Dystroglycan mediates homeostatic synaptic plasticity at GABAergic synapses

Horia Pribiag[1], Huashan Peng[1], Waris Ali Shah, David Stellwagen[2], and Salvatore Carbonetto[2]

Department of Neurology and Neurosurgery, Centre for Research in Neuroscience, Research Institute of McGill University Health Center, Montréal, QC, Canada H3G 1A4

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Dystroglycan (DG), a cell adhesion molecule well known to be essential for skeletal muscle integrity and formation of neuromuscular synapses, is also present at inhibitory synapses in the central nervous system. Mutations that affect DG function not only result in muscular dystrophies, but also in severe cognitive deficits and epilepsy. Here we demonstrate a role of DG during activity-dependent homeostatic regulation of hippocampal inhibitory synapses. Prolonged elevation of neuronal activity up-regulates DG expression and glycosylation, and its localization to inhibitory synapses. Inhibition of protein synthesis prevents the activity-dependent increase in synaptic DG and $GABA_A$ receptors ($GABA_A$Rs), as well as the homeostatic scaling up of GABAergic synaptic transmission. RNAi-mediated knockdown of DG blocks homeostatic scaling up of inhibitory synaptic strength, as does knockdown of like-acetylglucosaminyltransferase (LARGE)—a glycosyltransferase critical for DG function. In contrast, DG is not required for the bicuculline-induced scaling down of excitatory synaptic strength or the tetrodotoxin-induced scaling down of inhibitory synaptic strength. The DG ligand agrin increases GABAergic synaptic strength in a DG-dependent manner that mimics homeostatic scaling up induced by increased activity, indicating that activation of this pathway alone is sufficient to regulate $GABA_A$R trafficking. These data demonstrate that DG is regulated in a physiologically relevant manner in neurons and that DG and its glycosylation are essential for homeostatic plasticity at inhibitory synapses.

muscular dystrophy | excitation–inhibition balance | dystrophin | AMPA receptors | retardation

Muscular dystrophies are often associated with mild to severe cognitive deficits, epilepsy, and other neurological deficits (1–3). This is particularly evident in muscular dystrophies caused by mutations that affect glycosylation of the membrane glycoprotein α-dystroglycan (α-DG) (4). α-DG docks with transmembrane β-DG to form the functional core of the dystrophin-associated glycoprotein complex (DGC) that links adhesive proteins in the extracellular matrix to dystrophin (5). α-DG is heavily glycosylated and interacts via its carbohydrate side chains with laminin and laminin G-like domains in a variety of proteins including agrin, perlecan, slit, neurexin, and pikachurin (6–10). Key carbohydrate residues are added onto α-DG by several glycosyltransferases, most notably like-acetylglucosaminyltransferase (LARGE) (11). LARGE is necessary for functional glycosylation of α-DG (12), and is mutated in muscular dystrophies associated with severe cognitive deficits (4).

DG was first identified in the nervous system (13), where it is important during development for neuroblast migration (14), axon guidance (7), and ribbon synapse formation (8). At neuromuscular synapses, DG is required for the stabilization of acetylcholine receptors in the postsynaptic density and contributes to the accumulation of acetylcholinesterase (10, 15). However, the function of DG at central synapses remains essentially unknown. In the mature central nervous system (CNS), neuronal DGC components are exclusively colocalized with $GABA_A$ receptors ($GABA_A$Rs) in multiple brain regions (16–18), raising the possibility for a role in $GABA_A$R regulation. However, DG is dispensable for GABAergic synapse formation in hippocampal cultures (17), although adult mice lacking full-length dystrophin show reduced clustering of $GABA_A$Rs in the hippocampus and other brain regions (16, 19, 20). Because dystrophin localization at GABAergic synapses depends on DG (17), these findings suggest that DG may regulate the plasticity of mature GABAergic synapses. Homeostatic synaptic plasticity is widely thought to be essential for brain function and involves the reciprocal regulation of glutamatergic and GABAergic synapses to stabilize neuronal activity (21). Chronic elevation of neuronal activity is associated with an increase in synaptic $GABA_A$Rs (22, 23), but the mechanistic details are incompletely understood.

Here, we assess the roles of DG and α-DG glycosylation in regulating the expression of homeostatic synaptic plasticity at GABAergic synapses. We find that in mature hippocampal cultures, prolonged elevation of neuronal activity up-regulates DG expression and the coclustering of α-DG and $GABA_A$Rs. Inhibition of protein synthesis or knockdown of DG blocks homeostatic scaling up of GABAergic synaptic strength. Knockdown of the selective α-DG glycosyltransferase LARGE also blocks homeostatic scaling up, suggesting a role for ligand binding. Furthermore, exogenous application of agrin—a ligand for glycosylated α-DG—is sufficient to scale up GABAergic synaptic strength in a DG-dependent fashion. These data identify a mechanism whereby expression of glycosylated α-DG is linked to neuronal activity level and is essential for homeostatic scaling up of GABAergic synaptic strength by regulating $GABA_A$R abundance at the synapse.

Significance

Normal levels of brain activity result from a fine balance of excitation and inhibition, and disruption of this balance may underlie many neurological disorders. Physiologically, homeostatic synaptic plasticity maintains this balance, though the molecular underpinnings of this plasticity, necessary to explain brain dysfunction and define therapies, are not well understood. Here we have described regulation of inhibitory synaptic plasticity by dystroglycan, a cell adhesion molecule that forms a scaffold at inhibitory (GABAergic) synapses and homeostatically regulates the abundance of $GABA_A$ receptors in the postsynaptic density. These data may explain the epilepsy and cognitive deficits observed in individuals lacking functional dystroglycan.

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[1]H. Pribiag and H. Peng contributed equally to this work.

[2]To whom correspondence may be addressed. E-mail: david.stellwagen@mcgill.ca or sal.carbonetto@mcgill.ca.

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Results

Characterization of the DG Complex at Hippocampal GABAergic Synapses. We used immunohistochemical staining with mAb IIH6 antibody, which recognizes functionally glycosylated α-DG (24), and antibodies to $GABA_AR$ α1, to reveal that virtually all $GABA_AR$ α1 clusters colocalize with α-DG clusters in the CA3 stratum pyramidale of WT mice (Fig. S1 *B* and *C*). However, in *Mdx* mice—which do not express the full-length isoform of dystrophin (5) that anchors DG at the cell surface (25)—we observed substantially reduced expression of α-DG and fewer $GABA_AR$ α1 clusters in the CA3 region (Fig. S1*D*). Similarly, *Myd* mice, which have hypoglycosylated α-DG due to a mutation in the glycosyltransferase LARGE (26), also displayed reduced α-DG and α1 clusters in CA3 hippocampus (Fig. S1*E*).

We further examined the localization of DG using mature hippocampal cultures and found that α-DG colocalizes with β-DG and with $GABA_AR$ clusters apposed to GAD65 puncta, but that α-DG does not colocalize with AMPA receptor (AMPAR) clusters (Fig. S2*A*). We also observed a high degree of colocalization between α-DG and several proteins previously identified as part of the DGC at neuromuscular synapses, including agrin, ezrin, nNOS, and utrophin (15, 27) (Fig. S2*B*). Furthermore, using immunoprecipitation from brain lysates, we found that α-DG forms a supramolecular complex, not only with β-DG, but also with $GABA_AR$ subunits α1 and β2/3, as well as with the inhibitory synapse scaffolding protein gephyrin (Fig. S2*C*). Taken together, these findings suggest that the neuronal DGC is part of the postsynaptic scaffold at mature inhibitory synapses and may stabilize and/or regulate the abundance of $GABA_ARs$.

Activity-Dependent Homeostatic Regulation of α-DG and $GABA_ARs$. At neuromuscular synapses, DG is required for the stabilization of acetylcholine receptors in the postsynaptic density (15). In hippocampal neurons, however, previous work has reported that DG is not necessary for GABAergic synapse formation and is recruited after the appearance of $GABA_AR$ clusters (17), suggesting that, at least in vitro, DG is not required for the initial clustering of $GABA_ARs$. This suggests that DG may function subsequent to inhibitory synapse formation, much as it does at neuromuscular synapses (15), by contributing to activity-dependent regulation of the abundance of synaptic $GABA_ARs$. To investigate this possibility, we used a model of homeostatic synaptic plasticity in which chronic elevation of neuronal firing rate, either by blockade of inhibition with the $GABA_AR$ antagonist bicuculline (Bic) or by elevated extracellular $[K^+]$, results in a multiplicative "scaling up" of GABAergic synaptic strength (22, 28). This process occurs to a large extent through an increase in synaptic $GABA_ARs$ and contributes to the renormalization of neuronal firing rate (22, 23). Following bicuculline treatment (10 μM, 24 h) we observed not only an expected homeostatic increase in the total cluster area of $GABA_AR$ β3 subunits and an increase in the surface level of clustered $GABA_AR$ γ2 subunits, but also an increase in the total cluster area of functionally glycosylated α-DG [α-DG: 209.5 ± 19.9% of control (Ctrl); $GABA_AR$ β3: 142.5 ± 10.4% of Ctrl; surface $GABA_AR$ γ2: 147.3 ± 9.7% of Ctrl; Fig. 1 *A*–*D*]. In addition, using cell-surface biotinylation assays we determined that surface expression of glycosylated α-DG, β-DG, and $GABA_AR$ β3 are significantly up-regulated following bicuculline treatment (α-DG: 125.5 ± 5.2% of Ctrl; β-DG: 125.9 ± 7.4% of Ctrl; $GABA_AR$ β3: 143.6 ± 17.7% of Ctrl; Fig. 1 *E* and *F*). These results reveal a parallel homeostatic up-regulation of $GABA_ARs$ and DG, consistent with a function for DG in homeostatic plasticity at GABAergic synapses.

Protein Synthesis Is Necessary for Inhibitory Homeostatic Plasticity. Protein synthesis plays a crucial role in many forms of synaptic plasticity (29), including some forms of homeostatic synaptic plasticity. However, little is known regarding the role of protein synthesis in homeostatic scaling up at GABAergic synapses. We found that following bicuculline treatment (10 μM, 24 h) the total protein expression levels of glycosylated α-DG and β-DG were significantly increased (α-DG: 146.8 ± 13.6% of Ctrl; β-DG: 126.8 ± 10.3% of Ctrl; Fig. 1 *E* and *F*). However, consistent with previous reports (22), we did not observe a significant change in the total expression of $GABA_AR$ β3 in response to chronic activity elevation (100.9 ± 5.5% of Ctrl; Fig. 1 *E* and *F*). Given the colocalization and biochemical association of DG with synaptic $GABA_ARs$ (Fig. S2 *A* and *C*), these data suggest that the homeostatic increase in DG and $GABA_AR$ β3 clustering

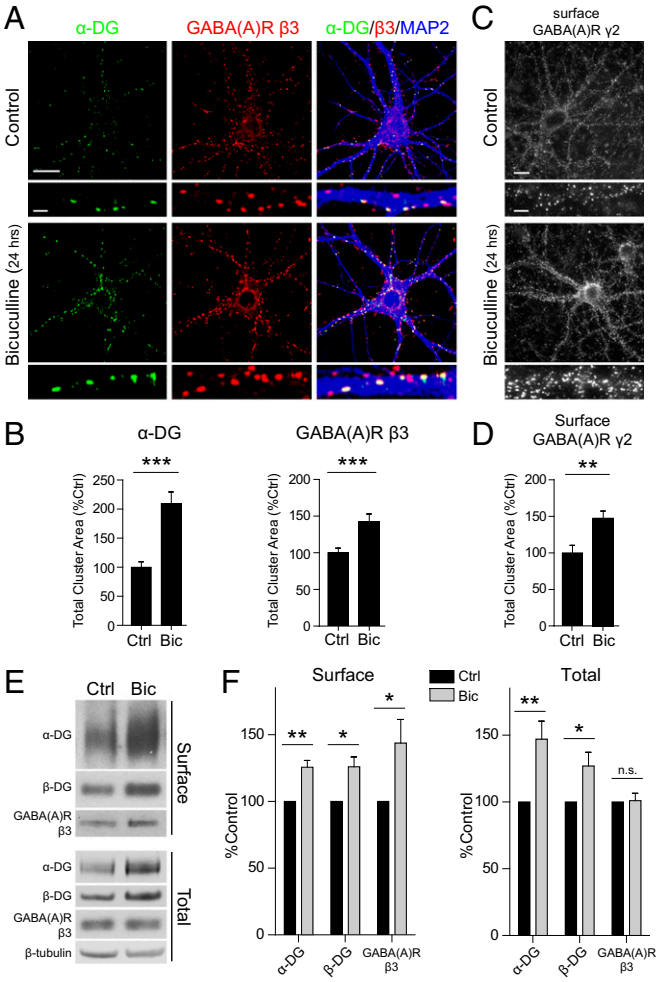

Fig. 1. Activity-dependent homeostatic regulation of DG and $GABA_ARs$. (*A* and *C*) Representative images of permeabilized immunostaining for glycosylated α-DG (mAb IIH6, green), $GABA_AR$ β3 (red), MAP2 (blue), and cell-surface immunostaining for $GABA_AR$ γ2, under control conditions and following 24 h of bicuculline (10 μM) treatment. (Scale bars: low magnification, 10 μm; high magnification, 2.5 μm.) (*B* and *D*) Quantification of total cluster area corresponding to experiments represented in *A* and *C*, showing an increase in α-DG, $GABA_AR$ β3, and surface $GABA_AR$ γ2 cluster areas following bicuculline (Bic) treatment ($n = 40$–41 images in each condition for α-DG and β3; $n = 20$ images in each condition for surface γ2; two-tailed Student *t* test, $***P < 0.001$, $**P < 0.01$). (*E*) Representative Western blots from cell-surface biotinylation assays probing control (Ctrl) and bicuculline treated (10 μM, 24 h) cultures for surface and total expression of glycosylated α-DG (mAb IIH6), β-DG, and $GABA_AR$ β3. (*F*) Quantification of experiments represented in *E*, showing an increase in surface α-DG, β-DG, and $GABA_AR$ β3, following bicuculline treatment, and an increase in the total (whole lysate) expression of α-DG and β-DG but not $GABA_AR$ β3 ($n = 7$–10 for surface, $n = 18$–21 for total; Wilcoxon signed-rank test, $**P < 0.01$, $*P < 0.05$; n.s., not significant).

and surface expression (Fig. 1 *A–F*) may be coordinated by an activity-dependent up-regulation of DG expression.

To determine whether protein synthesis is necessary for activity-dependent homeostatic regulation of α-DG and $GABA_AR$ β3 clusters, we preincubated cultures with the protein synthesis inhibitor cycloheximide (CHX, 100 μM) and then treated with bicuculline (10 μM, 6 h). CHX blocked the homeostatic increase in total area of both α-DG and $GABA_AR$ β3 clusters (α-DG: Bic 163.0 ± 14.0% of DMSO alone, CHX alone 112.4 ± 8.6% of DMSO alone, and CHX plus Bic 99.1 ± 5.7% of DMSO alone; β3: Bic 187.9 ± 9.6% of DMSO alone, CHX alone 118.0 ± 7.2% of DMSO alone, and CHX plus Bic 108.3 ± 5.6% of DMSO alone; Fig. 2 *A* and *B*). By monitoring miniature inhibitory postsynaptic currents (mIPSCs), we examined the role of protein synthesis in homeostatic scaling up at inhibitory synapses and found that preincubation with CHX blocked this form of plasticity (DMSO alone 34.1 ± 2.4 pA; Bic 49.7 ± 4.4 pA; CHX alone 38.7 ± 3.3 pA; and CHX plus Bic 33.8 ± 5.2 pA; Fig. S3). Together, these findings indicate that this form of plasticity requires de novo protein synthesis and are consistent with the proposition that one of the necessary proteins synthesized is DG.

DG Is Necessary for Homeostatic Scaling Up of GABAergic Synaptic Strength. To determine whether DG is an essential component in the recruitment of additional $GABA_ARs$ to inhibitory synapses during homeostatic scaling up, we recorded mIPSCs from bicuculline-treated (10 μM, 24 h) neurons, several days after transfection with a DG knockdown (KD) miRNA construct that coexpresses mRFP for visualization of transfected cells (Fig. S4 and Fig. 3*A*). DG KD had no effect on baseline mIPSC amplitude or frequency, suggesting that DG loss, at least in the short term, is not required for GABAergic synapse maintenance. However, DG KD abrogated scaling up of mIPSC amplitude induced by bicuculline treatment [nontargeting (NT) miRNA: Ctrl 32.6 ± 2.8 pA and Bic 50.5 ± 3.9 pA; DG KD miRNA: Ctrl 33.8 ± 3.3 pA and Bic 37.7 ± 3.3 pA; scaling factors vs. NT Ctrl: 1.74 ± 0.01 for NT Bic, 1.08 ± 0.01 for DG KD Ctrl, 1.20 ± 0.01 for DG KD Bic, and 1.07 ± 0.01 for NT Bic scaled; Fig. 3 *A–D*]. Because we used conditions where only 1–3% of the cells were transfected, the recorded cells were isolated from nontransfected cells, suggesting that DG requirement is cell autonomous. Conversely, a trend for increased mIPSC frequency following bicuculline treatment was maintained in DG KD neurons (NT miRNA: Ctrl 2.51 ± 0.52 Hz and Bic 3.93 ± 0.63 Hz; DG KD miRNA: Ctrl 2.14 ± 0.35 Hz and Bic 3.68 ± 0.57 Hz; Fig. 3 *A* and *B*), suggesting that the mIPSC frequency increase observed in this form of

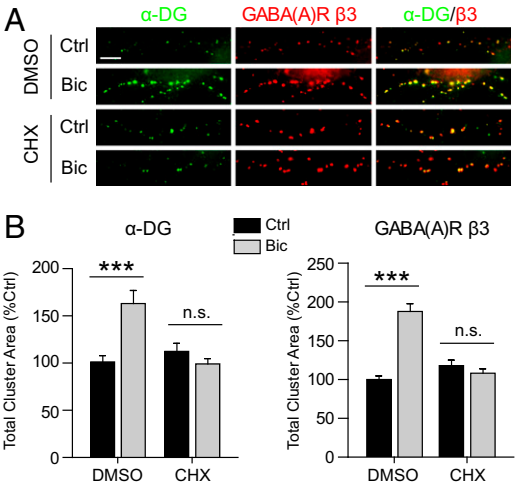

Fig. 2. Protein synthesis is required for homeostatic plasticity at inhibitory synapses. (*A*) Representative images of dendritic regions immunostained for glycosylated α-DG (mAb IIH6) and $GABA_AR$ β3, from control (Ctrl) and bicuculline treated (Bic, 10 μM, 6 h) neurons, preincubated either with DMSO (0.1%) or with the protein synthesis inhibitor cycloheximide (CHX, 100 μM). (Scale bar: 2.5 μm.) (*B*) Quantification corresponding to experiments represented in *A*, showing that cycloheximide blocks the increase in total cluster area of α-DG and $GABA_AR$ β3 induced by bicuculline treatment (n = 79–80 and 70–80 cells in each condition for α-DG and β3, respectively; two-way ANOVA, Tukey's post hoc test, ***$P < 0.001$; n.s., not significant).

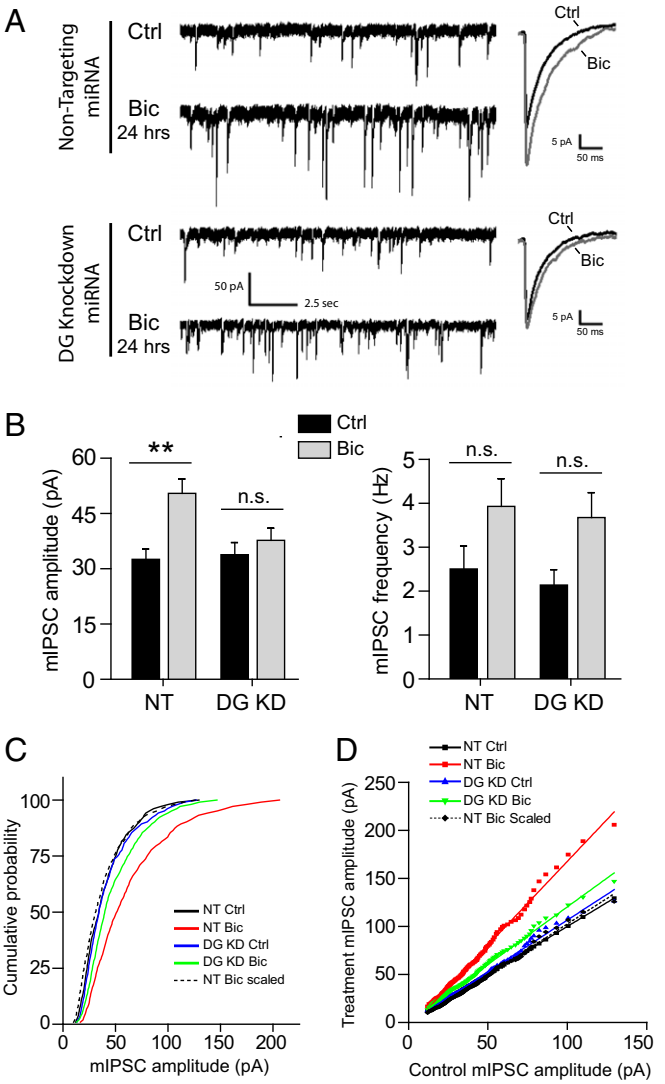

Fig. 3. Cell-autonomous expression of DG is required for homeostatic scaling up of inhibitory synaptic strength. (*A*) Representative traces of mIPSC recordings from control (Ctrl) and bicuculline-treated (Bic, 10 μM, 24 h) neurons transfected with either a nontargeting miRNA-mRFP construct or a miRNA-mRFP construct targeting DG for knockdown. Corresponding average mIPSC traces from representative neurons are shown (*Right*). (*B*) Group data of average mIPSC amplitude (*Left*) and frequency (*Right*) corresponding to experiments shown in *A*. mIPSC amplitude was significantly increased following bicuculline treatment in neurons expressing the nontargeting miRNA-mRFP construct (NT); this increase was absent in DG knockdown neurons (DG KD) (n = 9–14 cells in each condition; two-way ANOVA, Tukey's post hoc test, **$P < 0.01$; n.s., not significant). (*C* and *D*) Cumulative distribution and linear regression plots of mIPSC amplitudes showing that multiplicative scaling induced by bicuculline is blocked by DG knockdown [(*C*), $P < 0.001$ for NT Bic vs. NT Ctrl, $P = 0.99$ for DG KD Ctrl vs. NT Ctrl, $P = 0.38$ for DG KD Bic vs. DG KD Ctrl, and $P = 0.72$ for NT Bic scaled vs. NT Ctrl, Kolmogorov–Smirnov test; (*D*) $r^2 > 0.99$ for all fitted lines].

plasticity is due to DG-independent mechanisms acting to increase neurotransmitter release probability and/or functional synapse number. In summary, DG is required for the recruitment of additional $GABA_A$Rs during homeostatic scaling up of GABAergic synapses, although not for the maintenance of baseline levels of synaptic $GABA_A$Rs.

DG Is Not Required for Homeostatic Scaling Down of Excitatory or Inhibitory Synapses. We investigated the possibility that DG may influence other aspects of homeostatic synaptic plasticity, such as scaling down at glutamatergic synapses in response to elevated neuronal activity and scaling down at GABAergic synapses in response to reduced neuronal activity (21). Consistent with the absence of DG at excitatory synapses (Fig. S2*A*), we observed no effect of DG KD on either baseline excitatory synaptic strength or on scaling down in response to bicuculline treatment (10 μM, 24 h) (NT miRNA: Ctrl 12.3 ± 1.2 pA and Bic 9.02 ± 0.4 pA; DG KD miRNA: 12.1 ± 0.7 pA and Bic 9.0 ± 0.5 pA; Fig. S5). Furthermore, strengthening the notion that baseline inhibitory synaptic strength is set independently of DG, we observed no effect of DG KD on scaling down of mIPSC amplitude in response to prolonged blockade of neuronal activity with tetrodotoxin (TTX) (1 μM, 48 h) (NT miRNA: Ctrl 43.3 ± 3.5 pA and TTX 29.7 ± 2.2 pA; DG KD miRNA: Ctrl 40.5 ± 3.4 pA and TTX 27.1 ± 1.5 pA; Fig. S6). Therefore, DG appears to function selectively in potentiating inhibitory synaptic strength during prolonged periods of high neuronal activity.

Glycosylated α-DG Is Necessary for Homeostatic Scaling Up at GABAergic Synapses. Carbohydrate side chains are necessary for α-DG to bind to agrin and other ligands such as laminin and neurexin (6, 9, 24). LARGE is a glycosyltransferase required for O-linked glycosylation of α-DG (30, 31) and is able to compensate in some respects for deficiencies in other members of the glycosylation pathway (32). Perturbation of LARGE disrupts α-DG ligand binding and neuromuscular junctions in a manner similar to DG deficiency (30, 33). Our findings showing a decrease in $GABA_A$R clustering in the hippocampus of *Myd* mice (Fig. S1*E*) and an increase in glycosylated α-DG following bicuculline treatment of neurons in culture (Fig. 1 *E* and *F*) suggest that glycosylation-dependent ligand binding of α-DG could play a crucial role in homeostatic regulation of GABAergic synaptic strength. To test this possibility, we used siRNA silencing of LARGE expression (Fig. S7) to determine whether homeostatic plasticity at GABAergic synapses would be affected by reduced glycosylation of α-DG. Knockdown of LARGE blocked the activity-dependent scaling up of mIPSC amplitude (NT siRNA: Ctrl 30.7 ± 3.5 pA and Bic 43.0 ± 3.7 pA; LARGE KD siRNA: Ctrl 27.4 ± 2.2 pA and Bic 30.6 ± 3.6 pA; Fig. 4), while not affecting changes in frequency (nontargeting siRNA: Ctrl 1.59 ± 0.60 Hz and Bic 2.61 ± 0.41 Hz; LARGE KD siRNA: Ctrl 1.94 ± 0.34 Hz and Bic 2.81 ± 0.55 Hz). Knockdown of LARGE did not affect the basal distribution of mIPSCs, but did prevent multiplicative scaling (Fig. S8). Overall, this suggests that functional glycosylation and ligand binding of α-DG is necessary for scaling up of GABAergic synaptic strength.

Agrin Binding to DG Is Sufficient to Increase GABAergic Synaptic Strength. Agrin is a transmembrane protein involved in neuromuscular junction maturation (34) that is released following proteolytic cleavage triggered by neural activity in the hippocampus (35). Previous studies using cultured neurons have suggested that agrin may regulate dendrite extension and clustering of $GABA_A$Rs (36). Having determined that agrin is localized to GABAergic synapses (Fig. S2*B*), we tested if application of agrin was sufficient to drive changes at these synapses. Similar to chronic activity elevation with bicuculline, treating cultures with recombinant agrin (1 nM, 24 h) significantly increased total cluster area of both α-DG and $GABA_A$R β3 (α-DG: 135.8 ± 14.2% of Ctrl; $GABA_A$R β3: 204.8 ± 18.8% of Ctrl; Fig. 5 *A* and *B*). Agrin treatment was also sufficient to increase mIPSC amplitude in a manner that was dependent on cell-autonomous DG expression (NT miRNA: Ctrl 37.2 ± 2.2 pA and agrin 48.2 ± 3.0 pA; DG KD miRNA: Ctrl 35.0 ± 2.7 pA and agrin 38.5 ± 4.0 pA; Fig. 5 *C* and *D*), although it had no significant effect on mIPSC frequency (NT miRNA: Ctrl 3.9 ± 0.5 Hz and agrin 3.2 ± 0.6 Hz; DG KD miRNA: Ctrl 4.5 ± 0.6 Hz and agrin 4.0 ± 0.6 Hz; Fig. 5 *C* and *D*). The increase in mIPSC amplitude was multiplicative, suggesting an equal effect on all GABAergic synapses (Fig. S9). Together, these data indicate that ligand-induced signaling through DG, or ligand-induced clustering of DG, is sufficient to increase $GABA_A$R cluster size and GABAergic neurotransmission, suggesting that agrin may function upstream of DG in the activity-dependent pathway responsible for homeostatic scaling up of GABAergic synaptic strength.

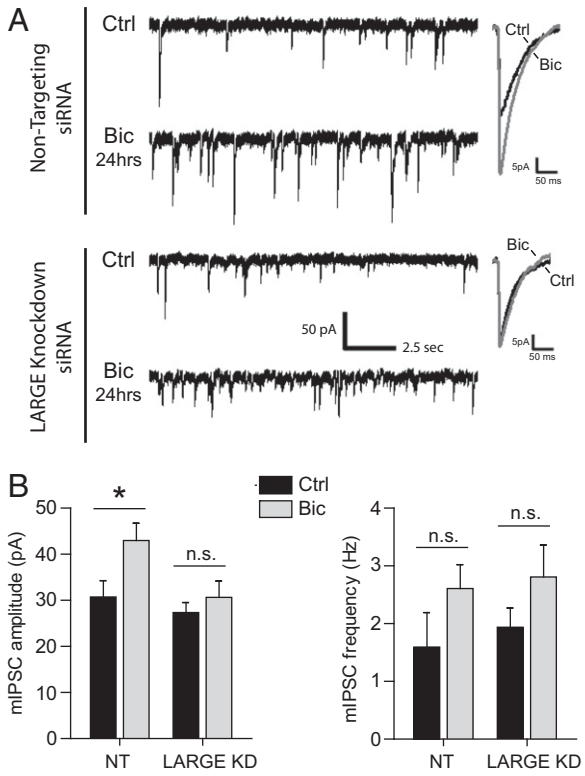

Fig. 4. The α-DG glycosylating enzyme LARGE is required for homeostatic scaling up of inhibitory synaptic strength. (*A*) Representative traces of mIPSC recordings from control (Ctrl) and bicuculline-treated (Bic, 10 μM, 24 h) neurons transfected with either nontargeting siRNA or with siRNA targeting LARGE glycosyltransferase for knockdown. Corresponding average mIPSC traces from representative neurons are shown (*Right*). (*B*) Group data of average mIPSC amplitude (*Left*) and frequency (*Right*) showing that the increase in mIPSC amplitude occurring in bicuculline-treated neurons transfected with nontargeting siRNA (NT) is blocked in neurons transfected with siRNA targeting LARGE (LARGE KD) (n = 11–15 cells in each condition; two-way ANOVA, Bonferroni post hoc test, $*P < 0.05$; n.s., not significant).

Discussion

Mutations in dystrophin or in enzymes glycosylating α-DG result in muscular dystrophies associated with cognitive and neurological deficits (2, 4). Here we show that in response to chronically elevated neuronal activity or acute agrin treatment, the surface expression and clustering of α-DG and $GABA_A$Rs is up-regulated and GABAergic synaptic strength is scaled up. Scaling up of GABAergic synaptic strength and the up-regulation of α-DG requires de novo protein synthesis. Crucially, expression of

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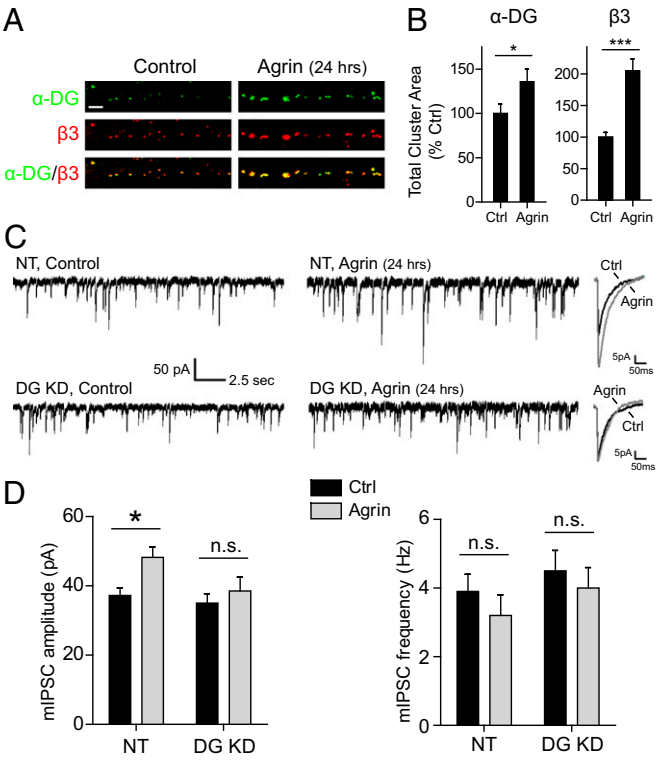

Fig. 5. Agrin treatment enhances clustering of α-DG and $GABA_AR$ β3, and scales up inhibitory synaptic strength through DG. (*A*) Representative dendritic regions from control and agrin-treated (1 nM, 24 h) neurons immunostained for glycosylated α-DG (mAb IIH6) and $GABA_AR$ β3. (Scale bar: 2.5 μm.) (*B*) Quantifications corresponding to experiments represented in *A*, showing an increase in the total cluster area of α-DG (*Left*) and $GABA_AR$ β3 (*Right*) ($n = 30$ cells in each condition; two-tailed Student *t* test, $***P < 0.001$, $*P < 0.05$). (*C*) Representative traces of mIPSC recordings from control and agrin-treated (1 nM, 24 h) neurons, transfected with either the nontargeting (NT) miRNA-mRFP construct (NT) or the miRNA-mRFP construct targeting DG for knockdown (DG KD). Corresponding average mIPSC traces from representative neurons are shown (*Right*). (*D*) Group data of average mIPSC amplitude (*Left*) and frequency (*Right*) showing a significant increase in mIPSC amplitude following agrin treatment, with no change in mIPSC frequency. The effect on amplitude is blocked by DG KD. ($n = 11$–13 cells in each condition; two-way ANOVA, Tukey's posthoc test, $*P < 0.05$, n.s., not significant).

DG and the α-DG glycosylating enzyme LARGE are required for activity-dependent scaling up of GABAergic synaptic strength. However, scaling down of excitatory and inhibitory synaptic strength occurs independently of DG. Together, these findings support a model whereby activity-dependent up-regulation of functional DG is necessary for the recruitment of $GABA_ARs$ during homeostatic synaptic plasticity.

Homeostatic synaptic plasticity in response to chronic increase in neuronal activity multiplicatively scales up inhibitory synaptic strength while scaling down excitatory synaptic strength (22, 23) to restore neuronal firing rate to normal levels (21, 22). Scaling up of inhibitory synapses is known to be initiated cell autonomously, resulting in an increase in the abundance and residence time of synaptic $GABA_ARs$ (22), as well as a BDNF-dependent increase in mIPSC frequency (23). We found that elevated activity also up-regulates the synaptic clustering and expression level of α- and β-DG. Regulation of $GABA_ARs$ appears to be restricted to increased localization at synapses, as their overall expression level is not altered significantly. Critically, we found that either global blockade of de novo protein synthesis or cell-autonomous knockdown of DG expression abrogated scaling up of mIPSC amplitude. Our findings are consistent with a model in which chronically elevated activity increases DG expression, thereby increasing the presence of DG at inhibitory synapses. In turn, DG acts as a scaffold to retain $GABA_ARs$ at synapses, thereby scaling up quantal amplitude. We cannot exclude the possibility that cycloheximide blocks this form of plasticity by blocking synthesis of some critical component other than DG, in which case existing levels of DG would be sufficient for synaptic retention of $GABA_ARs$. However, the necessity for DG in this form of plasticity, along with the biochemical association we observed between DG and $GABA_ARs$ support the notion that DG is instrumental in recruiting or stabilizing $GABA_ARs$ at synapses during scaling up. Whether DG would mediate other increases of inhibitory synaptic strength, such as inhibitory long-term potentiation (LTP), appears unlikely as the effects of agrin are relatively slow compared with the induction of LTP.

We did not observe a change in the baseline level of mIPSC amplitude or frequency in untreated cultures following knockdown of DG. In addition, TTX-induced scaling down of inhibitory synapses was independent of DG. Therefore, DG does not appear to play a significant role in setting the baseline level of GABAergic synaptic strength. Furthermore, baseline miniature excitatory postsynaptic current (mEPSC) amplitude was unaltered by DG KD, consistent with the absence of DG at excitatory synapses and suggesting that a normal basal excitation/inhibition ratio is maintained independently of DG. In vivo, however, in the absence of either full-length dystrophin or LARGE activity, we observed a considerable reduction of α1 $GABA_AR$ clustering in the hippocampus. The discrepancy between our in vitro and in vivo findings could be due to a higher level of bursting activity in vivo, which may resemble a partially scaled up state in cultures. Alternatively, our use of acute knockdown in vitro may not replicate the complete absence of full-length dystrophin and LARGE in *Mdx* and *Myd* mice during brain development. DG may also be localized to a subset of synapses proximal to the cell soma, thus limiting the basal effects, but up-regulated at all GABAergic synapses during scaling.

Glycosylation of α-DG is essential for binding ligands such as agrin, laminin, and neurexin. Our data indicate that LARGE-dependent α-DG glycosylation is necessary for $GABA_AR$ clustering in *Myd* mice in vivo and for homeostatic scaling up of GABAergic synapses in vitro. This suggests a necessary interaction of α-DG with a ligand, whereas binding of β-DG to neuroligin-2 and S-SCAM (37) is not sufficient, but is perhaps important for the targeting of DG to GABAergic synapses. The effect of agrin on neuronal synapses is controversial. In peripheral ganglia, agrin participates in cholinergic synapse formation (38) but in the CNS its effects and effectors are unclear (39). We found that acute treatment with exogenous agrin was sufficient to induce a multiplicative scaling of inhibitory synapses that resembles activity-dependent scaling up. However, agrin did not induce a trend for increased mIPSC frequency, suggesting that agrin may function in conjunction with BDNF during homeostatic plasticity (23). Consistent with DG specifically regulating postsynaptic expression of homeostatic scaling up, neither DG knockdown nor LARGE knockdown reduced the trend for increased mIPSC frequency in response to bicuculline treatment. The effect of agrin required DG expression, suggesting that agrin recruits additional $GABA_ARs$ to the synapse either by initiating intracellular signaling via DG or by binding to and stabilizing α-DG (and associated $GABA_ARs$) in clusters, much as it does in muscle cells (6, 40). Neuronal activity is known to induce neurotrypsin-dependent agrin cleavage from the cell surface at central synapses (35), which would increase during bicuculline treatment.

Dystrophin mutations in *Mdx* mice or in patients with Duchenne muscular dystrophy are often accompanied by cognitive deficits,

as well as a higher risk for epilepsy and autism spectrum disorder (ASD) (4, 41). Dystroglycanopathies display similar phenotypes including severe intellectual disability and epilepsy (4). Our findings of impaired GABAergic homeostatic plasticity following reduced α-DG or LARGE expression may help explain several of these cognitive impairments. For example, increased excitation/inhibition ratio has been implicated in ASD-like behavioral phenotypes (42). An inability to homeostatically scale up inhibitory synaptic strength during prolonged periods of neuronal spiking activity may also explain the susceptibility to epilepsy (43, 44). The contribution of the DGC to glutamatergic LTP remains unclear since the DGC is exclusively localized to GABAergic synapses. Enhanced LTP in *Mdx* mice (16) may be due to a facilitation of LTP induction owing to reduced inhibition, since restoring dystrophin expression in *Mdx* mice restores both $GABA_AR$ clustering and LTP to normal levels (19, 45).

In conclusion, we present the first evidence, to our knowledge, of physiological regulation of DG in the nervous system. Our data describe a protein synthesis-dependent pathway for homeostatic plasticity at GABAergic synapses that requires glycosylated dystroglycan to recruit or stabilize $GABA_ARs$ at the synapse. Identification of DG as a key component of homeostatic regulation of GABAergic synaptic strength provides an entry point into the contribution of DG to central synapse function.

Materials and Methods

Hippocampal Neuron Culture, Knockdown, and Treatments. Dissociated rat hippocampal neuron cultures were transfected with DG knockdown plasmids or LARGE siRNA at least 3 d before performing experiments at 17–21 days in vitro. See *SI Materials and Methods* for additional details.

Cell-Surface Biotinylation, Immunoprecipitation, and Western Blotting. See *SI Materials and Methods*.

Immunohistochemistry, Immunocytochemistry, and Image Analysis. See *SI Materials and Methods*.

Electrophysiology. Neurons were superfused with artificial cerebrospinal fluid solution containing (in mM): 115 NaCl, 5 KCl, 23 glucose, 26 sucrose, 4.2 Hepes, 2.5 $CaCl_2$, and 1.3 $MgCl_2$. For mIPSC recordings, pipettes were filled with an internal solution containing (in mM): 127 CsCl, 8 NaCl, 1 $CaCl_2$, 10 Hepes, 10 EGTA, 0.3 Na_3-GTP, and 2 Mg-ATP. To isolate mIPSCs, 200 nM TTX, 10 μM 2,3-Dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[*f*]quinoxaline-7-sulfonamide (NBQX) and 25 μM D-(-)-2-Amino-5-phosphonovaleric acid (D-APV) were added to the external solution, and recordings were obtained at $V_h = -70$ mV. See *SI Materials and Methods* for additional details.

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