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DISC1 Protein Regulates γ-Aminobutyric Acid, Type A ($GABA_A$) Receptor Trafficking and Inhibitory Synaptic Transmission in Cortical Neurons*

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Background: The impact of DISC1, a key protein driving the endophenotypes of major mental disorders, on GABAergic inhibition is unknown.
Results: DISC1 knockdown or overexpression altered $GABA_AR$-mediated synaptic transmission by regulating kinesin motor/microtubule-based $GABA_AR$ trafficking.
Conclusion: DISC1 exerts an important effect on $GABA_AR$ trafficking and synaptic inhibition.
Significance: Our observations may be relevant for the role of DISC1 in mental disorders associated with altered GABAergic inhibition.

Association studies have suggested that Disrupted-in-Schizophrenia 1 (DISC1) confers a genetic risk at the level of endophenotypes that underlies many major mental disorders. Despite the progress in understanding the significance of DISC1 at neural development, the mechanisms underlying DISC1 regulation of synaptic functions remain elusive. Because alterations in the cortical GABA system have been strongly linked to the pathophysiology of schizophrenia, one potential target of DISC1 that is critically involved in the regulation of cognition and emotion is the $GABA_A$ receptor ($GABA_AR$). We found that cellular knockdown of DISC1 significantly reduced $GABA_AR$-mediated synaptic and whole-cell current, whereas overexpression of wild-type DISC1, but not the C-terminal-truncated DISC1 (a schizophrenia-related mutant), significantly increased $GABA_AR$ currents in pyramidal neurons of the prefrontal cortex. These effects were accompanied by DISC1-induced changes in surface $GABA_AR$ expression. Moreover, the regulation of $GABA_ARs$ by DISC1 knockdown or overexpression depends on the microtubule motor protein kinesin 1 (KIF5). Our results suggest that DISC1 exerts an important effect on GABAergic inhibitory transmission by regulating KIF5/microtubule-based $GABA_AR$ trafficking in the cortex. The knowledge gained from this study would shed light on how DISC1 and the GABA system are linked mechanistically and how their interactions are critical for maintaining a normal mental state.

The *Disrupted-in-schizophrenia 1 (DISC1)* gene was originally identified in a unique Scottish pedigree in which all members with a major mental illness carried the inherited chromosomal translocation that interrupts the coding sequence of *DISC1*, resulting in the loss of DISC1 expression (1–4). Subsequent association studies have reported a link between genetic variations of *DISC1* and specific endophenotypes that are commonly associated with schizophrenia, depression, and bipolar disorder (5–9). DISC1 is not only highly expressed in the developing brain but also in various areas of the adult brain, including the cortex and hippocampus, and in both pyramidal neurons and interneurons (10). During early development, DISC1 regulates progenitor cell proliferation via glycogen synthase kinase 3β-regulated WNT-β-catenin activity and postmitotic neuronal migration via interacting with proteins in the dynein motor complexes that are associated with microtubules and the centrosome (11–13). In the adult brain, DISC1 regulates neurogenesis and orchestrates the tempo of neuronal integration (14, 15).

DISC1 is enriched in the postsynaptic density of excitatory synapses (16), where it regulates spine size, spine number, and AMPA receptor synaptic trafficking via a kalirin 7-Rac1-PAK pathway (17). DISC1 also regulates synapse maintenance through interacting with TRAF2 and NCK-interacting protein kinase (TNIK) (18) and exerts an important effect on NMDA receptor expression and function through a mechanism depending on the transcription factor cAMP response element-binding protein (19). DISC1 has also been found at inhibitory synapses (16). However, the role of DISC1 in regulating inhibitory synaptic transmission is largely unknown.

A convergent finding in individuals with schizophrenia is the reduced perisomatic inhibition of pyramidal neurons in the dorsolateral prefrontal cortex (PFC),[3] which leads to the diminished synchronized neuronal activity required for cognitive functions (20, 21). The $GABA_A$ receptor is the major mediator of fast synaptic inhibition in the mammalian brain. The dynamic regulation of $GABA_A$ receptor composition, trafficking, and function plays a key role in controlling network and cellular activity in normal and disease conditions (22, 23).

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[3] The abbreviations used are: PFC, prefrontal cortex; DIV, days *in vitro*; $GABA_AR$, $GABA_A$ receptor; mIPSC, miniature inhibitory postsynaptic current; VIAAT, vesicular inhibitory amino acid transporter.

Selective alterations in $GABA_A$ receptors, GABA content, and GABAergic local circuit neurons have been discovered in the PFC of schizophrenia and depression patients (21, 24). In this study, we examined the effect of DISC1 on $GABA_A$ receptors and GABAergic transmission in cortical pyramidal neurons, which may help our understanding of the role of DISC1 in regulating synaptic inhibition.

Experimental Procedures

Primary Neuronal Culture—Rat PFC cultures were prepared by modification of methods described previously (25). Briefly, the PFC was dissected from 18-day-old rat embryos, and cells were dissociated using trypsin and titrated through a Pasteur pipette. The neurons were plated on coverslips coated with poly-L-lysine in DMEM with 10% fetal calf serum at a density of 1×10^5 cells/cm^2. When neurons attached to the coverslip within 24 h, the medium was changed to Neurobasal with a B27 supplement (1.0% penicillin/streptomycin, 2% B27, and 0.5 mM Glutamine). Two to three days after plating, 5 μm cytosine arabinoside was added to inhibit glial growth. Subsequently, half of the medium was changed to a conditional medium once a week. Neurons were maintained for 2–3 weeks before being used for recordings.

Small Interfering RNA and Dominant Negative Constructs—To suppress the expression of DISC1 in cultured neurons, we used shRNA, a potent agent for sequence-specific gene silencing (26). DISC1 shRNA (5′-GGCAAACACTGTGAAGTGC-3′) or scramble shRNA (control shRNA) (5′-GGAGCAGACG-CTGAATTAC-3′) was inserted into the vector pSuper-Venus and driven by the H1-RNA polymerase III promoter. The construct also carried CMV promoter-driven enhanced GFP (12, 17, 19). The DISC1 shRNA-encoding plasmid (DISC1 shRNA) has been shown to induce a strong suppression of DISC1 expression (12). Cultured PFC neurons (DIV 15–16) were transfected with control shRNA or DISC1 shRNA using the Lipofectamine 2000 method. Full-length DISC1 (17) plasmid was used to overexpress DISC1. To suppress total KIF5 activity or overexpress KIF5, we co-transfected KLC1 siRNA (Santa Cruz Biotechnology, Santa Cruz, CA), dominant negative KIF5C, or the full-length heavy chains of KIF5C (a gift from Dr. Josef T. Kittler, University College London) (27) with control shRNA or DISC1 shRNA. Neurons were used in experiments 2–3 days after transfection.

Whole-cell Recordings of Ionic Currents—Recordings of whole cell $GABA_A$R-mediated currents used standard voltage clamp techniques (28). The internal solution consisted of 180 mM *N*-methyl-D-glucamine, 40 mM HEPES, 4 mM $MgCl_2$, 0.5 mM 1,2-bis(2-aminohenoxy) ethane *N*,*N*,*N*′,*N*′-tetraacetic acid, 12 mM phosphocreatine, 2 mM Na_2ATP, 0.2 mM Na_3GTP, and 0.1 mM leupeptin (pH 7.2–7.3, 265–270 mosmol/liter). The external solution consisted of 135 mM NaCl, 20 mM CsCl, 1 mM $MgCl_2$, 10 mM HEPES, 5 mM $BaCl_2$, 10 mM glucose, and 0.001 mM tetrodotoxin (pH 7.3–7.4, 300–305 mosmol/liter). Recordings were obtained using an Axopatch 200B amplifier controlled and monitored with a computer running pClamp 8 with a DigiData 1320 series interface. Electrode resistances were typically 2–4 MΩ in the bath. After seal rupture, series resistance (4–10 MΩ) was compensated (70–90%) and monitored periodically. The cell membrane potential was held at 0 mV. GABA (50 μM) was applied for 2 s every 30 s to minimize the desensitization-induced decrease of current amplitude. Drugs were applied using a gravity-fed "sewer pipe" system. The array of application capillaries (~150-μm inner diameter) was positioned a few hundred micrometers from the cell being recorded. Solution changes were affected by the SF-77B fast-step solution stimulus delivery device (Warner Instruments, Hamden, CT). Data analyses were performed with Clampfit (Axon Instruments, Sunnyvale, CA) and Kaleidagraph (Albeck Software, Reading, PA). Current density was calculated by taking the average peak amplitude (picoampere) of a single recording and dividing that value by the capacitance (picofarad).

Electrophysiological Recording of Synaptic Currents—Recording of miniature inhibitory postsynaptic currents (mIPSC) in cultured PFC neurons (DIV 14–16) used the whole-cell patch technique. Electrodes (3–5 MΩ) were filled with the following internal solution: 100 mM CsCl, 30 mM *N*-methyl-D-glucamine, 10 mM HEPES, 4 mM NaCl, 1 mM $MgCl_2$ 5 mM EGTA, 5 mM MgATP, 0.5 mM Na_2GTP, 12 mM phosphocreatine, 0.2 mM leupeptin, and 2 mM QX-314 (pH 7.2–7.3, 265–270 mosmol/liter). Oxygenated artificial cerebral spinal fluid (130 mM NaCl, 3 mM KCl, 5 mM $MgCl_2$, 1 mM $CaCl_2$, 26 mM $NaHCO_3$, 1.25 mM NaH_2PO_4, and 10 mM glucose (pH 7.4, 300 mosmol/liter) was used as the external solution. Tetrodotoxin (0.5 μM), D-AP5 (50 μM), and 6,7-dinitroquinoxaline-2,3-dione (10 μM) were added to cultures to block action potentials *N*-methyl-D-aspartic acid and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid/kainate receptors, respectively. The cell membrane potential was held at −70 mV. A mini analysis program (Synaptosoft, Leonia, NJ) was used to analyze the spontaneous synaptic events. For each different condition, mIPSC recordings of 8 min were used for analysis.

Immunocytochemical Staining—For the detection of surface $GABA_A$ receptors, the primary cultures (2.5×10^4 cells/cm^2, DIV 17–18) were fixed, blocked, and incubated with a monoclonal antibody against the $GABA_A$R β2/3 subunits (1:250, Millipore, catalog no. MAB341 or 1:250, NeuroMab, catalog no. 75-363) overnight at 4 °C. After washing, neurons were permeabilized and incubated with a polyclonal antibody against MAP2 (1:250; Santa Cruz Biotechnology, catalog no. sc-20172) or a polyclonal anti-VIAAT (1:1000, a gift from Dr. Stephen Moss, Tufts University) for 2 h at room temperature. After washing, the neurons were incubated with Alexa Fluor 568-conjugated secondary antibody (1:250, Invitrogen) and Alexa Fluor 647-conjugated secondary antibody (1:250, Invitrogen) for 2 h at room temperature. After washing in PBS three times, coverslips were mounted on slides with Vectashield mounting medium (Vector Laboratories, Burlingame, CA).

For the detection of $GABA_A$R at synapses, neurons (2.5×10^4 cells/cm^2, DIV 17–18) were fixed, permeabilized, blocked, and incubated with a monoclonal anti-$GABA_A$R β2/3 antibody (1:250, Millipore, catalog no. MAB341, Ref. 29) and a polyclonal anti-VIAAT antibody (1:1000) overnight at 4 °C. After washing, the neurons were incubated with Alexa Fluor 568-conjugated secondary antibody (1:250, Invitrogen) and Alexa Fluor 647-conjugated secondary antibody (1:250, Invitrogen) for 2 h at room temperature. After washing, the coverslips were mounted on slides with Vectashield mounting medium.

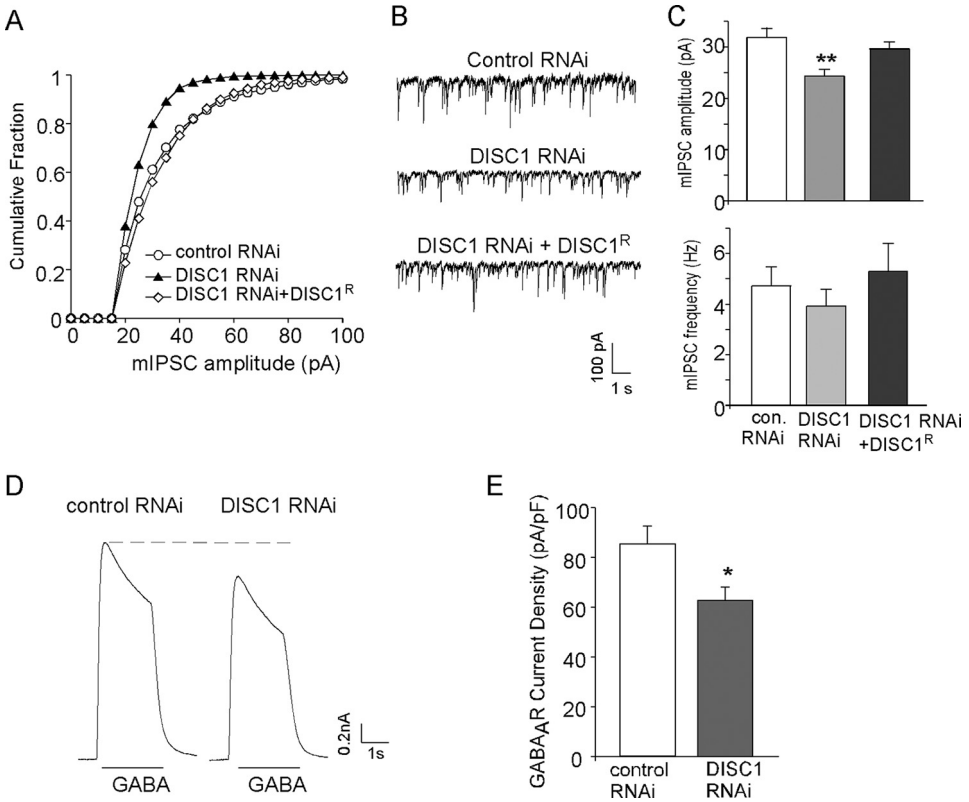

FIGURE 1. **DISC1 knockdown reduces mIPSC amplitude and $GABA_AR$ current density in cultured cortical pyramidal neurons.** *A*, cumulative plots of mIPSC amplitudes in neurons transfected with control shRNA, DISC1 shRNA, or DISC1 shRNA plus an shRNA-resistant full-length DISC1 (*DISC1*^{R}). Note that DISC1 knockdown caused a leftward shift in the distribution of mIPSC amplitudes, indicative of a reduction of mIPSC sizes. *B*, representative mIPSC traces taken from neurons in *A*. *C*, summary (mean ± S.E.) of mIPSC amplitudes and frequencies in neurons with different transfections. **, $p < 0.01$; Dunnett test. *con*, control. *D*, representative whole-cell $GABA_AR$ current traces in neurons transfected with control or DISC1 shRNA. *E*, summary (mean ± S.E.) of $GABA_AR$ current density in neurons with different transfections. *, $p < 0.05$, Student's *t* test.

Fluorescence images were obtained using a ×100 oil immersion objective with a cooled charge-coupled device camera mounted on a Nikon fluorescence microscope (Nikon Eclipse E600). Images were captured digitally using SPOT basic image capture software. All specimens were imaged under identical conditions and analyzed with identical parameters using ImageJ software. To define dendritic clusters, a single threshold was chosen manually so that clusters corresponded to puncta with the mean intensity equal to ~2-fold of the diffuse fluorescence mean intensity on the dendritic shaft. When $GABA_AR$ β2/3 and VIAAT clusters were identified, the co-localized clusters were highlighted as white points by an analysis tool in ImageJ. On each coverslip, the cluster density, cluster size, and cluster fluorescence intensity of three to five neurons (two to three dendritic segments 50 μm in length per neuron) were measured. Quantitative analyses were conducted blindly (without knowledge of experimental treatment).

Statistics—Student's *t* test or analysis of variance with post hoc Dunnett or Turkey tests (Kaleidagraph) were performed to detect statistical significance among groups with different treatments.

Results

DISC1 Knockdown or Overexpression Alters $GABA_AR$-mediated Currents—To examine the effect of DISC1 knockdown on GABAergic signaling, we measured mIPSC, a synaptic response to the quantal release of single GABA vesicles, in cultured PFC pyramidal neurons. Neurons (DIV 14–16) were transfected with a scrambled shRNA (control shRNA) or DISC1 shRNA, and recordings were performed 2 days after transfection. As shown in Fig. 1, *A–C*, DISC1 shRNA-transfected neurons had a significant reduction in mIPSC amplitude (control shRNA, 31.8 ± 1.8 pA, $n = 19$; DISC1 shRNA, 24.4 ± 1.3 pA, $n = 23$; $p < 0.01$), whereas there was no significant change in mIPSC frequency (control shRNA, 4.73 ± 0.74 Hz, $n = 19$; DISC1 shRNA, 3.93 ± 0.66 Hz, $n = 23$; $p > 0.05$). Transfecting the full-length shRNA-resistant DISC1 (DISC1-FL^R) rescued mIPSC amplitude (29.6 ± 1.4 pA, $n = 17$, $p > 0.05$), confirming that the effect of DISC1 shRNA on mIPSC is mediated by the specific loss of DISC1.

To test whether the DISC1 shRNA-induced reduction in mIPSC amplitude was due to alteration of receptor density and/or activity, we measured the GABA-elicited whole-cell current mediated by both synaptic and extrasynaptic $GABA_A$ receptors. Application of GABA (50 μM) evoked a partially desensitizing outward current in cultured PFC pyramidal neurons (held at 0 mV) that could be blocked by the $GABA_A$ receptor antagonist bicuculline (30 μM). As shown in Fig. 1, *D* and *E*, neurons transfected with DISC1 shRNA had a significantly smaller $GABA_AR$ current density (control shRNA, 85.3 ± 7.3 pA/pF, $n = 7$; DISC1 shRNA, 62.7 ± 5.5 pA/pF, $n = 7$; $p < 0.05$).

In addition to DISC1 knockdown, we also examined the impact of overexpressing full-length DISC1 (FL-DISC1) on GABAergic signaling. As shown in Fig. 2, *A* and *B*, transfecting

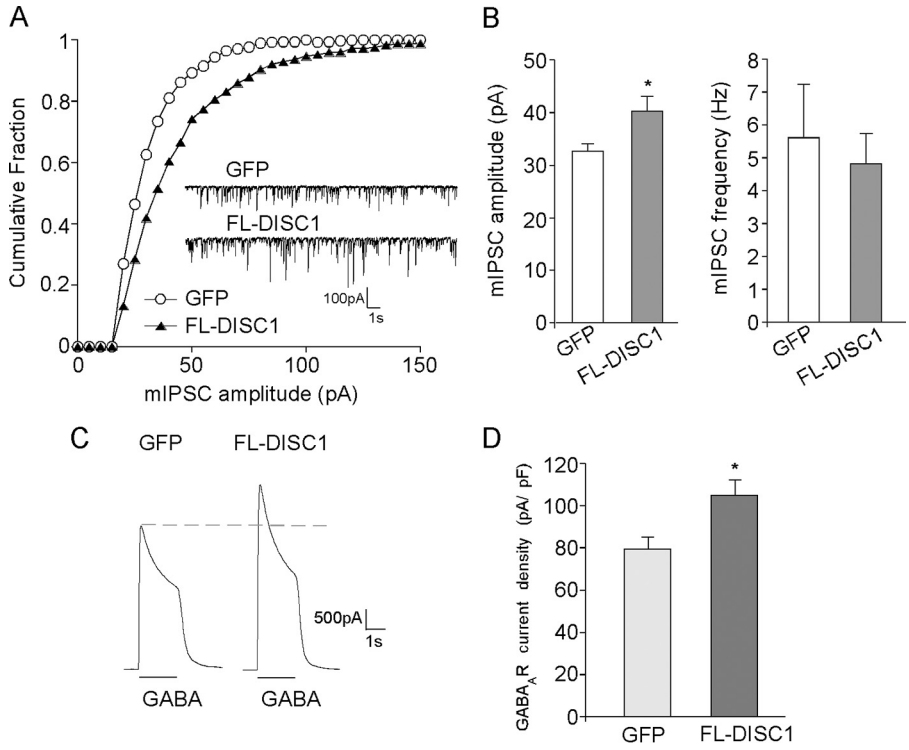

FIGURE 2. **DISC1 overexpression increases mIPSC amplitude and GABA$_A$R current density in cultured cortical pyramidal neurons.** *A*, cumulative plots of mIPSC amplitudes in neurons transfected with GFP or FL-DISC1. Note that DISC1 overexpression caused a rightward shift in the distribution of mIPSC amplitudes, indicative of an increase in the sizes of mIPSCs. *Inset*, representative mIPSC traces. *B*, summary (mean ± S.E.) of mIPSC amplitudes and frequencies in neurons with different transfections. *, $p < 0.05$; Student's *t* test. *C*, representative whole-cell GABA$_A$R current traces in neurons transfected with GFP or FL-DISC1. *D*, summary (mean ± S.E.) of GABA$_A$R current density in neurons with different transfections. *, $p < 0.05$; Student's *t* test.

GFP-tagged FL-DISC1 caused a significant increase in mIPSC amplitude in cultured PFC pyramidal neurons (GFP, 32.7 ± 1.4 pA, $n = 14$; FL-DISC1, 40.3 ± 2.8 pA, $n = 16$; $p < 0.05$). Whole-cell GABA$_A$R current density (picoampere/picofarad) was also increased significantly by full-length DISC1 (Fig. 2, *C* and *D*; GFP, 79.5 ± 5.8 pA/pF, $n = 14$; FL-DISC1, 104.9 ± 7.2 pA/pF, $n = 14$; $p < 0.05$). Together, these results indicate that DISC1 knockdown or overexpression leads to a decrease or increase in GABA$_A$R currents, respectively.

DISC1 Knockdown or Overexpression Alters GABA$_A$R Surface and Synaptic Distribution—Emerging evidence suggests that the trafficking of GABA$_A$Rs underlies dynamic changes in synaptic receptor numbers and inhibitory postsynaptic current amplitudes, providing an effective mechanism for regulating the strength and plasticity of synaptic inhibition (23, 30). Therefore, we examined whether the change in GABA responses by DISC1 knockdown or overexpression was associated with altered membrane trafficking of GABA$_A$Rs by using a quantitative surface immunostaining assay. Surface GABA$_A$R clusters were compared in PFC cultures transfected with control shRNA, DISC1 shRNA, or full-length DISC1. An antibody against the N-terminal extracellular domain of GABA$_A$R β2/3 subunits was used to detect surface GABA$_A$Rs, and immunostaining was performed under nonpermeabilized conditions. As shown in Fig. 3, *A* and *B*, DISC1 knockdown significantly decreased surface GABA$_A$R cluster density (number of clusters/30 μm dendrite; control shRNA, 21.7 ± 1.2, $n = 23$; DISC1 shRNA, 14.1 ± 0.8, $n = 19$; $p < 0.01$) and size (square micrometer; control shRNA, 0.19 ± 0.03, $n = 23$; DISC1 shRNA, 0.11 ± 0.01, $n = 19$; $p < 0.05$). In contrast, overexpression of FL-DISC1 significantly increased surface GABA$_A$R cluster density and size (Fig. 3, *C* and *D*) (cluster density: GFP, 19.3 ± 1.1, $n = 53$; FL-DISC1, 28.6 ± 1.2, $n = 51$; $p < 0.001$; cluster size: GFP, 0.27 ± 0.03, $n = 53$; FL-DISC1, 0.46 ± 0.03, $n = 51$; $p < 0.001$) without altering the expression of the inhibitory synapse marker VIAAT (27, 31) (Fig. 3, *C* and *D*) (cluster density: GFP, 14.3 ± 0.8, $n = 26$; FL-DISC1, 14.5 ± 1.5, $n = 26$; $p > 0.05$; cluster size: GFP, 0.64 ± 0.05, $n = 26$; FL-DISC1, 0.63 ± 0.05, $n = 26$; $p > 0.05$). These results suggest that the changes in surface GABA$_A$R expression are not due to the formation of new inhibitory synapses.

To examine synaptic GABA$_A$Rs, we co-stained the GABA$_A$R subunit with the inhibitory synapse marker VIAAT. GABA$_A$Rs opposed to VIAAT-labeled inhibitory synaptic terminals were considered postsynaptic GABA$_A$Rs. As shown in Fig. 3, *E* and *F*, DISC1 knockdown did not significantly change the density of total GABA$_A$R clusters (control shRNA, 47.6 ± 2.2, $n = 27$; DISC1 shRNA, 46.8 ± 2.8, $n = 28$; $p > 0.05$) or VIAAT clusters (control shRNA, 33.7 ± 1.6, $n = 27$; DISC1 shRNA, 31.6 ± 1.8, $n = 28$; $p > 0.05$) but significantly reduced synaptic GABA$_A$R cluster density (control shRNA, 33.0 ± 1.6, $n = 27$; DISC1 shRNA, 22.4 ± 1.4, $n = 28$; $p < 0.001$). These results suggest that DISC1 regulates GABAergic transmission mainly by changing the number of GABA$_A$Rs in the synapse.

DISC1 Regulation of GABA$_A$Rs Depends on the Microtubule Motor Protein KIF5—Next we investigated the potential mechanism underlying DISC1 regulation of GABA$_A$Rs at the cell surface. It has been shown that DISC1 can directly interact with

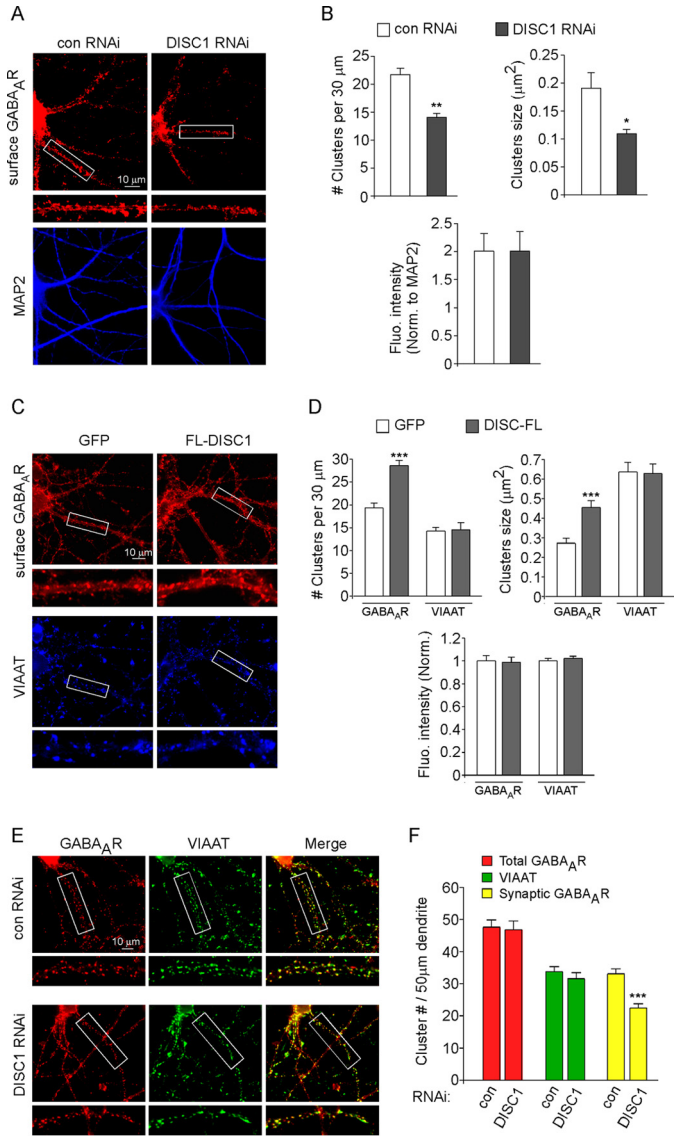

FIGURE 3. **DISC1 knockdown reduces surface and synaptic GABA$_A$R clusters, whereas DISC1 overexpression increases surface GABA$_A$R clusters.** *A* and *C*, immunocytochemical images of surface GABA$_A$R β2/3 subunits in cortical cultures transfected with a control (*con*) *versus* DISC1 shRNA (*A*) or GFP *versus* FL-DISC1 (*C*). Following surface GABA$_A$R β2/3 labeling, neurons were permeabilized and co-stained with MAP2 (*A*) or VIAAT (*C*). Enlarged versions of the *boxed regions* of dendrites are also shown. *B* and *D*, quantitative analysis of surface GABA$_A$R β2/3 or VIAAT clusters in cortical cultures with different transfections. ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$; Student's *t* test. *Fluo*, fluorescence; *Norm*, normalized. *E*, immunocytochemical images of control shRNA- *versus* DISC1 shRNA-transfected cortical neurons co-stained with GABA$_A$R β2/3 and VIAAT. Enlarged versions of the *boxed regions* of dendrites are also shown. *F*, quantitative analysis of the densities of total GABA$_A$R clusters, VIAAT clusters, and synaptic GABA$_A$R clusters (co-localized with VIAAT). ***, $p < 0.001$, Student's *t* test.

KIF5, a microtubule motor protein (32). Our previous studies have found that GABA$_A$Rs are trafficked to synapses along microtubules by KIF5 (27). Using co-immunoprecipitation assays, we have demonstrated that immunoprecipitated GABA$_A$Rs could readily bring down tubulin and KIF5 light chain (33), suggesting that GABA$_A$R-KIF5-tubulin form a complex *in vivo*. Therefore, it is possible that DISC1 may affect the interaction between GABA$_A$Rs and KIF motor proteins, influencing GABA$_A$R transport along dendritic microtubules and inhibitory synaptic transmission.

To test the role of KIF5 in GABA$_A$R trafficking and function, we transfected neurons with the siRNA against KIF5 light chain 1 (KLC1), which caused an effective suppression of KLC1 expression (34). KLCs are essential for the proper function or localization of KIF5 heavy chains (35). As shown in Fig. 4, *A* and *D*, KLC1 knockdown resulted in a significant reduction in mIPSC amplitude (control shRNA, 32.9 ± 1.4 pA, $n = 14$; KLC1 siRNA, 27.4 ± 1.4 pA, $n = 16$; $p < 0.01$) but not mIPSC frequency (control shRNA, 1.91 ± 0.6 Hz, $n = 17$; KLC1 siRNA, 0.96 ± 0.3 Hz, $n = 14$; $p > 0.05$). These results suggest that GABA$_A$Rs rely upon KIF5 for trafficking to the postsynaptic membrane.

To examine the role of KIF5 in DISC1 regulation of GABA$_A$Rs, we blocked the function of KIF by either KLC1 siRNA or a dominant-negative KIF5C construct (DN-KIF5C) that lacks the motor domain (27) and examined the effect of DISC1 knockdown or overexpression on mIPSC. As shown in Fig. 4, *B* and *D*, knockdown of KLC resulted in the occlusion of DISC1 shRNA effects on mIPSC amplitude (control shRNA + KLC1 siRNA, 27.6 ± 1.9 pA, $n = 6$; DISC1 shRNA + KLC1 siRNA, 27.1 ± 2.0 pA, $n = 4$; $p > 0.05$). Such an occlusion was also observed in the presence of DN-KIF5C (Fig. 4, *C* and *D*, control shRNA + DN-KIF5C, 26.5 ± 1.4 pA, $n = 9$; DISC1 shRNA + DN-KIF5C, 25.0 ± 2.1 pA, $n = 8$; $p > 0.05$). Blocking the function of KIF17, another kinesin family motor protein involved in NMDA receptor transport (36), failed to alter the reducing effect of DISC1 shRNA effects on mIPSC amplitude (Fig. 4*D*) (control shRNA + KIF17 antisense, 33.0 ± 1.9 pA, $n = 23$; DISC1 shRNA + KIF17 antisense, 25.5 ± 1.2 pA, $n = 13$; $p < 0.01$). In addition, the enhancing effect of FL-DISC1 on mIPSC amplitude was blocked in the presence of DN-KIF5C (Fig. 4, *E* and *F*) (DN-KIF5, 25.8 ± 0.8 pA, $n = 13$; FL-DISC1 + DN-KIF5, 25.1 ± 1.2 pA, $n = 20$; $p > 0.05$).

Finally, we examined whether overexpressing KIF5 could block the effect of DISC1 knockdown on mIPSC. As shown in Fig. 5, *A–D*, the reducing effect of DISC1 shRNA on mIPSC amplitude (control shRNA, 33.3 ± 2.3 pA, $n = 15$; DISC1 shRNA, 26.4 ± 1.0 pA, $n = 15$; $p < 0.05$) was prevented in the presence of full-length KIF5C (control shRNA + KIF5C, 30.7 ± 1.6 pA, $n = 15$; DISC1 shRNA + KIF5C, 30.7 ± 1.7 pA, $n = 13$; $p > 0.05$). There was no significant change in mIPSC frequency with any of these treatments (control shRNA, 5.35 ± 1.53 Hz, $n = 15$; DISC1 shRNA, 3.92 ± 0.95 Hz, $n = 15$; control shRNA + KIF5C, 3.96 ± 0.57 Hz, $n = 15$; DISC1 shRNA + KIF5C, 4.04 ± 0.79 Hz, $n = 13$; $p > 0.05$). Together, these results suggest that the kinesin motor protein KIF5 is necessary and sufficient for DISC1 regulation of GABA$_A$Rs.

Discussion

DISC1, a key protein driving the endophenotypes of major mental disorders (9), is located at both excitatory and inhibitory synapses (16). From the network of protein-protein interactions around DISC1, DISC1 is implicated in processes of cytoskeletal stability, intracellular transport, and synaptic activity (37). It has been found that DISC1 interacts with multiple proteins, such as the second messenger PDE4B, which hydrolyzes cAMP and regulates the PKA signaling pathway (4), the transcription factor ATF4/CREB2, which controls gene expression (38), and the motor protein kinesin 1, which mediates the

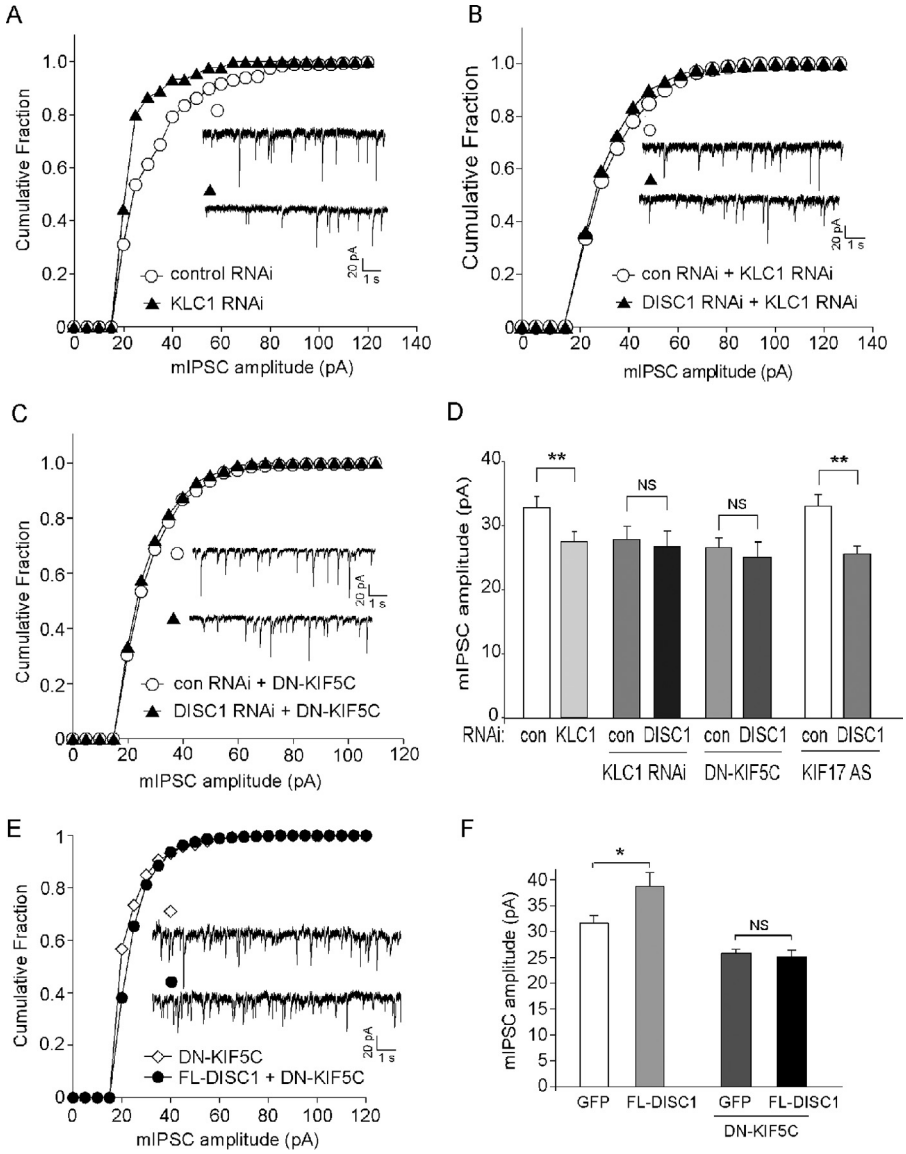

FIGURE 4. **DISC1 regulation of synaptic inhibition requires KIF5.** *A*, cumulative plots of mIPSC amplitudes in cultured cortical pyramidal neurons transfected with control or KLC1 siRNA. Note that KLC1 knockdown caused a leftward shift in the distribution of mIPSC amplitudes, indicative of a reduction of mIPSC sizes. *B* and *C*, cumulative plots of mIPSC amplitudes in neurons transfected with control (*con*) or DISC1 shRNA in the presence of KLC1 siRNA (*B*) or dominant-negative (*DN*) KIF5C (*C*). *E*, cumulative plots of mIPSC amplitudes in neurons with or without FL-DISC1 transfection in the presence of DN-KIF5C. *Insets* (*A–C* and *E*), representative mIPSC traces. *D* and *F*, summary (mean ± S.E.) of mIPSC amplitudes in neurons with different transfections. *, $p < 0.05$; **, $p < 0.01$; *NS*, not significant; analysis of variance.

microtubule-based transport of neuronal cargos (32). The role of DISC1 in regulating glutamate receptors and spine synapses has been documented (17–19). In this study, we revealed the role of DISC1 in regulating $GABA_A$ receptors and synaptic inhibition. DISC1 knockdown led to a significant decrease of $GABA_A$R-mediated synaptic and whole-cell current (Fig. 1), which was accompanied by reduced surface and synaptic $GABA_A$R clusters (Fig. 3), whereas DISC1 overexpression significantly increased $GABA_A$R currents (Fig. 2) and surface expression (Fig. 3). These data suggest that DISC1 facilitates the delivery of $GABA_A$Rs to the synaptic membrane.

The trafficking of $GABA_A$Rs begins in the endoplasmic reticulum, where they are assembled and then forward-trafficked to the Golgi network. This is followed by long-range transport along microtubules on dendrites and insertion into extrasynaptic sites, where they can diffuse laterally into synapses (23). The anterograde transport of neuronal cargos along microtubules relies on the kinesin superfamily of motor proteins, which typically consists of two identical heavy chains and two identical light chains (39–41). Kinesin heavy chain has a conserved N-terminal motor domain that is responsible for microtubule binding and a diverse C-terminal non-motor domain containing kinesin light chain binding domain and cargo binding domain. Previous studies by us and others have found that kinesin motor proteins are important for the microtubule-based transport of ionotropic glutamate receptors and the efficacy of excitatory synaptic transmission (34, 36, 42–45). The role of KIF5 motors in regulating $GABA_A$R trafficking and the strength of inhibitory synaptic transmission was later revealed by our studies (27, 33).

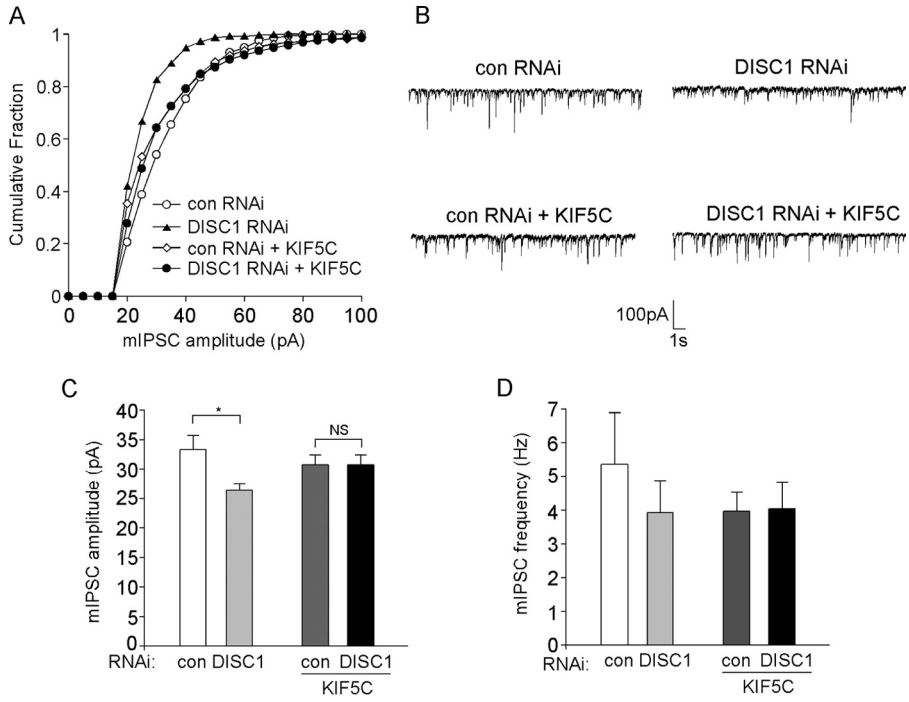

FIGURE 5. **Overexpression of KIF blocks the reducing effect of DISC1 knockdown on synaptic inhibition.** *A*, cumulative plots of mIPSC amplitudes in cultured cortical pyramidal neurons transfected with control (*con*) or DISC1 shRNA in the absence or presence of full-length KIF5C. *B*, representative mIPSC traces taken from neurons in *A*. *C* and *D*, summary (mean ± S.E.) of mIPSC amplitudes (*C*) or frequencies (*D*) in neurons with different transfections. *, $p < 0.05$; analysis of variance.

Because DISC1 can interact directly with KIF5 (32), we hypothesized that KIF5 may be involved in DISC1 regulation of $GABA_A$ receptor trafficking. Consistently, the reducing effect of DISC1 knockdown on GABAergic transmission was occluded by suppressing KIF5 (Fig. 4) and blocked by overexpressing KIF5 (Fig. 5), suggesting that KIF5 is necessary and sufficient for DISC1 regulation of synaptic inhibition.

Deficits in microtubule/KIF-based transport have been suggested to underlie the pathogenesis of a number of neurodegenerative diseases (46). Our results suggest that the loss of DISC1 function in mental disorder conditions may interfere with the interaction between $GABA_A$Rs and KIF motor proteins, leading to the disruption of $GABA_A$R transport along dendritic microtubules and impairment of inhibitory synaptic transmission. The diminished synaptic inhibition could contribute to the reduced capability to generate γ frequency-synchronized neuronal activity, leading to the impaired working memory functions shared by various mental illnesses (21).

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