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The Schizophrenia Susceptibility Gene *Dysbindin* Controls Synaptic Homeostasis

Dion K. Dickman and Graeme W. Davis *

Department of Biochemistry and Biophysics, University of California, San Francisco, 1550 4th street, Rock Hall 4th Floor North, San Francisco, CA 94158, USA

Abstract

The molecular mechanisms that achieve homeostatic stabilization of neural function remain largely unknown. To help understand how neural function is stabilized during development and throughout life we used an electrophysiology-based forward genetic screen and assessed the function of over 250 neuronally expressed genes for a role in the homeostatic modulation of synaptic transmission in *Drosophila*. This screen ruled out the involvement of numerous synaptic proteins and identified a critical function for *dysbindin*, a gene linked to schizophrenia in human. Dysbindin was essential, presynaptically, for the retrograde, homeostatic modulation of neurotransmission. Dysbindin functioned in a dose-dependent manner downstream or independently of calcium influx. Thus, Dysbindin is essential for adaptive neural plasticity, and may link altered homeostatic signaling with a complex neurological disease.

At glutamatergic synapses of species ranging from *Drosophila* to human, disruption of postsynaptic neurotransmitter receptor function can be precisely offset by an increase in presynaptic neurotransmitter release to homeostatically maintain normal postsynaptic excitation (1–3). The *Drosophila* neuromuscular junction (NMJ) is a glutamatergic synapse that is used as a model for this form of homeostatic signaling in the nervous system (1,4,5). Efficient homeostatic modulation of presynaptic release at the *Drosophila* NMJ can occur in ten min following bath application of philanthotoxin-433 (PhTx), which persistently and specifically inhibits postsynaptic glutamate receptors (fig. S1)(4).

We have systematically screened for mutations that block the rapid, PhTx-dependent induction of synaptic homeostasis (Fig. 1). Mutations in 276 genes were screened electrophysiologically (see Supporting Text). For each mutant, we calculated an average value for the amplitude of both the spontaneous miniature excitatory junctional potential (mEJP) and evoked excitatory junctional potential (EJP) following treatment of the dissected neuromuscular preparation with PhTx for 10 min (4). We isolated 14 mutants with average EJP amplitudes more than two standard deviations smaller than the distribution mean (Fig. 1C, red filled bars). From these candidates we identified 7 mutants that block synaptic homeostasis without an obvious effect on NMJ morphology or baseline synaptic transmission. We conclude that the molecular mechanisms of synaptic homeostasis can be

* to whom correspondence should be addressed: graeme.davis@ucsf.edu.

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genetically separated from the mechanisms responsible for normal neuromuscular development and baseline synaptic transmission.

A fraction of the mutants we assayed (19.5%) are previously published genetic lesions. This allows us to rule out the involvement of numerous genes and associated biochemical processes. Mutations that disrupt RNA-interference/micro-RNA processing, retrograde trans-synaptic signaling, synaptic transmission, active zone assembly, synaptic vesicle endocytosis and mitochondria all showed reliable homeostatic compensation (Fig. 1, D and E, and fig. S1). Therefore, synaptic homeostasis is a robust phenomenon, unperturbed by a broad spectrum of synaptic mutations. In addition, significant homeostatic compensation in *synaptojanin* and *endophilin* mutants argues against the involvement of synaptic vesicle endocytosis and indicates that the size of the recycling synaptic vesicle pool is not a limiting factor for synaptic homeostasis. These data also emphasize the importance and specificity of those mutations we identified that do block synaptic homeostasis. These include four ion channels, two of which are of unknown function, and two calcium-binding proteins of unknown function. Thus, homeostatic signaling at the NMJ may include previously unexplored mechanisms of synaptic modulation.

One mutation that was identified with a specific defect in homeostatic compensation is a transposon insertion that resides in the *Drosophila* homologue of *dysbindin* (CG6856; fig. S2). The *DTNBPI* (*dysbindin*) locus is linked with schizophrenia in humans (6–11). We have identified a transposon insertion within the *dysbindin* locus (pBac^{e01028}, referred to as *dysb^l*; fig. S2) that showed a complete absence of homeostatic compensation following application of PhTx (Fig. 2, A and B). A similar effect was observed when *dysb^l* was placed in trans to a deficiency that uncovers the *dysb* locus, indicating that the *dysb^l* mutant was a strong loss of function or null mutation (Fig. 2, A and B). No significant change in baseline synaptic transmission in *dysb^l* mutant animals (0.5 mM extracellular calcium) was observed. Thus, under these recording conditions, this mutation disrupted synaptic homeostasis without altering baseline neurotransmission (Fig. 2B). As a control, synaptic homeostasis was normal in animals in which the pBac^{e01028} transposon was precisely excised (Fig. 2, C and D).

The *dysb* gene is ubiquitously expressed in *Drosophila* embryos (fig. S2) consistent with widespread expression in vertebrates (12,13). Therefore, we generated and expressed a *dysbindin* transgene in the *dysb^l* mutant. Presynaptic expression of *dysb* fully restored homeostatic compensation in the *dysb^l* mutant background, whereas muscle-specific expression of *dysb* did not (Fig. 2, C and D, and fig. S3). Thus, Dysbindin is necessary presynaptically for the rapid induction of synaptic homeostasis.

We next asked whether Dysbindin is also required for the sustained expression of synaptic homeostasis. We generated double mutant animals harboring both the *dysb^l* mutation and a mutation in a gene encoding a postsynaptic glutamate receptor (*GluRIIA*). *GluRIIA* mutant animals normally show robust homeostatic compensation (14). However, homeostatic compensation was blocked in *GluRIIA; dysb^l* double mutant animals (Fig. 2E). Thus, *dysbindin* was also necessary for the sustained expression of synaptic homeostasis over several days of larval development.

Next we demonstrated that synapse morphology was qualitatively normal in *dysb* mutants (Fig. 3A) including both the shape of the presynaptic nerve terminal and the levels, localization and organization of synaptic markers including futsch-positive microtubules, synapsin and synaptotagmin (fig. S4). Bouton number and active zone density are also normal in *dysb* mutants (Fig. 3, C to E)(15). Thus, the disruption of synaptic homeostasis in *dysb^l* mutants is not a secondary consequence of altered or impaired NMJ development.

In the vertebrate nervous system, Dysbindin is associated with synaptic vesicles (16). We examined the localization of a Venus-tagged *dysb* transgene (*ven-dysb*) that rescues the *dysb*¹ mutant (Fig. 2, C and D). Ven-Dysb showed extensive overlap with synaptic vesicle associated proteins when expressed in neurons (Fig. 3B). Thus, Dysbindin functions presynaptically, potentially at or near the synaptic vesicle pool.

To further define the function of Dysbindin, we investigated baseline synaptic transmission in the *dysb* mutant in greater detail. At 0.5 mM extracellular calcium, synaptic transmission in *dysb*¹ mutant animals was indistinguishable from wild type (Fig. 2, A and B). However, when extracellular calcium was reduced (0.2 – 0.4 mM Ca²⁺), baseline synaptic transmission was significantly impaired in *dysb* compared to wild type (Fig. 4A) and this defect was rescued by presynaptic expression of *dysb* (fig. S5). Thus, there is an alteration of the calcium dependence of synaptic transmission in the *dysb* mutant. Indeed, at reduced extracellular calcium, both paired-pulse facilitation and facilitation that occurs during a prolonged stimulus train were increased in *dysb* mutants (Fig. 4B and fig. S6).

In vertebrates, the levels of *dysb* expression correlate with parallel changes in extracellular glutamate concentration (17). Therefore, we tested whether *dysb* overexpression might increase presynaptic release. In wild-type animals overexpressing *dysb* in neurons, synaptic transmission is normal at low extracellular calcium (0.2 and 0.3 mM Ca²⁺) but was enhanced at relatively higher extracellular calcium (0.5 mM Ca²⁺; Fig. 4C and fig. S5). The complementary effects of *dysb* loss-of-function and overexpression confirm that Dysbindin has an important influence on calcium-dependent vesicle release.

The presynaptic Ca_v2.1 calcium channel, encoded by *cacophony* (*cac*), is required for synaptic vesicle release at the Drosophila NMJ (18). *cac* mutations decrease presynaptic calcium influx (19) and also block synaptic homeostasis (4). We therefore tested for a genetic interaction between *dysb* and *cac* during synaptic homeostasis. Because homozygous *cac* and *dysb* mutations individually block synaptic homeostasis, analysis of double mutant combinations would not be informative. We resorted to an analysis of heterozygous mutant combinations and gene overexpression (5). Synaptic homeostasis was suppressed by a heterozygous mutation in *cac* (Fig. 4D). However, this suppression was not enhanced by the presence of a heterozygous mutation in *dysb* (Fig. 4 and fig. S7). In addition, neuronal overexpression of *cac* did not restore homeostatic compensation in *dysb* mutant animals and the enhancement of presynaptic release caused by neuronal *dysb* overexpression still occurs in a heterozygous *cac* mutant background (Fig. 4, E and F). Thus, Dysbindin may function downstream or independently of Cac during synaptic homeostasis.

To further explore the relationship between Dysbindin and Cac, we asked whether *dysb* mutations might directly influence presynaptic calcium influx. The spatially averaged calcium signal in *dysb*¹ was indistinguishable from wild type, indicating no difference in presynaptic calcium influx (fig. S8). Thus, Dysbindin appears to function downstream or independently of calcium influx to control synaptic homeostasis.

Through a systematic electrophysiological analysis of more than 250 mutants we were able to rule out the involvement of numerous synaptic proteins and biochemical processes in the mechanisms of synaptic homeostasis and demonstrate that this phenomenon is separable from the molecular mechanisms that specify structural and functional synapse development. We then identify Dysbindin as an essential presynaptic component within a homeostatic signaling system that regulates and stabilizes synaptic efficacy. Dysbindin functions downstream or independently of the presynaptic Ca_v2.1 calcium channel in the mechanisms of synaptic homeostasis.

Emerging lines of evidence suggest that glutamate hypofunction could be related to the etiology of schizophrenia (20–24). Likewise, reduced levels of *dysbindin* expression were associated with schizophrenia (25,26). The *sandy* mouse, which lacks Dysbindin, has a decreased rate of vesicle release (~30% decrease), a correlated decrease in vesicle pool size and an increased thickness of the postsynaptic density (27). We confirm a modest, facilitatory function for Dysbindin during baseline transmission. However, numerous mutations with similar or more severe defects in baseline transmission show normal synaptic homeostasis (Fig. 1, D and E). By contrast, loss of Dysbindin completely blocks the adaptive, homeostatic modulation of vesicle release, suggesting that the potential contribution of *dysbindin* mutations to schizophrenia may be derived from altered homeostatic plasticity as opposed to decreased baseline glutamatergic transmission.

Supplementary Material

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Figure 1. Electrophysiology-based screen for homeostatic mutations

(A) Flow diagram of screen strategy and outcome. (B) Histogram of average mEJP amplitude per genotype after PhTx application. Wild-type average mEJP (blue arrow) and wild-type average mEJP after PhTx application (black arrow) are indicated. (C) Histogram of average EJP amplitudes per genotype after PhTx application. Arrows as in (B). Red columns indicate values greater than 2 standard deviations from the mean. (D,E) Homeostatic increases in quantal content observed in published genetic mutations. Data are normalized to the same genotype without PhTx treatment in D, and to wild-type values in E. Full genotypes, n values, and references are shown in Tables S1,2.

Figure 2. Dysbindin is required presynaptically for synaptic homeostasis

(**A**) Mutations in *dysbindin* block the homeostatic increase in quantal content following PhTx application. Data are normalized to each genotype in the absence of PhTx. (**B**) Representative traces for data in (A). (**C**) Precise excision of the *e01028* transposon (revertant) restores compensation. Neuronal-specific expression of *dysb* (with or without a *venus* tag; *c155-GAL4/+; UAS-dysb/+; dysb¹*) restores compensation. Muscle-specific expression (*UAS-dysb/+; mhc-GAL4, dysb¹/dysb¹*) does not. (**D**) Representative traces for data in (C). (**E**) Sustained homeostatic compensation is blocked in *GluRIIA^{SP16}; dysb¹* double mutants.

Figure 3. NMJ morphology and Dysbindin localization

(A) Wild-type (w^{1118}) and $dysb^I$ mutant NMJs co-immunostained for anti-HRP (red; motoneuron membrane) and anti-DLG (green; postsynaptic muscle membrane). (B) $ven-dysb$ expressed in neurons co-localizes with the synaptic vesicle associated protein synapsin (green). (C) Quantification of bouton number at muscles 4 and 6/7 comparing wild-type ($n=19$) and $dysb$ ($n=17$) ($p>0.41$; Student's t-test). (D) Quantification of nc82 puncta per NMJ comparing wild-type ($n=15$) and $dysb$ ($n=16$) ($p>0.97$; Student's t-test). (E) Density of nc82 puncta comparing wild-type ($n=15$) and $dysb$ ($n=16$) ($p>0.32$; Student's t-test). Scale bar is 5 microns.

Figure 4. Dysbindin modulates the calcium-dependence of vesicle release

(A) Quantal content as a function of extracellular calcium concentration. (B) Enhanced paired-pulse facilitation in *dysb¹* mutants. (C) Quantal content is increased by neuronal overexpression of *dysb* (*dysb OE*; *c155-GAL4/Y*; *UAS-dysb/+*). (D) Homeostatic compensation is calculated as in Fig. 1E. Compensation is suppressed in *cac^{S/+}* ($n=15$) compared with wild type ($n=25$; $p<0.001$; Student's t-test) or *dysb^{1/+}* ($n=17$; $p<0.001$; Student's t-test) but is not further suppressed in *cac^{S/+}; dysb^{1/+}* mutants ($n=19$; $p>0.27$; Student's t-test). (E) Compensation remains blocked when *cac* is neuronally overexpressed in a *dysb¹* mutant ($n=9$, *dysb; cac OE*; *c155-GAL4/Y*; *UAS-cacGFP/+*; *dysb¹*). (F) Quantal content is increased by neuronal overexpression of *dysb* ($n=17$, *dysb OE*; *c155-GAL4/Y*; *UAS-dysb/+*; $p<0.0002$; Student's t-test) and when *dysb* is overexpressed in a *cac^S* mutant ($n=19$, *cac^{S/+}; dysb OE*; *cac^S/c155-GAL4*; *UAS-dysb/+*; $p<0.006$; Student's t-test).