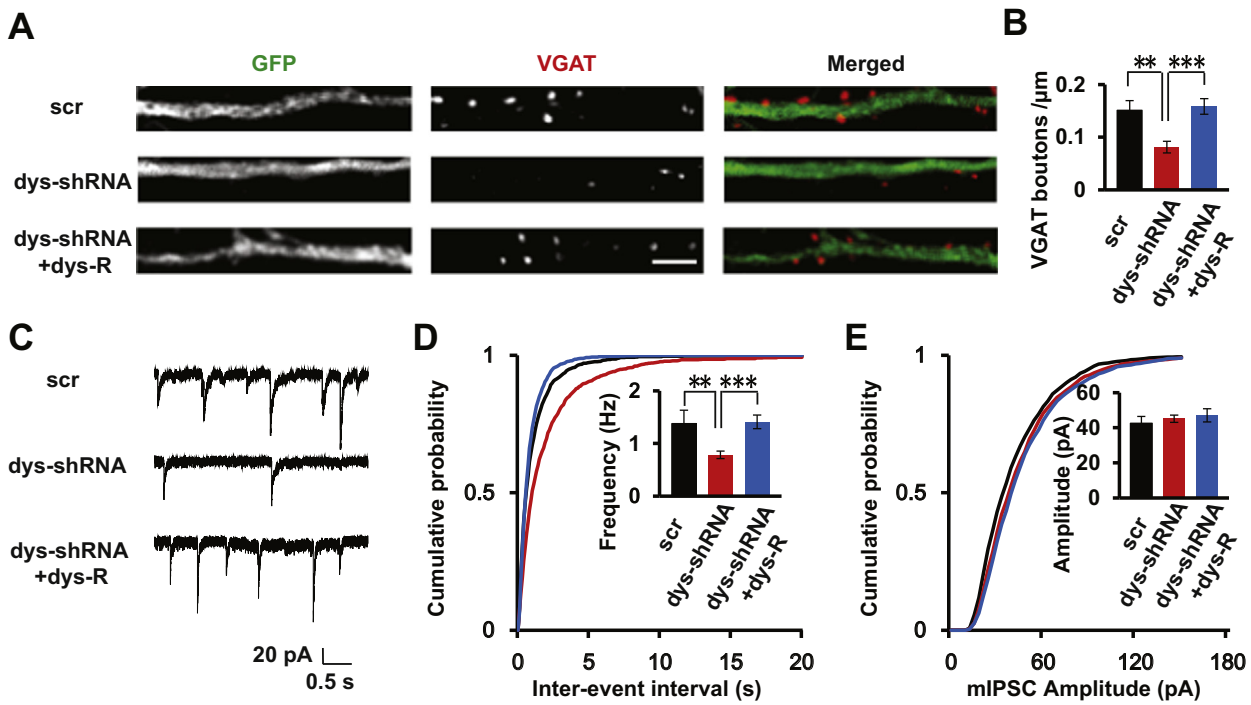

Figure 2. Dysbindin-1 knockdown reduces the numbers of inhibitory synapses in rat cultured cortical neurons. **(A)** Representative images of cultured excitatory cortical neurons transfected with scrambled short hairpin RNA (shRNA) (scr), dysbindin-1 shRNA (dys-shRNA), or dysbindin-1 shRNA with rescue (dys-shRNA + dys-R). Green fluorescent protein (GFP)-positive neurons were analyzed at day-in-vitro (DIV) 14 by immunofluorescence using antibodies against vesicular gamma-aminobutyric acid transporter (VGAT). Scale bar = 5 µm. **(B)** Summary graph of the effects of dysbindin-1 knockdown on inhibitory synapse density (scr vs. dys-shRNA), quantified using VGAT boutons, and rescued by shRNA-resistant dysbindin-1 (dys-shRNA + dys-R) (n = 12, 16, and 16, respectively). **(C–E)** Miniature inhibitory postsynaptic currents (mIPSCs) were recorded from DIV13 to DIV15 cortical neurons expressing scr, dys-shRNA, or dys-shRNA + dys-R (n = 15, 42, and 17, respectively). Shown are sample traces **(C),** cumulative plot and histograms of mIPSC frequency **(D),** and amplitude **(E)**. All histogram data are shown as means ± SEM. Statistical significance was assessed using Student t test with the Bonferroni correction (**$p < .01$; ***$p < .001$).

dysbindin-1 (dys-R) that harbors four silent mutations within the sequence targeted by the shRNA (Supplemental Figures S2A and S2B). Western blot analysis confirmed the resistance of dys-R to dys-shRNA in vitro (Supplemental Figures S2A and S2B). The effect of dysbindin-1 knockdown on the density of inhibitory synapses was rescued by the co-expression of dys-R (dys-shRNA + dys-R: .158 ± .015 boutons/µm) (Figure 2A, B). These data indicate that dysbindin-1 knockdown in excitatory neurons reduces the number of inhibitory synapses formed.

Given the reduction in the number of inhibitory synapses due to dysbindin-1 knockdown in the excitatory neurons, we recorded mIPSCs via whole-cell patch-clamp recordings of cortical pyramidal neurons that were transfected with either scr or dys-shRNA. The neurons expressing dys-shRNA displayed a significant reduction in the frequency (but not the amplitude) of the mIPSCs compared with the neurons expressing scr (scr: 1.38 ± .25 Hz, 42.6 ± 3.9 pA; dys-shRNA: .78 ± .07 Hz, 45.2 ± 2.1 pA; $p < .01$) (Figure 2C–E). This reduction in the mIPSC frequency was restored by co-expressing dys-R (dys-shRNA + dys-R: 1.41 ± .13 Hz, 47.1 ± 3.8 pA; $p = .59$) (Figure 2C–E). Taken together, our data strongly indicate that endogenously expressed dysbindin-1 in excitatory neurons regulates the number of inhibitory synaptic inputs.

To unequivocally rule out any effect of the endogenously expressed dysbindin-1 in interneurons, we performed two additional sets of experiments. First, we used a bicistronic construct expressing only GFP in the interneurons using the GAD65 promoter and analyzed the complexity, length, and branch numbers of the dendrites. We found no significant difference between the groups (Supplemental Figures S5A and S5B). Additionally, the loss of dysbindin-1 expression from interneurons did not affect the density of their presynaptic GABA-containing synaptic vesicles as evaluated with GAD67 immunostaining (Supplemental Figure S5C and S5D). Together, these results indicate that the reduction in dysbindin-1 expression in interneurons did not change morphology or the number of GABA-containing vesicles.

Reduced BDNF Secretion in Neurons Upon Dysbindin-1 Knockdown

We observed that dysbindin-1 knockdown in excitatory neurons reduced the number of inhibitory synapses on the excitatory neurons. What mediates this transneuronal modulation? Recent studies have shown that dysbindin-1 interacts with and regulates the trafficking and exocytosis of secretory vesicles (31–33). Therefore, we hypothesized that dysbindin-1 knockdown impaired the release from excitatory neurons of a

trophic factor that is critical for interneuron synapse development and/or formation on excitatory neurons. Intriguingly, BDNF may represent the only trophic factor that is a plausible candidate for this transneuronal activity (25,34). First, gene ablation studies have shown that signaling mediated by BDNF and tropomyosin receptor kinase B (TrkB), a cognate receptor of BDNF, regulates inhibitory synapse formation and the maturation of inhibitory neurons in the cortex. Furthermore, BDNF knockdown in an individual excitatory neuron in the cerebral cortex results in a reduction in inhibitory synapses on that neuron (27). However, inhibitory neurons must rely on the BDNF that is secreted from excitatory neurons because inhibitory neurons do not express BDNF messenger RNA (35). Based on these findings, we sought to determine whether dysbindin-1 knockdown in cortical pyramidal neurons reduces BDNF secretion.

First, we monitored the cellular localization of dysbindin-1 with respect to BDNF in neurons. In cortical neurons, GFP-tagged dysbindin-1 puncta co-localized with TdTomato-tagged BDNF signals, which were also overlapped with immunoreactivity of chromogranin B, a marker protein for large-dense core vesicles (LDCVs) (Supplemental Figure S6). We next examined the temporal and spatial relationship between dysbindin-1 and BDNF within neurons using confocal live-cell imaging. Live-cell imaging revealed that punctate spots of GFP-dysbindin-1 traveled together with TdTomato-BDNF-harboring vesicles along the dendrite (Movie S1) as well as an axon (Movie S2) in cortical neurons. These data suggested a potential role of dysbindin-1 in regulating the trafficking and exocytosis of BDNF secretory vesicles.

Next, to directly visualize BDNF secretion from neurons, we took advantage of a pH-sensitive variant of EGFP (superecliptic pHluorin [SEP]), the fluorescence of which is quenched at low pH, and we fused this SEP to the C-terminal end of BDNF (BDNF-SEP) (Figure 3A) (36). The distribution of BDNF-SEP, as revealed via co-immunostaining for EGFP, SMI312 (an axonal marker), and microtubule-associated protein 2 (a dendritic marker), was similar to that of endogenous BDNF stained with a BDNF-specific antibody, displaying punctate expression patterns in axons, dendrites, and cell bodies (Supplemental Figure S7). The neuronal processes that were not stained with the microtubule-associated protein 2 antibody were further confirmed to be axons; these processes were co-immunostained with an antibody against the axon-specific microtubule-associated protein SMI312 (Supplemental Figure S7). Reconstructed images in the axial plane of an axon, a dendrite, and a soma further supported the granular nature of the punctate fluorescent staining (Figure 3B), as is consistent with previous reports (Supplemental Figure S7).

To detect BDNF secretion via exocytosis, we performed time-lapse total internal reflection fluorescence imaging, which enables the selective excitation of fluorophores in the evanescent field above the cover glass (≤100 nm) (29,37). When expressed in cultured cortical neurons, BDNF-SEP puncta were sparsely distributed in axons, dendrites, and cell bodies (Figure 3B). Bath application of ammonium chloride solution (50 mmol/L, pH 7.4) (38), which deacidifies the vesicular lumen and thereby unquenches BDNF-SEP, resulted in a marked increase in the fluorescence intensity of each punctum and revealed many BDNF-SEP puncta in each neuron (Supplemental Figure S8), suggesting that BDNF-SEP was quenched in a majority of the vesicles.

Next, we monitored the activity-induced exocytosis of BDNF-SEP-containing vesicles in cultured cortical neurons by applying a high concentration of potassium ions (K^+) (60 mmol/L) to depolarize the neurons. Before high K^+ stimulation, we detected discrete, randomly appearing puncta of BDNF-SEP fluorescence (Figure 3B). These randomly appearing BDNF-SEP puncta indicated spontaneous neuronal BDNF exocytosis (39). After high K^+ treatment, BDNF-SEP fluorescence was significantly increased in the control neurons (Figure 3B; Movie S3). Additionally, the high K^+ treatment increased the number of BDNF exocytotic events, as quantified by the appearance of new fluorescent puncta at the cell surface (Figure 3C). In contrast, the increase in the number and intensity of BDNF-SEP puncta induced by high K^+ treatment was dramatically reduced in neurons expressing dys-shRNA (Figure 3C). Additionally, we monitored the activity-dependent exocytosis of BDNF-SEP in PC12 cells and found that expressing dys-shRNA markedly reduced BDNF-SEP exocytosis (Figure 3D, E). Taken together, these data suggest that dysbindin-1 knockdown reduces activity-dependent BDNF secretion from neurons.

BDNF Application Rescues Dysbindin-1 shRNA-Mediated Inhibitory Synapse Defects

The reduction in BDNF secretion from dysbindin-1 knockdown neurons suggested that BDNF could act as a crucial trophic factor in regulating the formation and/or maintenance of interneuron synapses. If this hypothesis were correct, the application of exogenous BDNF would rescue the reduced numbers of inhibitory synapses detected in cortical neurons after dysbindin-1 knockdown.

To test this hypothesis, we exogenously applied recombinant BDNF (100 ng/mL) for 4 days to cortical neurons transfected with either scr or dys-shRNA. Dysbindin-1 knockdown in excitatory neurons reduced the inhibitory synapse density, as assessed using the number of VGAT-positive clusters (scr: .134 ± .024; dys-shRNA: .076 ± .009 boutons/μm) (Figure 4A, B). Intriguingly, the application of exogenous BDNF rescued this reduction in the inhibitory synapse density in dys-shRNA-expressing neurons (BDNF with dys-shRNA: .156 ± .015 boutons/μm; $p < .001$) (Figure 4A, B). We confirmed this result using GAD65 immunostaining as an alternative method to quantify the inhibitory synapse density and found a similar change (Supplemental Figure S9). To assess the specificity of this BDNF-mediated effect, we treated dys-shRNA-expressing neurons with recombinant nerve growth factor (100 ng/mL), which has multiple synaptic effects (Supplemental Figure S10). Treatment with nerve growth factor failed to rescue the inhibitory synapse reduction in dys-shRNA-expressing neurons, indicating that the rescue of the reduction in inhibitory synapse density in dysbindin-1 knockdown neurons was specific to BDNF.

To further determine whether exogenous BDNF treatment rescues the deficits in inhibitory synaptic transmission in dys-shRNA-expressing neurons, we measured the mIPSCs from dys-shRNA-expressing neurons treated with either vehicle or BDNF (100 ng/mL). The application of BDNF (but not of the vehicle) rescued the reduction in frequency of mIPSCs recorded from dys-shRNA-expressing excitatory neurons

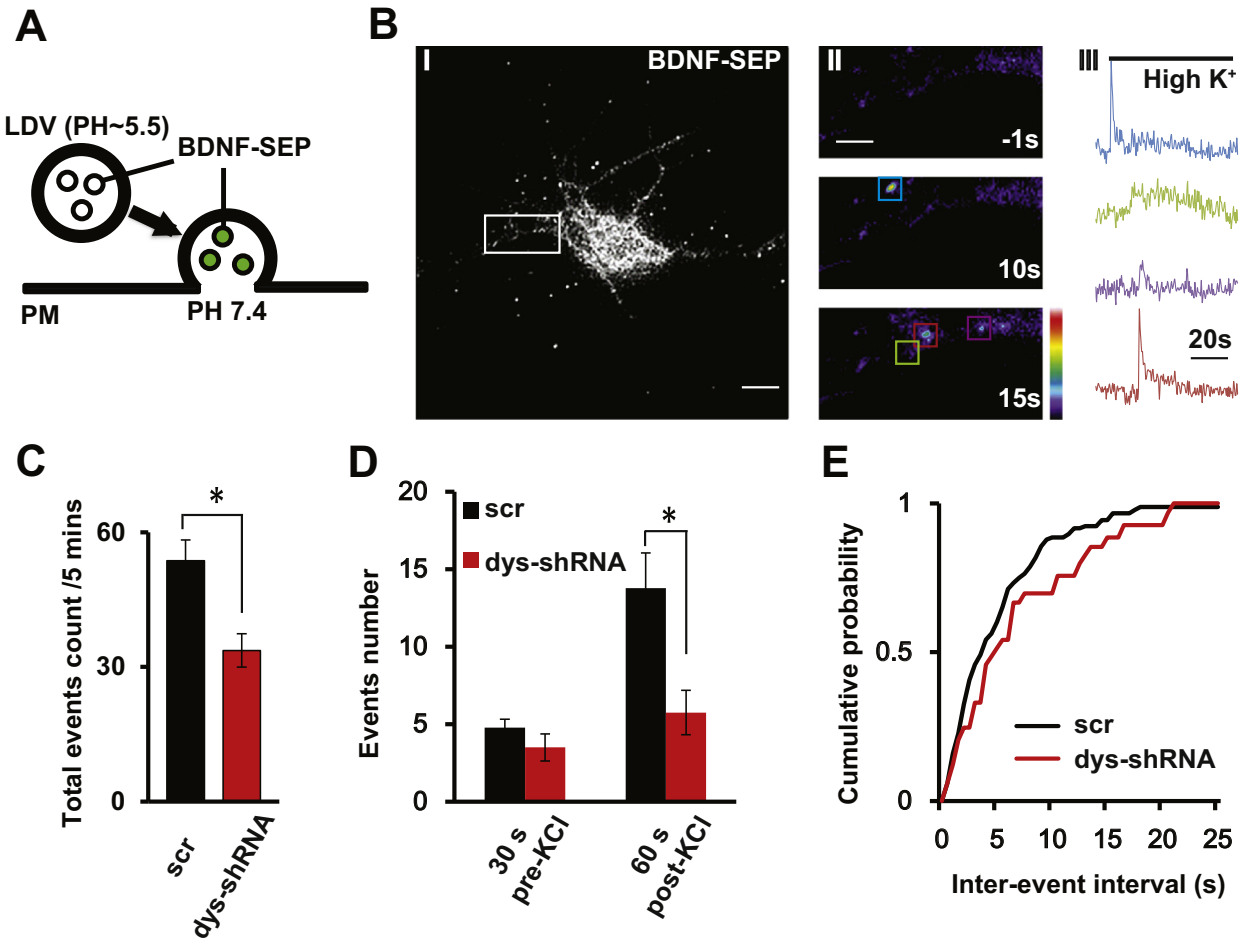

Figure 3. Dysbindin-1 knockdown reduces brain-derived neurotrophic factor (BDNF) secretion in neuronal cells and rat cultured cortical excitatory neurons. **(A)** Schematic illustration of BDNF-super-ecliptic pHluorin (SEP) fluorescence changing at different pH during BDNF secretion. **(B)** Total internal reflection fluorescence microscopic image of BDNF puncta in cultured cortical neurons transfected with BDNF-SEP (I, scale bar = 20 μm) and magnified views of BDNF puncta in boxed area of panel I at 1 second before and 10 seconds and 15 seconds after potassium chloride (KCl) stimulation, respectively (II, scale bar = 5 μm). Panel III, sample traces showing fluorescence change with time at boxed areas in panel **(B)**, representing different events. **(C)** Histograms of total event numbers during 5 minutes after KCl stimulation in cultured cortical neurons transfected with scrambled short hairpin RNA (shRNA) (scr), dysbindin-1 shRNA (dys-shRNA) (n = 10 and 12, respectively). **(D)** Histograms and **(E)** cumulative plots of events number during 1 minute after KCl stimulation in PC12 cells transfected with scr or dys-shRNA (n = 6 and 5, respectively). All histogram data are shown as means ± SEM. Statistical significance was assessed using Student t test (*$p < .05$). K^+, potassium ions; LDV, large dense core vesicle; PM, plasma membrane.

(scr: 1.87 ± .30 Hz, 47.1 ± 3.5 pA; dys-shRNA: .73 ± .12 Hz, 54.24 ± 2.37 pA; dys-shRNA with BDNF: 1.63 ± .30 Hz, 56.2 ± 3.7 pA; $p < .01$) (Figure 4C–E). Taken together, we conclude that BDNF selectively rescues the reduction in the number of functional inhibitory synapses in cortical excitatory neurons induced by dysbindin-1 knockdown.

Rescued Inhibitory Synaptic Transmission Upon BDNF Application in Ex Vivo Organotypic Hippocampal Slices

To investigate the effects of the lack of endogenous dysbindin-1 expression on excitatory pyramidal neuron function ex vivo, we transfected our shRNA constructs into organotypic hippocampal slices via biolistic gene gun (27). The transfected neurons were marked with simultaneously introduced EGFP (Figure 5A). To induce an extremely sparse distribution of the transfected neurons, steel mesh was attached to the muzzle of the gene guns (40). Typically, fewer than 10 EGFP-expressing neurons were detected among the vast number of neurons within each slice culture (Figure 5A).

Next, we measured the inhibitory synaptic transmission in hippocampal pyramidal neurons expressing either scr or dys-shRNA. Analysis of the mIPSCs revealed marked decreases in the frequency of mIPSCs in dys-shRNA-expressing pyramidal neurons compared with scr-expressing pyramidal neurons (scr: 5.504 ± .476 Hz, 40.32 ± 1.70 pA; dys-shRNA: 3.6 ± .51 Hz, 34.6 ± 3.4 pA; $p < .05$) (Figure 5C, D). Intriguingly, these inhibitory synaptic deficits were rescued by BDNF application (100 ng/mL, 4 days) (dys-shRNA: 3.6 ± .51 Hz, 34.6 ± 3.4 pA; dys-shRNA with BDNF: 5.984 ± .561 Hz, 32.5 ± 2.12 pA; $p < .01$) (Figure 5C, D), as indicated by the

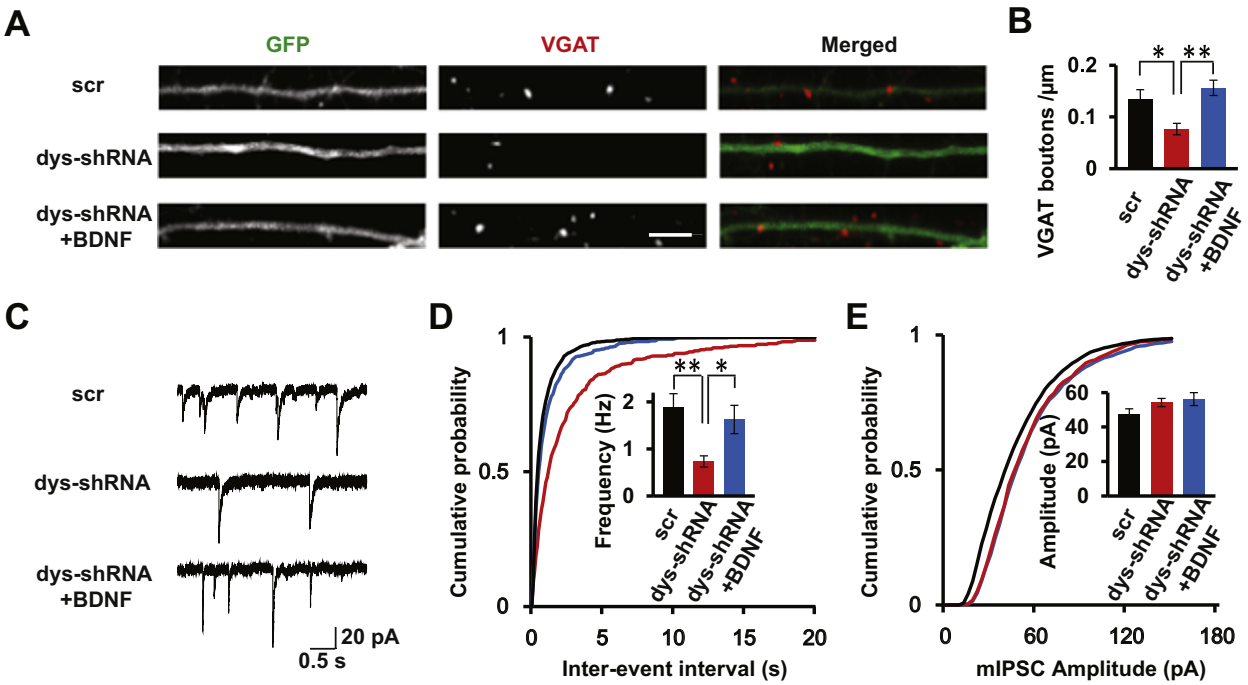

Figure 4. Brain-derived neurotrophic factor (BDNF) application rescues defects in inhibitory synapses induced by dysbindin-1 (dys) short hairpin RNA (shRNA) (dys-shRNA). **(A)** Representative images of rat cultured excitatory cortical neurons transfected with scrambled shRNA (scr) or dys-shRNA and treated with or without BDNF. Green fluorescent protein (GFP)-positive neurons were analyzed on day-in-vitro (DIV) 14 by immunofluorescence using antibodies against vesicular gamma-aminobutyric acid transporter (VGAT). Scale bar = 5 μm. **(B)** Summary graph of BDNF-mediated rescue of defects in inhibitory synapses caused by dysbindin-1 shRNA (scr vs. dys-shRNA vs. dys-shRNA + BDNF, n = 14, 23, and 15, respectively). **(C–E)** Miniature inhibitory postsynaptic currents (mIPSCs) were recorded from DIV13 to DIV15 cortical neurons treated with or without BDNF. Shown are sample traces **(C),** cumulative plot and histograms of mIPSC frequency **(D),** and amplitude **(E)** (scr vs. dys-shRNA vs. dys-shRNA + BDNF, n = 20, 18, and 13, respectively). All histogram data are shown as means ± SEM. Statistical significance was assessed using Student t test with the Bonferroni correction (*$p < .05$; **$p < .01$).

significant shifts in the cumulative distribution curves for the mIPSC interevent interval.

DISCUSSION

Since the dysbindin-1 gene was identified, extensive molecular, cell biological, animal model, and human genotype-phenotype studies have been conducted to address the multiple functions and mechanisms of dysbindin-1 activity in the brain. Because dysbindin-1 is thought to mediate a range of intermediate phenotypes that underlie schizophrenia (21,41,42), elucidating the precise biological functions of dysbindin-1 have received intensive study. Disruption of dysbindin-1 is thought to confer glutamatergic dysfunction (43). However, in mice, a loss-of-function mutation in dysbindin-1 (dys-MT) disrupts inhibitory neurotransmission in both the hippocampus and the prefrontal cortex (19–21) via an unknown mechanism. In the present study, we discovered an unexpected role of dysbindin-1 for the exocytosis of BDNF from excitatory neurons that consequently transneuronally reduced the number of functional inhibitory synapses formed on the excitatory neurons.

Dysbindin-1, BDNF, and Their Roles in Inhibitory Synapse Development

BDNF is a neurotrophic factor that regulates synapse development and plasticity in the mammalian brain (44,45). Interestingly, BDNF regulates the maturation of interneurons, the dysfunction of which has been associated with schizophrenia (46). For example, in BDNF knockout mice, the number of interneurons is significantly reduced compared with that in wild-type mice (47,48). Conversely, in transgenic mice overexpressing BDNF, the maturation of interneurons was significantly accelerated; this effect was accompanied by an increase in spontaneous network activity (49,50). Similarly, the application of exogenous BDNF to interneuron cultures resulted in accelerated formation of reciprocal connections via enhanced TrkB signaling (51).

Intriguingly, GABAergic interneurons do not express BDNF messenger RNA and thus cannot produce BDNF themselves (52). Therefore, from whence do these neurons receive the BDNF that is necessary for their function? Kohara *et al.* (26) reported that in co-cultures of BDNF-KO neurons with GFP-labeled wild-type neurons, interneurons receive BDNF released from excitatory neurons (26). They later found that BDNF ablation in a single excitatory neuron induced a local reduction in the number of inhibitory synapses to that BDNF-knockout excitatory neuron (27). Likewise, enhanced BDNF exocytosis in synaptotagmin-IV knockout mice (53,54) and reduced BDNF exocytosis in calcium-dependent activator protein for secretion 2 knockout mice have been shown to result in increased and decreased GABAergic synaptic transmission, respectively (55). These findings suggest that BDNF (presumably released from excitatory neurons) shapes and

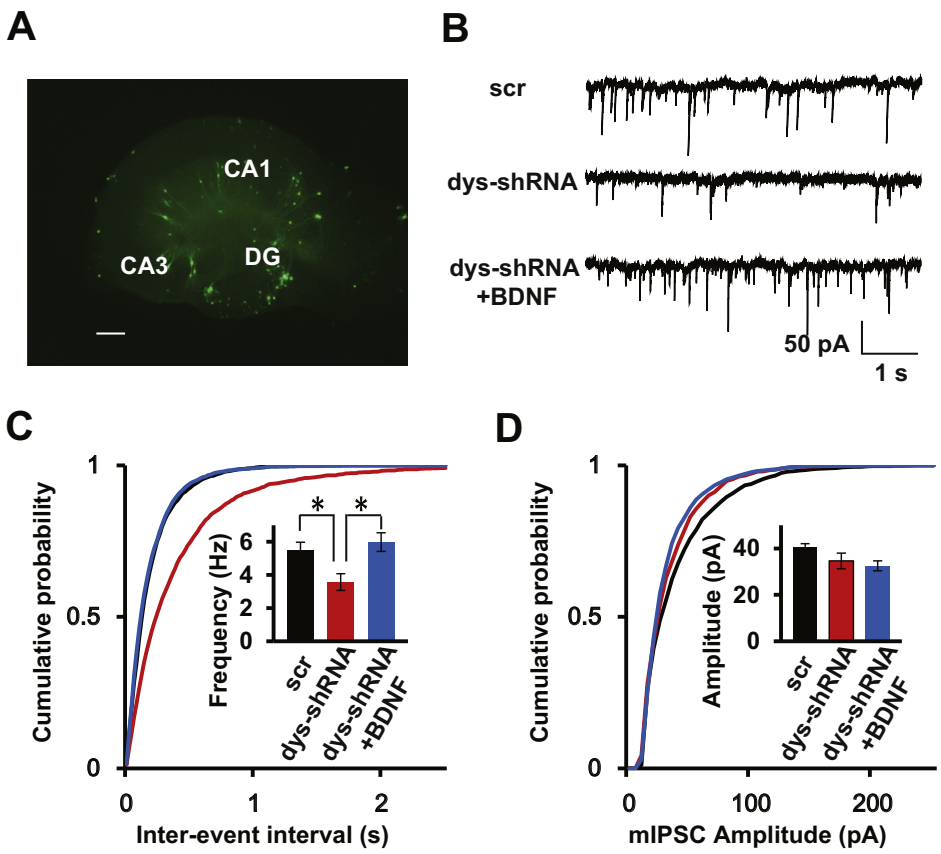

Figure 5. Dysbindin-1 knockdown reduces inhibitory synaptic transmission, which is restored by exogenous brain-derived neurotrophic factor (BDNF) in ex vivo rat hippocampal organotypic slices. **(A)** Representative image of a cultured organotypic slice transfected with dysbindin-1 (dys) short hairpin RNA (shRNA) (dys-shRNA). **(B–D)** Miniature inhibitory postsynaptic currents (mIPSCs) were recorded from transfected neurons expressing scrambled shRNA (scr), dys-shRNA, or dys-shRNA + BDNF (n = 9, 12, and 12, respectively) in organotypic slices. Shown are sample traces **(B),** cumulative plot and histograms of mIPSC frequency **(C),** and amplitude **(D)**. All histogram data are shown as means ± SEM. Statistical significance was assessed using Student t test with the Bonferroni correction (*$p < .05$). CA, cornu ammonis; DG, dentate gyrus.

regulates local inhibitory synapse formation and/or maintenance. Therefore, it is conceivable that any defect in the cellular and molecular machinery that regulates BDNF trafficking and release from excitatory neurons could affect inhibitory synapse formation on these excitatory neurons.

Here, we demonstrate that the loss of dysbindin-1 from cortical excitatory neurons leads to a reduction in functional inhibitory synapses, as demonstrated previously in the dys-MT mouse cortex and hippocampus (Figures 1 and 2) (20,21). However, the loss of dysbindin-1 from inhibitory neurons did not result in any defects in dendritic morphology or formation of axonal synaptic boutons (Supplemental Figure S4). This was potentially due to the lack of expression of dysbindin-1 in GABAergic neurons (22). Additionally, we show that the impairment in activity-dependent BDNF release from dysbindin-1 knockdown cortical neurons and neuronal cells (Figure 3) and, intriguingly, the inhibitory synapse defects exhibited by dysbindin-1 knockdown neurons were rescued by BDNF application (Figures 4 and 5). Our data reveal novel transneuronal signaling between excitatory and inhibitory neurons and provide new insight into the cellular and molecular mechanisms that link these abnormalities in BDNF secretion to psychiatric disorders.

Potential Mechanisms of Reduced BDNF Exocytosis in Dysbindin-1 Knockdown Neurons

Dysbindin-1A is a component of the biogenesis of lysosome-related organelles complex 1 (BLOC-1) complex, and several studies have demonstrated that the loss of dysbindin-1 is accompanied by the loss of all BLOC-1 complex constituents (11,22,56,57). Although our dysbindin-1 shRNA is designed to target conserved regions in both dysbindin-1A and dysbindin-1C isoforms, co-expression of the dysbindin-1A isoform rescued dysbindin-1 shRNA associated interneuron synaptic defects, indicating that the dysbindin-1A isoform might affect BDNF exocytosis. Further rescue experiments using dysbindin-1C isoform or isoform-specific shRNAs may elucidate which of the dysbindin-1 isoforms contribute to BDNF release and transneuronal interneuron synapse maintenance.

In addition, dysbindin-1 and three other subunits of BLOC-1 (pallidin, snapin, and muted) interact with adaptor protein complex 3 (AP-3) and are also expressed in AP-3-containing vesicles in neurons (58). AP-3 has generally been considered to function in endocytic rather than biosynthetic pathways; however, a recent RNA interference-based screen identified that AP-3 functions in the formation, trafficking, and regulated exocytosis of LDCVs, which contain the BDNF that is secreted from neurons (59). Additionally, the loss of AP-3 affects the number, morphology, and density of LDCVs in neurons (59,60). Furthermore, mice lacking the neuronal isoform of AP-3 (ap-3μ knockout mice) exhibited the reduced inhibitory neurotransmission and abnormal propagation of neuronal excitability that were detected in dys-MT mice (61). More importantly, the BDNF content was reduced in the hippocampus of BLOC-1-deficient mice (62). Taken together,

we speculate that defects in the dysbindin-1 interactome may impair BDNF release via AP-3-mediated LDCV function, but this possibility also requires further investigation.

Targeting Interneuron and BDNF/TrkB Signaling for Treatment of Schizophrenia

Dysbindin-1 protein expression is widely distributed throughout the brain, but it is concentrated in synapses in brain regions that are commonly affected in schizophrenia, such as the hippocampus, the striatum, and the prefrontal cortex (11). Interestingly, decreases in one or more dysbindin-1 isoforms have been detected in the cortex and the hippocampus of schizophrenic patients (11,15,63). Furthermore, dys-MT mice exhibited behavioral deficits resembling schizophrenia intermediate phenotypes; thus, dys-MT mice represent a good animal model of schizophrenia.

Impaired synaptic connectivity is a predominant hypothesis of the pathology of schizophrenia and has been suggested by the results of human postmortem studies (64). However, there is insufficient information about the cellular and molecular mechanisms underlying this potentially impaired synaptic connectivity in schizophrenia. It is widely believed that the impaired synaptic connectivity between GABAergic and glutamatergic neurons may cause network imbalance and the eventual impairment of brain function that is commonly observed in schizophrenic patients (4,64–66). Here, we provide cellular and molecular evidence that interneuron dysfunction may be the primary cause of the overall synapse deficits associated with dysfunction of two genes implicated in the pathophysiology of this illness (67). If these suppositions are correct, it may be possible to ameliorate the impaired GABAergic synapse formation and the subsequent synaptic imbalance between excitatory and inhibitory signaling within local cortical circuits by enhancing or restoring the levels of BDNF signaling. Therefore, it will be exciting to evaluate the ability of BDNF mimetic peptides and small molecules that enhance BDNF translation (68,69) in an attempt to restore inhibitory synapse function in dys-MT mice (70).

ACKNOWLEDGMENTS AND DISCLOSURES

This work was supported by the Singapore Ministry of Education Academic Research Fund (MOE2012-T2-1-021) and Duke-National University of Singapore Signature Research Program Block Grant (to HSJ).

QY, FY, and HSJ designed the research; QY, FY, YX, ST, NH, MR, ZH, KM, JSN, PJK, and HSJ performed the research; WH, KN, and DRW provided reagents, materials, and analysis tools and edited the manuscript; QY, FY, YX, ST, and HSJ analyzed the data; and HSJ wrote the paper.

We thank Dr. Marina Ceccarini at Centro Nazionale Malattie Rare for providing dysbindin-1 antibody and we also thank Dr. Zheng Li at the National Institute for Mental Health for providing dysbindin-1 mutant mice. We thank Hasini Ganegala for her excellent technical assistance. We thank Drs. Shirish Shenolikar and Zeng Li for their critical comments on the manuscript.

All authors report no biomedical financial interests or potential conflicts of interest.

ARTICLE INFORMATION

From the Molecular Neurophysiology Laboratory (QY, YX, ST, NH, JSN, PJK, HSJ), Signature Program in Neuroscience and Behavioral Disorders, Duke-National University of Singapore Graduate Medical School, Singapore, Singapore; Lieber Institute for Brain Development (FY, MR, ZH, KM, DRW), Johns Hopkins Medical Campus, Baltimore, Maryland; Singapore Bioimaging Consortium (WH), Singapore, Singapore; Institute for Developmental Research (KN), Aichi Human Service Center, Kasugai, Japan; and Department of Physiology (HSJ), Yong Loo Lin School of Medicine, National University of Singapore, Singapore, Singapore.

QY and FY contributed equally to this work.

Address correspondence to H. Shawn Je, Ph.D., Duke-NUS Graduate Medical School, 8 College Road, Singapore 169857, Singapore; E-mail: shawn.je@duke-nus.edu.sg.

Received Oct 24, 2014; revised Aug 12, 2015; accepted Aug 12, 2015.

Supplementary material cited in this article is available online at http://dx.doi.org/10.1016/j.biopsych.2015.08.019.

REFERENCES

1. Cline H (2005): Synaptogenesis: A balancing act between excitation and inhibition. Curr Biol 15:R203–R205.
2. DeFelipe J (1993): Neocortical neuronal diversity: Chemical heterogeneity revealed by colocalization studies of classic neurotransmitters, neuropeptides, calcium-binding proteins, and cell surface molecules. Cereb Cortex 3:273–289.
3. Nakazawa K, Zsiros V, Jiang Z, Nakao K, Kolata S, Zhang S, Belforte JE (2012): GABAergic interneuron origin of schizophrenia pathophysiology. Neuropharmacology 62:1574–1583.
4. Lewis DA, Hashimoto T, Volk DW (2005): Cortical inhibitory neurons and schizophrenia. Nat Rev Neurosci 6:312–324.
5. McGrath J, Saha S, Chant D, Welham J (2008): Schizophrenia: A concise overview of incidence, prevalence, and mortality. Epidemiol Rev 30:67–76.
6. Akbarian S, Huang HS (2006): Molecular and cellular mechanisms of altered GAD1/GAD67 expression in schizophrenia and related disorders. Brain Res Rev 52:293–304.
7. Gonzalez-Burgos G, Hashimoto T, Lewis DA (2010): Alterations of cortical GABA neurons and network oscillations in schizophrenia. Curr Psychiatry Rep 12:335–344.
8. Straub RE, Jiang Y, MacLean CJ, Ma Y, Webb BT, Myakishev MV, *et al*. (2002): Genetic variation in the 6p22.3 gene DTNBP1, the human ortholog of the mouse dysbindin gene, is associated with schizophrenia. Am J Hum Genet 71:337–348.
9. Riley B, Kuo PH, Maher BS, Fanous AH, Sun J, Wormley B, *et al*. (2009): The dystrobrevin binding protein 1 (DTNBP1) gene is associated with schizophrenia in the Irish Case Control Study of Schizophrenia (ICCSS) sample. Schizophr Res 115:245–253.
10. Ayalew M, Le-Niculescu H, Levey DF, Jain N, Changala B, Patel SD, *et al*. (2012): Convergent functional genomics of schizophrenia: From comprehensive understanding to genetic risk prediction. Mol Psychiatry 17:887–905.
11. Talbot K, Ong WY, Blake DJ, Tang J, Louneva N, Carlson GC, *et al*. (2009): Dysbindin-1 and its protein family. In: Lajtha A, Javitt D, Kantrowitz J, editors. Handbook of Neurochemistry and Molecular Neurobiology. New York: Springer, 107–241.
12. Talbot K, Cho DS, Ong WY, Benson MA, Han LY, Kazi HA, *et al*. (2006): Dysbindin-1 is a synaptic and microtubular protein that binds brain snapin. Hum Mol Genet 15:3041–3054.
13. Talbot K, Eidem WL, Tinsley CL, Benson MA, Thompson EW, Smith RJ, *et al*. (2004): Dysbindin-1 is reduced in intrinsic, glutamatergic terminals of the hippocampal formation in schizophrenia. J Clin Invest 113:1353–1363.
14. Weickert CS, Rothmond DA, Hyde TM, Kleinman JE, Straub RE (2008): Reduced DTNBP1 (dysbindin-1) mRNA in the hippocampal formation of schizophrenia patients. Schizophr Res 98:105–110.
15. Tang J, LeGros RP, Louneva N, Yeh L, Cohen JW, Hahn CG, *et al*. (2009): Dysbindin-1 in dorsolateral prefrontal cortex of schizophrenia cases is reduced in an isoform-specific manner unrelated to dysbindin-1 mRNA expression. Hum Mol Genet 18:3851–3863.
16. Karlsgodt KH, Robleto K, Trantham-Davidson H, Jairl C, Cannon TD, Lavin A, Jentsch JD (2011): Reduced dysbindin expression mediates N-methyl-D-aspartate receptor hypofunction and impaired working memory performance. Biol Psychiatry 69:28–34.
17. Jentsch JD, Trantham-Davidson H, Jairl C, Tinsley M, Cannon TD, Lavin A (2009): Dysbindin modulates prefrontal cortical glutamatergic

circuits and working memory function in mice. Neuropsychopharmacology 34:2601–2608.
18. Talbot K (2009): The sandy (sdy) mouse: A dysbindin-1 mutant relevant to schizophrenia research. Prog Brain Res 179:87–94.
19. Carlson GC, Talbot K, Halene TB, Gandal MJ, Kazi HA, Schlosser L, *et al*. (2011): Dysbindin-1 mutant mice implicate reduced fast-phasic inhibition as a final common disease mechanism in schizophrenia. Proc Natl Acad Sci U S A 108:E962–E970.
20. Ji Y, Yang F, Papaleo F, Wang HX, Gao WJ, Weinberger DR, Lu B (2009): Role of dysbindin in dopamine receptor trafficking and cortical GABA function. Proc Natl Acad Sci U S A 106:19593–19598.
21. Papaleo F, Yang F, Garcia S, Chen J, Lu B, Crawley JN, Weinberger DR (2012): Dysbindin-1 modulates prefrontal cortical activity and schizophrenia-like behaviors via dopamine/D2 pathways. Mol Psychiatry 17:85–98.
22. Wang H, Yuan Y, Zhang Z, Yan H, Feng Y, Li W (2014): Dysbindin-1C is required for the survival of hilar mossy cells and the maturation of adult newborn neurons in dentate gyrus. J Biol Chem 289: 29060–29072.
23. Yamada MK, Nakanishi K, Ohba S, Nakamura T, Ikegaya Y, Nishiyama N, Matsuki N (2002): Brain-derived neurotrophic factor promotes the maturation of GABAergic mechanisms in cultured hippocampal neurons. J Neurosci 22:7580–7585.
24. Kohara K, Kitamura A, Morishima M, Tsumoto T (2001): Activity-dependent transfer of brain-derived neurotrophic factor to postsynaptic neurons. Science 291:2419–2423.
25. Woo NH, Lu B (2006): Regulation of cortical interneurons by neurotrophins: From development to cognitive disorders. Neuroscientist 12: 43–56.
26. Kohara K, Kitamura A, Adachi N, Nishida M, Itami C, Nakamura S, Tsumoto T (2003): Inhibitory but not excitatory cortical neurons require presynaptic brain-derived neurotrophic factor for dendritic development, as revealed by chimera cell culture. J Neurosci 23:6123–6131.
27. Kohara K, Yasuda H, Huang Y, Adachi N, Sohya K, Tsumoto T (2007): A local reduction in cortical GABAergic synapses after a loss of endogenous brain-derived neurotrophic factor, as revealed by single-cell gene knock-out method. J Neurosci 27:7234–7244.
28. Ito H, Morishita R, Shinoda T, Iwamoto I, Sudo K, Okamoto K, Nagata K (2010): Dysbindin-1, WAVE2 and Abi-1 form a complex that regulates dendritic spine formation. Mol Psychiatry 15:976–986.
29. Mabb AM, Je HS, Wall MJ, Robinson CG, Larsen RS, Qiang Y, *et al*. (2014): Triad3A regulates synaptic strength by ubiquitination of Arc. Neuron 82:1299–1316.
30. Shcheglovitov A, Shcheglovitova O, Yazawa M, Portmann T, Shu R, Sebastiano V, *et al*. (2013): SHANK3 and IGF1 restore synaptic deficits in neurons from 22q13 deletion syndrome patients. Nature 503: 267–271.
31. Gokhale A, Larimore J, Werner E, So L, Moreno-De-Luca A, Lese-Martin C, *et al*. (2012): Quantitative proteomic and genetic analyses of the schizophrenia susceptibility factor dysbindin identify novel roles of the biogenesis of lysosome-related organelles complex 1. J Neurosci 32:3697–3711.
32. Larimore J, Tornieri K, Ryder PV, Gokhale A, Zlatic SA, Craige B, *et al*. (2011): The schizophrenia susceptibility factor dysbindin and its associated complex sort cargoes from cell bodies to the synapse. Mol Biol Cell 22:4854–4867.
33. Mead CL, Kuzyk MA, Moradian A, Wilson GM, Holt RA, Morin GB (2010): Cytosolic protein interactions of the schizophrenia susceptibility gene dysbindin. J Neurochem 113:1491–1503.
34. Autry AE, Monteggia LM (2012): Brain-derived neurotrophic factor and neuropsychiatric disorders. Pharmacol Rev 64:238–258.
35. Rocamora N, Pascual M, Acsady L, de Lecea L, Freund TF, Soriano E (1996): Expression of NGF and NT3 mRNAs in hippocampal interneurons innervated by the GABAergic septohippocampal pathway. J Neurosci 16:3991–4004.
36. Miesenbock G, De Angelis DA, Rothman JE (1998): Visualizing secretion and synaptic transmission with pH-sensitive green fluorescent proteins. Nature 394:192–195.
37. Merrifield CJ, Perrais D, Zenisek D (2005): Coupling between clathrin-coated-pit invagination, cortactin recruitment, and membrane scission observed in live cells. Cell 121:593–606.
38. Kennedy MJ, Davison IG, Robinson CG, Ehlers MD (2010): Syntaxin-4 defines a domain for activity-dependent exocytosis in dendritic spines. Cell 141:524–535.
39. Matsuda N, Lu H, Fukata Y, Noritake J, Gao H, Mukherjee S, *et al*. (2009): Differential activity-dependent secretion of brain-derived neurotrophic factor from axon and dendrite. J Neurosci 29:14185–14198.
40. Lee SJ, Escobedo-Lozoya Y, Szatmari EM, Yasuda R (2009): Activation of CaMKII in single dendritic spines during long-term potentiation. Nature 458:299–304.
41. Feng YQ, Zhou ZY, He X, Wang H, Guo XL, Hao CJ, *et al*. (2008): Dysbindin deficiency in sandy mice causes reduction of snapin and displays behaviors related to schizophrenia. Schizophr Res 106: 218–228.
42. Hattori S, Murotani T, Matsuzaki S, Ishizuka T, Kumamoto N, Takeda M, *et al*. (2008): Behavioral abnormalities and dopamine reductions in sdy mutant mice with a deletion in Dtnbp1, a susceptibility gene for schizophrenia. Biochem Biophys Res Commun 373:298–302.
43. Chen XW, Feng YQ, Hao CJ, Guo XL, He X, Zhou ZY, *et al*. (2008): DTNBP1, a schizophrenia susceptibility gene, affects kinetics of transmitter release. J Cell Biol 181:791–801.
44. Lu B, Je HS (2003): Neurotrophic regulation of the development and function of the neuromuscular synapses. J Neurocytol 32:931–941.
45. Lu B, Woo NH (2006): Trophic factors in synaptic plasticity and memory. In: Albright T, Bloom F, Gage F, Spitzer N, Squire L, editors. New Encyclopedia of Neuroscience. New York: Elsevier.
46. Konopaske GT, Sweet RA, Wu Q, Sampson A, Lewis DA (2006): Regional specificity of chandelier neuron axon terminal alterations in schizophrenia. Neuroscience 138:189–196.
47. Jones KR, Farinas I, Backus C, Reichardt LF (1994): Targeted disruption of the BDNF gene perturbs brain and sensory neuron development but not motor neuron development. Cell 76:989–999.
48. Altar CA, Boylan CB, Fritsche M, Jackson C, Hyman C, Lindsay RM (1994): The neurotrophins NT-4/5 and BDNF augment serotonin, dopamine, and GABAergic systems during behaviorally effective infusions to the substantia nigra. Exp Neurol 130:31–40.
49. Huang ZJ, Kirkwood A, Pizzorusso T, Porciatti V, Morales B, Bear MF, *et al*. (1999): BDNF regulates the maturation of inhibition and the critical period of plasticity in mouse visual cortex. Cell 98:739–755.
50. Aguado F, Carmona MA, Pozas E, Aguilo A, Martinez-Guijarro FJ, Alcantara S, *et al*. (2003): BDNF regulates spontaneous correlated activity at early developmental stages by increasing synaptogenesis and expression of the K+/Cl- co-transporter KCC2. Development 130:1267–1280.
51. Berghuis P, Dobszay MB, Sousa KM, Schulte G, Mager PP, Hartig W, *et al*. (2004): Brain-derived neurotrophic factor controls functional differentiation and microcircuit formation of selectively isolated fast-spiking GABAergic interneurons. Eur J Neurosci 20:1290–1306.
52. Ernfors P, Wetmore C, Olson L, Persson H (1990): Identification of cells in rat brain and peripheral tissues expressing mRNA for members of the nerve growth factor family. Neuron 5:511–526.
53. Dean C, Liu H, Staudt T, Stahlberg MA, Vingill S, Buckers J, *et al*. (2012): Distinct subsets of Syt-IV/BDNF vesicles are sorted to axons versus dendrites and recruited to synapses by activity. J Neurosci 32: 5398–5413.
54. Dean C, Liu H, Dunning FM, Chang PY, Jackson MB, Chapman ER (2009): Synaptotagmin-IV modulates synaptic function and long-term potentiation by regulating BDNF release. Nat Neurosci 12:767–776.
55. Shinoda Y, Sadakata T, Nakao K, Katoh-Semba R, Kinameri E, Furuya A, *et al*. (2011): Calcium-dependent activator protein for secretion 2 (CAPS2) promotes BDNF secretion and is critical for the development of GABAergic interneuron network. Proc Natl Acad Sci U S A 108:373–378.
56. Larimore J, Zlatic SA, Gokhale A, Tornieri K, Singleton KS, Mullin AP, *et al*. (2014): Mutations in the BLOC-1 subunits dysbindin and muted generate divergent and dosage-dependent phenotypes. J Biol Chem 289:14291–14300.

57. Li W, Zhang Q, Oiso N, Novak EK, Gautam R, O'Brien EP, *et al*. (2003): Hermansky-Pudlak syndrome type 7 (HPS-7) results from mutant dysbindin, a member of the biogenesis of lysosome-related organelles complex 1 (BLOC-1). Nat Genet 35:84–89.
58. Taneichi-Kuroda S, Taya S, Hikita T, Fujino Y, Kaibuchi K (2009): Direct interaction of dysbindin with the AP-3 complex via its mu subunit. Neurochem Int 54:431–438.
59. Asensio CS, Sirkis DW, Edwards RH (2010): RNAi screen identifies a role for adaptor protein AP-3 in sorting to the regulated secretory pathway. J Cell Biol 191:1173–1187.
60. Kantheti P, Qiao X, Diaz ME, Peden AA, Meyer GE, Carskadon SL, *et al*. (1998): Mutation in AP-3 delta in the mocha mouse links endosomal transport to storage deficiency in platelets, melanosomes, and synaptic vesicles. Neuron 21:111–122.
61. Nakatsu F, Okada M, Mori F, Kumazawa N, Iwasa H, Zhu G, *et al*. (2004): Defective function of GABA-containing synaptic vesicles in mice lacking the AP-3B clathrin adaptor. J Cell Biol 167: 293–302.
62. Larimore J, Ryder PV, Kim KY, Ambrose LA, Chapleau C, Calfa G, *et al*. (2013): MeCP2 regulates the synaptic expression of a Dysbindin-BLOC-1 network component in mouse brain and human induced pluripotent stem cell-derived neurons. PLoS One 8: e65069.
63. Talbot K, Louneva N, Cohen JW, Kazi H, Blake DJ, Arnold SE (2011): Synaptic dysbindin-1 reductions in schizophrenia occur in an isoform-specific manner indicating their subsynaptic location. PLoS One 6:e16886.
64. Lewis DA, Gonzalez-Burgos G (2008): Neuroplasticity of neocortical circuits in schizophrenia. Neuropsychopharmacology 33: 141–165.
65. Goldman-Rakic PS (1995): Cellular basis of working memory. Neuron 14:477–485.
66. Le Magueresse C, Monyer H (2013): GABAergic interneurons shape the functional maturation of the cortex. Neuron 77:388–405.
67. Weickert CS, Ligons DL, Romanczyk T, Ungaro G, Hyde TM, Herman MM, *et al*. (2005): Reductions in neurotrophin receptor mRNAs in the prefrontal cortex of patients with schizophrenia. Mol Psychiatry 10:637–650.
68. Longo FM, Yang T, Knowles JK, Xie Y, Moore LA, Massa SM (2007): Small molecule neurotrophin receptor ligands: Novel strategies for targeting Alzheimer's disease mechanisms. Curr Alzheimer Res 4: 503–506.
69. Price RD, Milne SA, Sharkey J, Matsuoka N (2007): Advances in small molecules promoting neurotrophic function. Pharmacol Ther 115: 292–306.
70. Nestler EJ, Hyman SE (2010): Animal models of neuropsychiatric disorders. Nat Neurosci 13:1161–1169.