Schizophrenia susceptibility gene *dysbindin* regulates glutamatergic and dopaminergic functions via distinctive mechanisms in *Drosophila*

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The dysfunction of multiple neurotransmitter systems is a striking pathophysiological feature of many mental disorders, schizophrenia in particular, but delineating the underlying mechanisms has been challenging. Here we show that manipulation of a single schizophrenia susceptibility gene, *dysbindin*, is capable of regulating both glutamatergic and dopaminergic functions through two independent mechanisms, consequently leading to two categories of clinically relevant behavioral phenotypes. Dysbindin has been reported to affect glutamatergic and dopaminergic functions as well as a range of clinically relevant behaviors in vertebrates and invertebrates but has been thought to have a mainly neuronal origin. We find that reduced expression of *Drosophila* dysbindin (Ddysb) in presynaptic neurons significantly suppresses glutamatergic synaptic transmission and that this glutamatergic defect is responsible for impaired memory. However, only the reduced expression of Ddysb in glial cells is the cause of hyperdopaminergic activities that lead to abnormal locomotion and altered mating orientation. This effect is attributable to the altered expression of a dopamine metabolic enzyme, Ebony, in glial cells. Thus, Ddysb regulates glutamatergic transmission through its neuronal function and regulates dopamine metabolism by regulating Ebony expression in glial cells.

dystrobrevin binding protein 1 | glutamate | glia

Schizophrenia is a debilitating mental disorder with intricate etiology and multidimensional pathophysiological and clinical features. Pathophysiologically, multiple neurotransmitter systems, including glutamate, dopamine, GABA, and serotonin, are disturbed in this morbid condition (1). Accordingly, schizophrenia has multiple clinical characteristics, including positive symptoms, negative symptoms, and cognitive impairments (2). However, how an imbalance of multiple neurotransmitter systems evolves and how the pathophysiological abnormalities give rise to the clinical features remain to be elucidated. Despite the complicated pathophysiology and associated symptoms, evidence is mounting that genetic factors contribute substantially to the development and expression of schizophrenia (3). Human genetic studies have identified a plethora of candidate genes linked to susceptibility for this disease (4). Notably, many susceptibility genes act by regulating the function or homeostasis of multiple neurotransmitter systems (5). Therefore, genetic manipulation of these susceptibility genes will help disclose the mechanisms underlying the genetic regulation of neurotransmitter systems as well as related behaviors and in turn will increase our understanding of the pathogenesis of the disease per se.

For this purpose, the current study began with an analysis of a *Drosophila* mutant of *dysbindin* (*Ddysb*), an ortholog of the human schizophrenia susceptibility gene, *dystrobrevin binding protein 1* (*DTNBP1,* also known as *"dysbindin"*). The *DTNBP1*-encoded dysbindin-1 was identified initially as a member of the dystrophin-associated protein complex (6) and later as a member of the biogenesis of lysosome-related organelles complex 1 (BLOC-1) (7). Despite some controversial results, the association between multiple variants in *DTNBP1* and schizophrenia has been replicated by several independent studies (8, 9). Furthermore, reduced dysbindin expression has been reported in the prefrontal cortex and hippocampus of schizophrenia patients (10, 11). Although it is a null mutant, the spontaneously occurring deletion in the mouse homolog of *DTNBP1* (7) leads to a range of schizophrenia-related pathophysiological and behavioral phenotypes, including reduced glutamatergic transmission, abnormalities in dopaminergic activities, locomotor hyperactivity, social withdrawal, and memory compromise (12, 13).

This study of the *Drosophila* mutant demonstrates that a 30–40% reduction in Ddysb expression is capable of recapitulating major features of schizophrenia-related pathophysiological changes observed in the null mutant of the so-called "*sandy*" mouse. Genetic manipulation allowed us to reveal distinct functions of Ddysb in neurons and in glial cells. Disruption of Ddysb function in neurons is responsible for hypoglutamatergic transmission and subsequent memory defects, whereas disruption of Ddysb function in glial cells causes hyperdopaminergic activity and locomotor hyperactivity via the reduction of the protein Ebony.

Results

Characterization of the *Ddysb* Mutant. The *Ddysb* mutant studied here is a pBac^{e01028} mutant carrying a piggyBac transposon in the 3′ UTR of *Ddysb* (Fig. 1*A*). Western blotting revealed that Ddysb expression in the adult head was reduced by ∼40% in this mutant (Fig. 1*B*). Immunohistochemistry also showed a consistent reduction of Ddysb signals in the mutant brain (Fig. S1*A*). In accordance with a recent report (14), these results suggest that pBac^{e01028} is a hypomorphic allele of *Ddysb* and thus is referred to hereafter as "*dysb*^{1}."

We first looked at pathophysiology-related changes, i.e., glutamatergic and dopaminergic activities. The two-electrode voltage-clamp method was used to assay excitatory junctional currents (EJCs) of glutamatergic synaptic transmission at the well-characterized larval neuromuscular junction (NMJ) (15). The *dysb*^{1} mutant showed significantly attenuated EJCs at various extracellular calcium concentrations (Fig. 1*C*) without significant morphological changes; this result is consistent with a recent independent study (14). To assay how dopamine activity might be affected, we measured the dopamine level in the heads of adult flies using ELISA (16). The dopamine concentration in *dysb*^{1} mutant heads was around twofold of that in WT flies (Fig. 1*D*). Therefore, reduced *Ddysb* expression leads to both hypoglutamatergic and hyperdopaminergic activities.

For pathophysiology-relevant behavioral phenotypes, we focused mainly on learning task and locomotor activity, because

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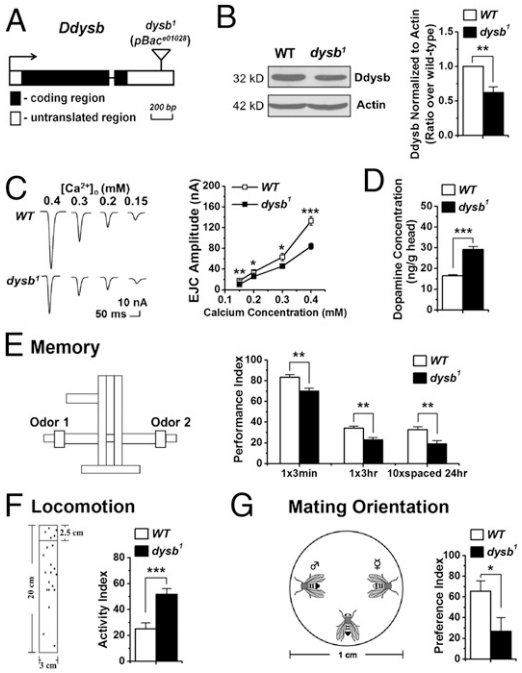

Fig. 1. Characterization of the *Ddysb* mutant. (*A*) Transposon insertion site. (*B*) Representative Western blots and group data showing reduced expression of Ddysb in the head of the *dysb*^{1} mutant (*t* test; $P = 0.007$; $n = 5$). (*C*) The *dysb*^{1} mutant shows decreased EJC amplitude (*t* test; $P = 0.002$, 0.02, 0.03, and 2.1E-6 for calcium concentrations of 0.15, 0.2, 0.3, and 0.4 mM, respectively; $n = 7$–14). (*D*) Dopamine concentration in whole-head extracts of adult flies is elevated significantly in the *dysb*^{1} mutant (*t* test; $P = 1.9E-6$; $n = 8$–10). (*E*) The *dysb*^{1} mutant shows memory defects in the Pavlovian olfactory aversive conditioning (*t* test; $P = 0.004$, 0.002, and 0.009; $n = 7$–8). The T-maze for memory test is illustrated. (*F*) *dysb*^{1} mutants show dramatically increased locomotor activity (*t* test; $P = 2E-4$; $n = 17$). The experimental paradigm for the locomotion test is illustrated on the left. (*G*) The *dysb*^{1} mutant shows mating disorientations (*t* test; $P = 0.02$; $n = 28$–32). The mating preference assay is illustrated. Data are means ± SEM. $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

both are often assayed in rodent models of schizophrenia (17). For the learning task, adult flies were trained with Pavlovian olfactory aversive conditioning in which flies learn to avoid an odor previously associated with electric shock (Fig. 1*E*, *Left*) (18, 19). Memory performance immediately after one-session training was lower in *dysb*^{1} mutants than in WT flies. Memory retention at later time points, either 3 h after single-session training or 24 h after repetitive training, was also compromised in the *dysb*^{1} mutant (Fig. 1*E*, *Right*), but the sensorimotor responses necessary for performing the learning task were not altered (Fig. S1 *B* and *C*). For locomotor activity, we used a climbing assay to record how high flies climbed in response to light taps on the container (Fig. 1*F*, *Left*) (20). The *dysb*^{1} mutant exhibited remarkably elevated activity compared with WT flies of the same age (Fig. 1*F*, *Right*).

During the climbing assay, we observed that *dysb*^{1} males showed abnormal male–male courtship behavior. Mutant males courted females, as WT flies do (Fig. S1*D*), but also oriented the typical courtship ritual toward another male. To quantify this abnormal mating orientation, we adopted a mating preference assay, in which the testing male was allowed to choose between a decapitated WT male and female as a mating partner (Fig. 1*G*, *Left*) (21). WT males display a strong bias toward female flies, but this courtship tendency was reduced significantly in the *dysb*^{1} mutants (Fig. 1*G*, *Right*).

Thus *dysb*^{1} mutants showed hypoglutamatergic and hyperdopaminergic activities as well as memory defects, locomotor hyperactivity, and mating disorientation. The genetic specificity of the observed phenotypes was confirmed through rescuing and RNAi silencing experiments presented later.

Tissue-Specific Regulation of Changes in Neurotransmitter Systems. While determining the genetic specificity of the observed phenotypes, we discovered tissue-specific functions of Ddysb using the Gal4/Upstream Activation System (Gal4/UAS) binary system (22). *Ddysb* RNAi (*DdysbIR*) and *UAS-Ddysb* flies were used for *Ddysb* knockdown and overexpression (Fig. S2). At the NMJ, we first determined whether glutamatergic transmission was supported by a presynaptic or postsynaptic function of Ddysb. Transgenic RNAi was targeted to either presynaptic neurons or postsynaptic muscle cells. We found that silencing Ddysb functions pan-neuronally [*embryonic lethal abnormal vision* (*elav*)-*Gal4/Y;DdysbIR-1/*$^+$], instead of in the muscle cells (*DdysbIR-1/*$^+$*; muscle Gal4* (*C57*)*/*$^+$), caused a similarly reduced EJC amplitude in the *dysb*^{1} mutant (Fig. 2*A*). To confirm further the presynaptic role of Ddysb, we performed a rescuing experiment, showing that pan-neuronal expression of the *UAS-Ddysb* transgene in the *dysb*^{1} mutant background (*elav-Gal4/Y;UAS-Ddysb/*$^+$*;dysb*^{1}) successfully restored the EJC to the WT level (Fig. 2*B*). Moreover, pan-neuronal overexpression of *Ddysb* by two copies in the WT background (*elav-Gal4;UAS-Ddysb*) led to increased EJC amplitude (Fig. S3*A*). Thus, Ddysb regulates the glutamatergic synaptic transmission presynaptically in a dosage-dependent manner.

Unexpectedly, the neuronal expression of *Ddysb* in *dysb*^{1} mutant (*UAS-Ddysb/*$^+$*;elav-Gal4*$_{(III)}$ *dysb*^{1}*/dysb*^{1}) did not rescue the dopamine level in the adult head (Fig. 2*C*, *Left*). This result prompted us to test the possibility that Ddysb might have non-neuronal functions. We found that expression of *Ddysb* with a glia-specific driver, Reversed polarity (Repo)-Gal4 (*UAS-Ddysb/*$^+$*; Repo-Gal4 dysb*^{1}*/dysb*^{1}) suppressed the elevated dopamine level in the *dysb*^{1} mutant (Fig. 2*C*, *Right*). Therefore, Ddysb appears to regulate glutamatergic transmission and dopamine level through different mechanisms. This result led us to explore whether and how such distinct roles affect relevant behavioral phenotypes.

Tissue-Specific Regulation of Different Behaviors. Pan-neuronal expression of *Ddysb* in the mutant background (*UAS-Ddysb/*$^+$*;elav-Gal4*$_{(III)}$ *dysb*^{1}*/dysb*^{1}) rescued the short-term memory defect in the *dysb*^{1} mutant (Fig. 2*D*, *Left*), whereas glial expression (*UAS-Ddysb/*$^+$*;Repo-Gal4 dysb*^{1}*/dysb*^{1}) failed to do so (Fig. 2*D*, *Right*).

Furthermore, pan-neuroanl overexpression of *Ddysb* (*elav-Gal4/*$^+$*;UAS-Ddysb/*$^+$) led to a similar defect in memory (Fig. S3*B*). Thus, all the data consistently supported the notion that intact Ddysb function in neurons is required for memory formation.

In contrast to the memory deficit, locomotor hyperactivity and the mating disorientation in the *dysb*^{1} mutant were suppressed by expressing *Ddysb* in glia (*UAS-Ddysb/*$^+$*;Repo-Gal4 dysb*^{1}*/dysb*^{1}) but not in neurons (*UAS-Ddysb/*$^+$*;elav-Gal4*$_{(III)}$ *dysb*^{1}*/dysb*^{1}) (Fig. 2 *E* and *F*). The specificity of this glial rescue was confirmed further via targeted RNAi silencing. Knocking down *Ddysb* pan-neuronally (*elav-Gal4/Y;DdysbIR-1/*$^+$) had no effect on the mating orientation of males. However, expressing *DdysbIR* either ubiquitously (*Actin-Gal4/DdysbIR-1*) or specifically in glia (*DdysbIR-1/*$^+$*; Repo-Gal4/*$^+$) resulted in dramatically increased male–male courtship behavior, as shown in the *dysb*^{1} mutant (Fig. S3*C*).

Human Dysbindin Rescues the Phenotypes in *dysb*^{1} Mutant. Ddysb shares 28% amino acid identity with human dysbindin-1 (Hdysb) (9), but it remains unknown whether the functions of dysbindin are conserved and whether the Hdysb also has the tissue-specific effects revealed above. Thus, we generated *UAS-Hdysb* transgenic flies (Fig. S4). Pan-neuronal expression of *Hdysb* successfully restored the glutamatergic transmission (*elav-Gal4/Y;UAS-Hdysb/*$^+$*; dysb1*) and associative memory (*UAS-Hdysb/*$^+$*;elav-Gal4*$_{(III)}$ *dysb1/dysb1*) in the *dysb*^{1} mutant (Fig. 2 *G* and *I*), whereas glial expression of *Hdysb* (*UAS-Hdysb/*$^+$*;Repo-Gal4 dysb*^{1}*/dysb*^{1}) suppressed the elevated brain dopamine level and hyperactivity (Fig. 2 *H* and *J*). Thus, the evidence suggests considerable conservation between human and *Drosophila* dysbindin in function.

Endogenous Expression of Ddysb in Glial Cells. Earlier studies on *dysbindin* focused largely on its neuronal function (8). However,

the experiments described above revealed an underestimated role of Ddysb in glia that could affect dopamine signaling, locomotion, and sex orientation. This finding led us to map the distribution of Ddysb in glial cells. We first attempted to visualize Ddysb in glial cells through immunohistochemical staining of Ddysb (in red) over GFP-labeled glia (*UAS-mCD8::GFP/*$^{+}$*;Repo-Gal4/*$^{+}$). The presence of endogenous Ddysb in glia was observed in the optic lobes and the central brain (merged yellow for Ddysb in glia; white arrows in Fig. 3*A*). To validate the detected signals, we expressed a Venus fluorescence protein (VFP)-tagged Ddysb (14) in GFP-labeled glia (*UAS-mCD8::GFP/*$^{+}$*;UAS-venus-dysb/Repo-Gal4*). A similar pattern of Ddysb expression was observed (merged yellow at the cell body region; white arrows in Fig. 3*B*). However, we noted that a large fraction of VFP-Ddysb (red dots and white arrowheads in the lower panels of Fig. 3*B* and Fig. S5) was distributed over glial processes that were not merged with the GFP-tagged membrane protein, suggesting that the endogenous level of glial Ddysb was underestimated (Fig. 3*A*).

Although its expression in glia is scattered, Ddysb plays a critical role in development in addition to the physiological and behavioral roles described above. Silencing *Ddysb* specifically in glia with two copies of *DdysbIR* (*DdysbIR-1/DdysbIR-2;Repo-Gal4/*$^{+}$) resulted in pupal lethality significantly higher than in the parental controls (Fig. S6). Driving even a single copy of RNAi by an astrocyte-like glial Gal4 (NP3233) resulted in embryonic lethality similar to that seen with universal knockdown of *Ddysb* (Table S1).

Acute Induction of *Ddysb* Rescues the Phenotypes in *dysb*^{1} Mutants. Because Ddysb might play a role in the development of the nervous system (as discussed above, and also see ref. 23 and 24), we wanted to determine the developmental contribution to the phenotypes observed. To this end, we tested the effects of acutely manipulated expression of *Ddysb*. Acute induction of *Ddysb* through a heat-shock Gal4 (hs-Gal4) driver (*UAS-Ddysb/*$^{+}$*;hs-Gal4 dysb*^{1}*/dysb*^{1}) (Fig. S7) rescued the short-term memory defect (Fig. 4*A*) and suppressed the increased brain dopamine concentration (Fig. 4*B*) and the mating disorientation (Fig. 4*C*) in the *dysb*^{1} mutant. These results indicate that, apart from developmental functions, Ddysb also plays an acute physiological role in regulating neurotransmitters and behaviors in adult animals.

Hypoglutamate Is Responsible for Memory Defect. Because both glutamatergic transmission at the NMJ and the memory capacity in the adult fly were restored by expressing WT *Ddysb* pan-neuronally in the *dysb*^{1} mutant background, we were interested in whether the function of Ddysb in glutamatergic transmission is related to its function in memory ability. First, we found that feeding flies with glycine, a NMDA receptor agonist and also a drug used to treat negative syndromes in schizophrenia (25), restored memory performance to the WT level in the adult fly,

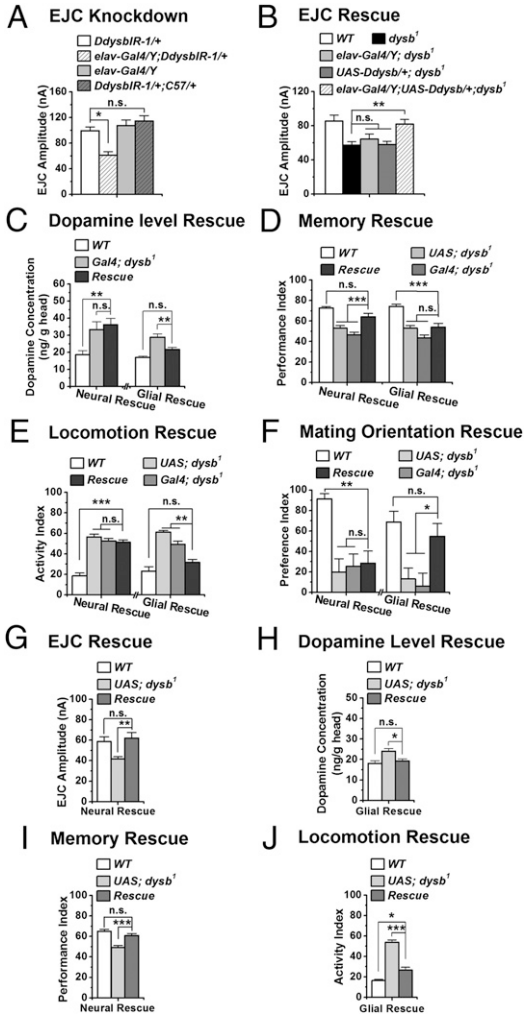

Fig. 2. Tissue-specific regulation of changes in different neurotransmitter systems and behaviors. (*A*) EJC amplitude was reduced significantly by expression of the *Ddysb* RNAi presynaptically (*elav-Gal4/Y; DdysbIR-1/*$^{+}$) but not postsynaptically (*DdysbIR-1/*$^{+}$*;C57/*$^{+}$) (ANOVA; n = 9–16). The calcium concentration for recording is 0.4 mM. (*B*) Neuronal expression of *UAS-Ddysb* (*elav-Gal4/Y; UAS-Ddysb/*$^{+}$*;dysb*^{1}) rescued the EJC deficit in the *dysb*^{1} mutant (ANOVA; n = 16–22). (*C*) Glial expression of *Ddysb* (*UAS-Ddysb/*$^{+}$*;Repo-Gal4 dysb*^{1}*/dysb*^{1}), but not neuronal expression of *Ddysb* (*UAS-Ddysb/*$^{+}$*;elav-Gal4*$_{(III)}$ *dysb*^{1}*/dysb*^{1}), restored the brain dopamine concentration to normal levels (ANOVA; n = 5–10). (*D*) Pan-neural expression of *Ddysb* (*UAS-Ddysb/*$^{+}$*;elav-Gal4*$_{(III)}$ *dysb*^{1}*/dysb*^{1}) is sufficient to rescue the 3-min associative memory deficit in the *dysb*^{1} mutant. However, glia-specific expression of *Ddysb* (*UAS-Ddysb/*$^{+}$*;Repo-Gal4 dysb*^{1}*/dysb*^{1}) failed to do so (ANOVA; n = 4–14). (*E* and *F*) Locomotor hyperactivity and mating disorientation were suppressed in *dysb*^{1} mutants expressing *UAS-Ddysb* in glia (*UAS-Ddysb/*$^{+}$*;Repo-Gal4 dysb*^{1}*/dysb*^{1}) but not in neurons (*UAS-Ddysb/*$^{+}$*;elav-Gal4*$_{(III)}$ *dysb*^{1}*/dysb*^{1}). (ANOVA; n = 8–10 for locomotor test; n = 22–51 for mating test.) Data are means ± SEM. (*G* and *I*) Pan-neuronal expression of Hdysb rescued the EJC amplitude (*elav-Gal4/Y;UAS-Hdysb/*$^{+}$*;dysb*^{1}) (ANOVA; n = 9–10) and the 3-min associative memory deficit (*UAS-Hdysb/*$^{+}$*;elav-Gal4*$_{(III)}$ *dysb*^{1}*/dysb*^{1}) (ANOVA; n = 16) in the *dysb*^{1} mutant. Calcium concentration for recording is 0.4 mM. (*H* and *J*) Glial expression of Hdysb (*UAS-Hdysb/*$^{+}$*;Repo-Gal4 dysb*^{1}*/dysb*^{1}) restored the brain dopamine level (ANOVA; n = 4–11) and partially suppressed the hyperactivity in the *dysb*^{1} mutant (ANOVA; n = 10). Data are means ± SEM. *P < 0.05; **P < 0.01; ***P < 0.001; n.s., not significant (P > 0.05).

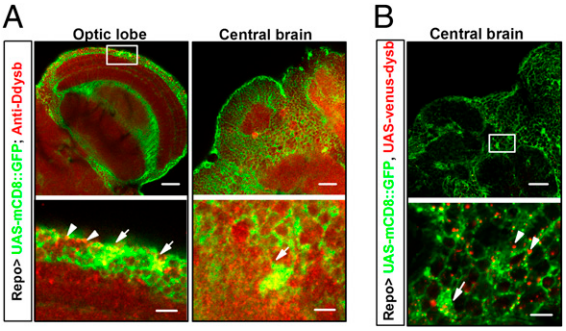

Fig. 3. Endogenous expression of Ddysb in glial cells. Lower row shows enlarged views of boxed areas in upper row. (*A*) Ddysb is expressed extensively in both neurons and glia. Immunosignals of anti-Ddysb antibody (red), representing endogenous Ddysb, partially overlap (yellow) with Repo-Gal4–driven mCD8::GFP (green) at the glial cell bodies (white arrows).The dotted red signals also aggregate at the boundaries of neurons and glial processes (white arrowheads). (*B*) Coexpression of *UAS-mCD8::GFP* (green) and *UAS-venus-dysb* (red) in glia (*UAS-mCD8::GFP/*$^{+}$*;UAS-venus-dysb/Repo-Gal4*). The immunosignals of Repo-Gal4–driven-Ddysb (red) localize at the glial cell body region (white arrow) and the boundaries of neurons and glial processes (white arrowheads), similar to the distribution of endogenous Ddysb in glia. (Scale bars: 20 μm *Upper*; 5 μm *Lower*.)

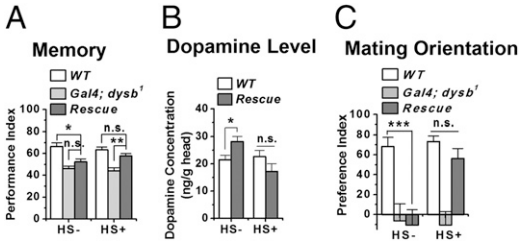

Fig. 4. Acute induction of *Ddysb* rescues the phenotypes in the *dysb*^{1} mutant. Acute induction of Ddysb expression ubiquitously by a heat-shock promoter–Gal4 driver in the mutant background (*UAS-Ddysb*$^+$*;hs-Gal4 dysb*^{1}*/dysb*^{1}) rescued the 3-min associative memory deficit (*A*) (ANOVA; n = 6–12) and restored the brain dopamine level (*B*) (t test; P = 0.15; n = 7–14) and normal mating orientation (*C*) (ANOVA; n = 19–24). Data are means ± SEM. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; n.s., not significant ($P > 0.05$).

suggesting that the hypoglutamatergia in the *dysb*^{1} mutant is responsible for its memory deficit (Fig. 5*A*). To confirm the specificity of the pharmacological rescuing effect, we then expressed *Ddysb* with a glutamatergic neuron-specific Gal4 driver (vesicular glutamate transporter Gal4, VGluT-Gal4) in the mutant background (*VGluT-Gal4/*$^+$*;UAS-Ddysb/*$^+$*;dysb*^{1}), which sufficiently restored the short-term memory to the normal level (Fig. 5*D*). Furthermore, overexpression of *Ddysb* in glutamatergic neurons (*VGluT-Gal4/*$^+$*;UAS-Ddysb/*$^+$) led to a defect in memory (Fig. S8) similar to that seen with pan-neuronal overexpression. Thus, all the data consistently show that the intact function of Ddysb in glutamatergic transmission is required for memory formation.

Elevated Dopamine Is Responsible for Hyperactivity and Mating Disorientation. Because the memory defect was associated specifically with Ddysb functions in glutamatergic neurons, we then tested whether glia-dependent hyperdopaminergic activity, locomotor hyperactivity, and mating disorientation are related. In fact, there are reports that hyperdopaminergia in the fruit fly can lead to male–male courtship (26) and hyperactivity (27). We fed flies with two inhibitors of dopamine signaling: the vesicular monoamine transporter inhibitor reserpine (28) and the tyrosine hydroxylase (TH) inhibitor α-methyl-p-tyrosine (AMPT) (29). Compared with the drug-free group, both reserpine and AMPT significantly suppressed locomotor hyperactivity and mating disorientation in the *dysb*^{1} mutant but at the tested concentration had no significant effects on WT flies (Fig. 5 *B* and *C*).

The specificity of the pharmacological assay was confirmed by a genetic interaction experiment between *Ddysb* and *ebony*, which encodes a glia-specific β-alanyl biogenic amine synthetase that metabolically inactivates biogenic amines in the nervous system. The dopamine sequestered by glia is inactivated by Ebony to form *N*-β-alanyldopamine (NBAD) (30). Defective Ebony increases brain dopamine level and perturbs multiple behaviors including courtship (26, 31). As expected, we revealed a genetic interaction between *Ddysb* and *ebony*. The locomotor hyperactivity and mating disorientation were observed in double-heterozygous mutants (*ebony*^{1}*/dysb*^{1}), although single heterozygotes (*ebony*^{1}*/*$^+$ or *dysb*^{1}*/*$^+$) showed no phenotypes (Fig. 5 *E* and *F*). Therefore, evidence from pharmacological rescue and genetic interaction corroborates the contribution of elevated dopamine signaling to locomotor hyperactivity and mating disorientation.

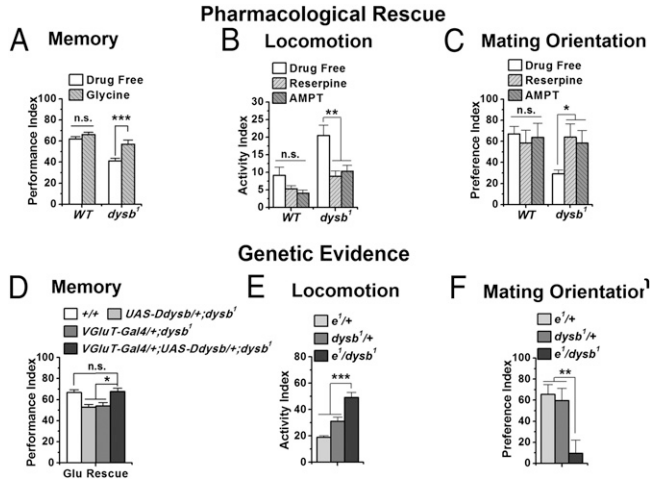

Fig. 5. Hypoglutamate and hyperdopamine levels are responsible for different behavioral phenotypes. (*A*) Feeding flies with glycine, an NMDA receptor agonist, restored the 3-min associative memory in the *dysb*^{1} mutant (t test; P = 0.0007; n = 9–12) without significantly affecting WT flies (t test; P = 0.17; n = 12). (*B* and *C*) Feeding flies with reserpine, a vesicular monoamine transporter inhibitor, and AMPT, a TH inhibitor, suppressed the increased locomotion and disorientated mating behavior in the *dysb*^{1} mutant (ANOVA; n = 9–12 for locomotor test; n = 20–57 for mating test) but did not significantly influence WT flies. (*D*) Expression of *Ddysb* specifically in the glutamatergic neurons (*VGluT-Gal4/*$^+$*;UAS-Ddysb/*$^+$*; dysb*^{1}) is sufficient to rescue the 3-min associative memory deficit in the *dysb*^{1} mutant (ANOVA; n = 14). (*E* and *F*) Genetic complementation test between *dysb*^{1} *and ebony*^{1} (e^1) Compared with parental controls (e^1*/*$^+$ and *dysb*^{1}*/*$^+$), the double heterozygotes (e^1*/dysb*^{1}) showed significant locomotor hyperactivity (ANOVA; n = 15–16) and mating disorientation (ANOVA; n = 34–36). Data are means ± SEM. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; n.s., not significant ($P > 0.05$).

Ddysb Regulates Dopamine Level via Ebony in Glia. To gain insights into how Ddysb regulates dopaminergic activity in glial cells, we examined the transcription profiles of major genes involved in the synthetic and metabolic pathway of dopamine in *Drosophila* (Fig. 6*A* and refs. 32 and 33). RT-PCR results showed that, among the genes examined, the transcription levels of *pale* [the genetic locus for TH, the rate-limiting enzyme in dopamine biosynthesis (Fig. 6*A* and ref. 32)], and *tan* [encoding Tan, the enzyme for hydrolysis of NBAD to dopamine (Fig. 6*A* and ref. 34)] were decreased dramatically in *dysb*^{1} mutants (Fig. 6*B*). However, the changes in expression of these two genes could not be responsible for hyperdopaminergic activity, because (*i*) both TH and Tan are expressed specifically in neurons; and (*ii*) their reduction is a change in the opposite direction of producing hyperdopaminergic activity. In addition to these two genes, expression of *ebony* is also reduced significantly at both mRNA and protein levels in the *dysb*^{1} mutant (Fig. 6 *B* and *C*). As mentioned earlier, a reduction in Ebony function leads to hyperdopaminergic activity (26). The genetic interaction between *Ddysb* and *ebony* (Fig. 5 *E* and *F*) corroborates nicely with biochemical data, indicating that a reduction in Ddysb activity leads to reduced Ebony activity, which in turn leads to hyperdopaminergic activity.

Further immunohistochemical analysis of the adult brain confirmed the changes in Ebony. The Ebony signals were decreased in both the cell body and in the processes region of the Ebony-expressing glial cells (Fig. 6*D*), with more visible reduction in the processes.

Discussion

The current study investigated functions of Ddysb to explore how the altered expression of a single schizophrenia susceptibility gene relates to the pathophysiology and clinically relevant phenotypes. The function of this gene is highly conserved from *Drosophila* to vertebrates and even to humans. The observed pattern of *Ddysb* expression in the *Drosophila* brain (Fig. 3*A* and Fig. S1*A*) is very similar to that reported in the vertebrate brain: widespread and enriched in neurons (6). Loss-of-function mutations and RNAi knockdown of *Ddysb* in *Drosophila* produced phenotypes similar to those observed in the *sandy* mouse, including attenuated glutamatergic transmission, hyperdopaminergic activity, memory defects, and locomotor hyperactivity. Moreover, the human *DTNBP1* gene was capable of rescuing *dysb*^{1} mutant phenotypes in *Drosophila* (Fig. 2 *G*–*J*). With the help of genetic tools exclusively available in *Drosophila*, however, we gained surprising insights, as outlined below.

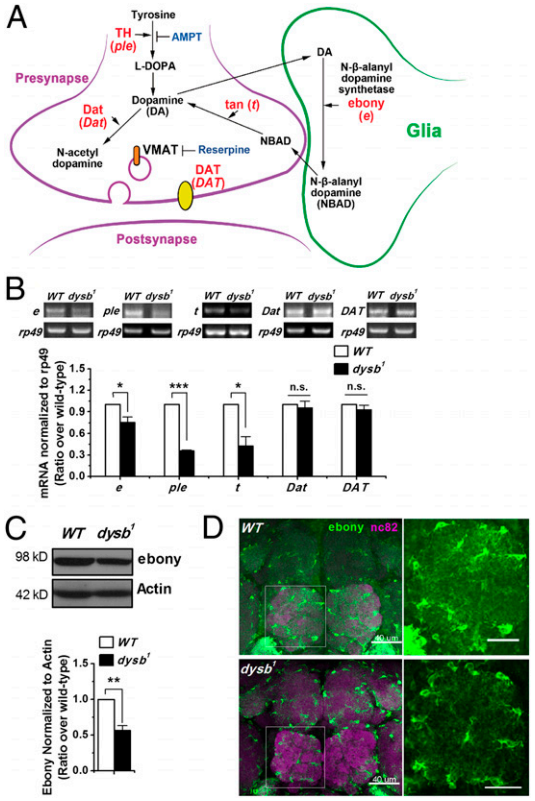

Fig. 6. Ddysb regulates dopamine level via Ebony in glial cells. (*A*) Dopamine (DA) synthetic and metabolic pathway. The actions of proteins, their encoding genes, and inhibitors are shown. DAT (dopamine transporter) is a dopamine transmembrane transporter. Dat (dopamine *N* acetyltransferase) is an *N*-acetyltransferase to metabolize dopamine to *N*-acetyl-dopamine. VMAT (vesicular monoamine transporter) is a synaptic vesicle amine transmembrane transporter. (*B*) Representative RT-PCR and group data show decreased mRNA levels of *ebony* (*e*), *pale* (*ple*), and *tan* (*t*) in the *dysb*^{1} mutant (*t* test; P = 0.02, 1.7E-5, and 0.02; *n* = 4), whereas the transcripts of *DAT* and *Dat* did not have significant changes compared with WT. (*C*) Representative Western blot and group data showing decreased Ebony protein level in the *dysb*^{1} mutant (*t* test; *P* = 0.002; *n* = 7). (*D*) Ebony is expressed in glial cells. Neuropils were stained with mAb nc82 (magenta). Ebony immunosignals (green) were weaker in the *dysb*^{1} mutant than in the WT. (Scale bars: 40 μm *Left*; 20 μm *Right*.) *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; n.s., not significant ($P > 0.05$).

First, although Ddysb is widely expressed in the brain, restoring Ddysb in glutamatergic neurons alone was sufficient to rescue hypoglutamatergic transmission and memory defects. Second, Ddysb's functions in glial cells are essential for normal dopaminergic activity and associated behaviors, including locomotion and mating orientation. Third, all observed pathophysiological and behavioral phenotypes were rescued with acute genetic or pharmacological treatments in adults.

We devoted special attention to validating the phenotypes observed, including maintaining an isogenic background for all genotypes (*Materials and Methods*), balancing the behavioral assays (19), and confirming the manifested phenotypes by different genetic manipulations (mutations, genetic rescuing, and RNAi knockdown). In the following sections, we address the significance and implications of our findings.

Glutamatergic Functions and Memory Defects. An increasing number of studies suggest that genetic variation in *DTNBP1* in normal human populations affects verbal and visual memories as well as working memory (35, 36). This association is supported by studies on the *sandy* mouse, which is defective in a range of memory tasks, including spatial memory, novel object recognition, and contextual fear conditioning (12, 13). However, the physiological causes of such memory defects are not clearly defined.

In this study, we show that altered *Ddysb* function in glutamatergic neurons alone is responsible for attenuated glutamatergic transmission and for the memory defect. It is interesting that this memory defect is not a developmental phenotype and could be rescued acutely both by feeding flies with the NMDA receptor agonist glycine (Fig. 5*A*) and by expressing *Ddysb* only in glutamatergic neurons (Fig. 5*D*). Such a result is consistent with reports showing that NMDA receptors in the *Drosophila* brain are involved in memory formation (37, 38).

Glial Functions and Regulation of Dopaminergic Activity. Before the current study, the expression and function of dysbindin were considered to occur primarily, if not exclusively, in neurons (8). However, recent reports have demonstrated that in mouse and rat brains the expression level of dysbindin in glia is comparable with, if not higher than, its expression in neurons (23, 39), although its glial functions remained to be determined. Genetic tools available for *Drosophila* allowed us not only to define the function of dysbindin in glia but also to gain insight into the underlying mechanisms.

Anatomically, we showed that immunohistochemical signals of Ddysb (in red) were detected in glial cells labeled by GFP-tagged membrane proteins, with sparse Ddysb distribution in cell bodies and the majority of glial Ddysb signals in glial processes or in thin layers surrounding individual neuronal cell bodies (Fig. 3*A*). This observation was supported by the distribution pattern of VFP-tagged Ddysb in GFP-labeled glial cells (Fig. 3*B* and Fig. S5).

Evidence supporting a functional role of Ddysb in glia is very strong. We showed that the escalated dopamine level in the *dysb*^{1} mutant could be rescued by targeted expression of the *Ddysb* or human *DTNBP1* transgene only in glial cells but not in neurons (Fig. 2 *C* and *H*). In addition, the hyperdopaminergia-elicited behaviors, including locomotor hyperactivity and mating disorientation, were rescued only through targeted glial expression of *Ddysb* or human *DTNBP1* transgenes (Fig. 2 *E*, *F*, and *J*). More convincingly, knocking down *Ddysb* universally or in glia but not in neurons resulted in embryonic or pupal lethality, respectively (Fig. S6 and Table S1).

Our further investigation suggests that mutations of *Ddysb* cause hyperdopaminergic activity by down-regulating the expression of Ebony. Our biochemical data profiling mRNA and protein expression corroborated well with genetic observations, supporting the idea that Ebony plays critical role in mediating the effects of Ddysb in glial cells. It is likely that this Ddysb/Ebony-produced hyperdopaminergic activity somehow leads to reduced TH and Tan expression in neurons through a negative feedback mechanism for maintaining the homeostasis of dopaminergic activity.

How Ddysb regulates expression of Ebony remains to be determined. One possibility comes from reports that human dysbindin can function as a nucleocytoplasmic shuttling protein that regulates the transcription of several genes either directly or by binding with other transcription-related factors (40–42). Here, we analyzed the Ddysb protein sequence with the PSORT II Prediction WWW Server (http://psort.ims.u-tokyo.ac.jp/form2.html) and found that the probability that Ddysb localizes to the nucleus is 94.1%. Thus, it is plausible that Ddysb in glia plays a role in regulating gene transcription.

Alternatively, Ddysb might regulate the dopamine level in glial cells by affecting the stability of the Ebony protein. The dysbindin-containing BLOC-1 complex is a component of the endosomal protein sorting and compartmental machinery (43, 44). Abnormalities in Ebony protein sorting may lead to abnormalities in ubiquitylation, protein instability, or malfunction of the enzyme.

Implications for Disease. Although the possibility of generating fly models of schizophrenia has been raised recently (45), the intent of this study is not to model schizophrenia in *Drosophila*. Instead, we are interested in whether and how a single mild genetic alteration, similar to those observed in cases of schizophrenia, gives rise to complex phenotypes at the neurotransmitter regulation and behavioral levels. Our study led to two interesting observations.

First, we were surprised to see that a rather mild 30–40% reduction in *Ddysb* expression led to significant alterations in both glutamatergic transmission and dopaminergic activity. Most schizophrenia susceptibility genes reported to date are identified not from mutations but from single-nucleotide polymorphisms or haplotypes, which are believed to produce only mild alterations at the gene expression level (5). It therefore is debatable how strong the contribution of an individual genetic variant is and whether multiple genetic components acting in concert are needed for the effects. Here we show that a mild reduction of at least one of the susceptibility genes is sufficient to cause complex changes in multiple neurotransmitter systems through very different mechanisms. Our findings suggest that these susceptibility genes might play such critical roles in neurotransmitter regulation that a mild change in expression is sufficient to cause detectable behavioral phenotypes.

Second, although a developmental role of dysbindin has been reported earlier (23, 24) and is supported, as mentioned above (Fig. S6 and Table S1), both neurotransmitter and behavioral phenotypes examined in this study could be rescued through acute treatments (Figs. 4 and 5 *A–C*). Schizophrenia is considered a neurodevelopmental disorder (46), a notion that is supported by animal model studies of development and by genetic mouse models of neurodevelopmental candidate genes and susceptibility genes (47). However, this study suggests that, to some extent, some of the genetically relevant phenotypes are reversible or could be treated in adults.

Materials and Methods

Fly stocks and detailed procedures for the generation of constructs and antibody, germ-line transformation, Western blot, RT-PCR, electrophysiology, drug treatments, dopamine level determination, immunohistochemistry, heat-shock regimen, and behavior assays including Pavlovian olfactory conditioning, the locomotor activity test, mating orientation test, and statistical analysis are described in *SI Materials and Methods*.

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