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Regulation of *Drosophila* Synaptic Function and Plasticity by a Schizophrenia Susceptibility Network.

By

Ariana Paone Mullin

Doctor of Philosophy

Graduate Division of Biological and Biomedical Science

Neuroscience

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B.S., Trinity College, Hartford, CT, 2009

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Abstract

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By Ariana Paone Mullin

Neurodevelopmental disorders (NDDs) are genetically complex, arising from single or multiple gene defects, and include schizophrenia, intellectual disability, and autism spectrum disorder. Many NDDs, particularly those associated with large chromosomal deletions, either share common genetic variations or it is postulated that the associated gene products converge into a common molecular or cellular pathway. However, the way multiple loci interact to modify phenotypic outcomes remains poorly understood. Additionally, current studies focus on monogenic NDDs because of their straightforward study and conceptualization, despite the involvement of multiple loci. Currently, there are no studies exploring the interactions of multiple genes or gene products associated with these disorders and their effects at the synapse. Here, I use a biochemically curated interaction network centered around the schizophrenia susceptibility gene *dysbindin (dysb),* the Drosophila ortholog of the human gene *DTNBP1.* In this study, I examined the phenotypes associated with mutations in the schizophrenia susceptibility gene *dysbindin* (*dysb*), in isolation or in combination with null alleles in the *dysb* network component *Blos1*. In humans, the *Blos1* ortholog *Bloc1s1* encodes a polypeptide that assembles, with dysbindin, into the octameric BLOC-1 complex. Here, I biochemically confirmed BLOC-1 presence in *Drosophila* neurons, and measured synaptic output and complex adaptive behavior in response to BLOC-1 perturbation. Homozygous loss-of-function alleles of *dysb*, *Blos1,* or compound heterozygotes of these alleles impaired neurotransmitter release, synapse morphology, and homeostatic plasticity at the larval neuromuscular junction, and impaired olfactory habituation. This multiparameter assessment indicated that phenotypes were differentially sensitive to genetic dosages of loss-of-function BLOC-1 alleles. Further, I identified the N-Ethylmaleimide Sensitive Factor (NSF) as a factor sensitive to BLOC-1 deficiency. I used NSF to test the hypothesis that molecular and genetic interactors converge into a functionally-defined pathway. My findings suggest that modification of a second genetic locus in a defined neurodevelopmental regulatory network does not follow a strict additive genetic inheritance, but rather, precise stoichiometry within the network determines phenotypic outcomes. Additionally, I demonstrate that a biochemically curated interactome can be used to direct investigation of pathways associated to complex genetic diseases, such as schizophrenia and related neurodevelopmental disorders. Together, this work supports the investigation of neurodevelopmental disorders through the assessment of multiple endophenotypes in response to polygenic experimental manipulations to better approximate complex disease states.

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“And now, let the wild rumpus start!”

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**Key Terms and Abbreviations**

NDD Neurodevelopmental Disorders

AP-3 Adaptor Protein complex- 3

GWAS Genome Wide Association Study

BLOC-1 Biogenesis of Lysosome-related Organelles Complex-1

dysb1  *Drosophila* dysbindin mutant allele; piggy-back insertion; hypomorph

dysbDF *Drosophila* dysbindin ‘deficiency’ allele; gene deletion

dysbRV  *Drosophila* dysbindin ‘revertant’ allele; precise excision of dysb1 insertion

Blos1EY *Drosophila* blos1 null allele; p-element insertion in the fly line EY0629

Blos1ex2­­ *Drosophila* blos1 mutant allele, deletion in blos1 ∆ -56-38

Blos1ex65 *Drosophila* blos1 mutant allele, deletion in blos1 ∆ -56-1033

NMJ Neuromuscular junction

EJP Excitatory Junctional Potential

mEJP miniature Excitatory Junctional Potential

RP Reserve Pool

RRP Readily Releasably Pool

SNARE SNAP receptor protein

SNAP soluble NSF attachment protein

NSF N-Ethylmaleimide Sensitive Factor

PhTx Philanthotoxin

STH Short-term olfactory habituation

**Homologous Human Genes and Encoded Proteins in *Drosophila***

|  |  |  |  |
| --- | --- | --- | --- |
| Human Gene | Human Protein | *Drosophila* Gene | *Drosophila* Protein |
| *FMR1* | fmr1, FMRP | *dfmr1* | dFMR1 |
| *DTNBP1, BLOC1S8* | Dysbindin, BLOC1s8 | *dysbindin, dysb* | dysbindin, dysb  |
| *BLOC1S1, BLOS1* | BLOC1s1, BLOS1 | *blos1* | blos1 |
| *NSF* | NSF | *comt, Nsf,dNsf1* | dNsf-1 |