Biological Psychiatry: Celebrating 50 Years

Dysfunction of Habituation Learning: A Novel Pathogenic Paradigm of Intellectual Disability and Autism Spectrum Disorder

Ying Cheng and Peng Jin

Intellectual disability (ID) and autism spectrum disorder (ASD) are two neurodevelopmental disorders with a prevalence of more than 1.5% in developed countries. ID is characterized by impaired social, cognitive, and adaptive skills and refers to those with an IQ lower than 70; ASD encompasses a spectrum of neurodevelopmental problems, such as functional deficits in communication skills, impaired linguistic and social aptitude, and repetitive or stereotypical behaviors (1). In many cases, a single gene mutation can effectively result in ID. Most patients with severe ID have a genetic disorder, and the cause is usually a mutation or variant at the genomic level (1). However, our knowledge of the genetic causes of ASD is still largely lacking. Although genetic factors are the main contributors for the development of ASD, as suggested by both twin and family studies, ASD presents as a much more complicated polygenic disorder. ASD is often thought to be caused by the combined dysfunction of ASD risk genes and epigenetic components (2). Thus far, monogenic causes, such as the mutations in *FMR1* (which causes fragile X syndrome) and *MECP2* (which causes oRett syndrome) that lead to autistic phenotypes, account for only a small fraction of ASDs (1,3). Notably, high comorbidity is often observed between ID and ASD: it has been reported that approximately 70% of autistic individuals also present with ID (4), suggesting possible genetic similarities between the two disorders. Although several hundred genes have been found to be associated with ID/ASD, the extreme heterogeneity and poorly understood biology of these disorders remain barriers for identifying the specific underlying disease pathogenic mechanisms.

Habituation of neural response is one of simplest form of nonassociative learning and can serve as an important indicator of learning. Habituation is conserved across species, is essential to selective attention, and is recognized as a prerequisite for cognitive functions. It has been reported that reduced habituation may be a mechanism for the social impairment that is associated with ID and ASD (1,5). Although it is critical to understand the underlying mechanism, investigating the function of human habituation genes on a large scale is technically difficult. In the current issue of *Biological Psychiatry*, Fenckova *et al.* (6) take on this important challenge by using large-scale inducible RNA interference screening in *Drosophila melanogaster* (known as the fruit fly) to investigate the role of fruit fly orthologs of 286 human ID genes, and they identify nearly 100 ID genes that regulate habituation learning. Further analyses demonstrated that some of these "habituation-deficient" genes were also implicated in ASD and highlighted the role of Ras/MAPK (mitogen-activated protein kinase) signaling in regulating adaptive habituation response. Given the significant overlap in both clinical features and risk genes between ID and ASD, this study of monogenic causes of ID provides an alternative approach to reveal the common pathogenic pathways shared between ID and ASD.

Drosophila is an attractive model organism and has many practical features that are suitable for genetic study. Its genome contains approximately 75% of the known human disease gene orthologs, enabling large-scale genetic functional studies. To identify novel habituation–associated genes in ID *Drosophila* models, Fenckova *et al.* (6) first generated more than 500 neuron-specific knockdown lines that represented 286 human ID genes (*Drosophila* orthologs). The knockdown and control flies were then used to perform the light-off jump habituation assay to test for deficits. Fenckova *et al.* (6) demonstrated that 98 human ID genes are functionally important for controlling habituation learning by verifying that knockdown of these genes did not influence the health or jump response of the fruit flies. Enrichment analyses indicated that these genes were significantly involved in biological processes related to synaptic function and clinical features related to macrocephaly. Given that impaired habituation and macrocephaly have been associated with ASD, Fenckova *et al.* (6) next investigated the relationship between *Drosophila* habituation and human ASD. By comparing with the ASD risk genes obtained from two independent databases, the Simons Simplex Collection cohort and the Simons Foundation Autism Research Initiative database, they found that a significant fraction of habituation-deficient ID genes were associated with ASD. More interestingly, Simons Simplex Collection individuals carrying mutations in these genes have a higher trend to exhibit atypical behaviors, mainly because of the impairment of one subdomain—inappropriate and stereotypical speech. These results indicate that habituation deficits are relevant to ASD-implicated genes and may reflect certain behavioral subdomains of ASD.

The circuitry in the human brain is complicated and consists of distinct layered networks of excitatory and inhibitory neurons, such as the cortical excitatory glutamatergic pyramidal cells and inhibitory gamma-aminobutyric acidergic neurons. The balanced excitation and inhibition ratio is essential to maintain neuronal function, and an altered excitation and inhibition ratio has been demonstrated to be associated with—and thought to cause—a broad spectrum of neuropsychiatric disorders, including ID and ASD (7). For

SEE CORRESPONDING ARTICLE ON PAGE 294

https://doi.org/10.1016/j.biopsych.2019.06.012
ISSN: 0006-3223

instance, defects in synaptic function have been proposed to be commonly involved in autism pathogenesis, and the models for mutations associated with ASD in humans consistently point to impaired synaptic connectivity and alterations in the excitation and inhibition ratio (8). It seems that the "autistic neurons" exhibit altered activity of excitatory synapses relative to the level of inhibition, skewing the overall excitation and inhibition ratio (8). In autistic neurons, alterations of plasticity-related proteins available to active synapses may cause changes in synaptic connectivity, disrupting network performance and leading to cognitive impairment. Mutations in monogenic disorders with a high morbidity of autism could cause dysfunction of synaptic protein synthesis, which could be a mechanism that potentially contributes to autism pathogenesis.

One fundamental question to support the importance of habituation learning in the current study is: what is the neurophysiological substrate? To answer this question, Fenckova *et al.* (6) performed the pathway enrichment analysis for those habituation-deficient genes in ID-specific molecular interactome and identified Ras/MAPK signaling as the core pathway associated with habituation deficits. The Ras/MAPK signaling pathway was originally identified in cancer research and has now been demonstrated to play critical roles in synaptic plasticity and abnormal behaviors in neuropsychiatric disorders (9). Both knockdown of negative regulators of Ras and constitutively active Ras resulted in strong habituation deficits in *Drosophila*, suggesting a similar genetic mechanism for habituation deficits in *Drosophila* and causes of RASopathies in humans. Moreover, the genetic evidence revealed that increased Ras-mediated signaling in gamma-aminobutyric acidergic neurons, not cholinergic neurons, is a key contributor for habituation deficits. Interestingly, Fenckova *et al.* (6) found that decreased activity of Ras/MAPK signaling can also lead to habituation deficits, of which the neurophysiological substrate was identified to be excitatory cholinergic neurons. Unfortunately, whether this difference is caused by the developmental effects or acute circuit plasticity is not fully addressed by Fenckova *et al* (6). Nevertheless, the dual function of Ras/MAPK signaling in excitatory and inhibitory neurons indicates the complexity of the mechanism involved in neuronal plasticity and related neurological disease, and this discovery may provide a better understanding for the treatment of RASopathies in humans.

In summary, the present study takes advantage of the speed and cost-effectiveness of *Drosophila* inducible RNA interference screening and demonstrates that habituation is a widely affected mechanism in *Drosophila* models of ID and ASD. These results provide further insight into the identification of the various contributors of ID/ASD pathology. The treatment of neuropsychiatric disorders, including ID and ASD, will need to have a focus on specific dysregulated pathways or networks because these disorders are too heterogeneous to be treated at the level of a single gene. *Drosophila*, as a powerful genetic tool, has shown its value in preclinical drug discovery. For example, in a previous study of large-scale in vivo drug screening conducted in *Drosophila*, application of gamma-aminobutyric acid could successfully rescue glutamate toxicity in *Fmr1* mutants, a *Drosophila* model of fragile X syndrome (10). If more substantial evidence can be provided to prove the clinical relevance of habituation learning in the future, cross-species habituation assessment could serve as a great tool for risk gene screening and drug target testing for neuropsychiatric disorders.

Acknowledgments and Disclosures

The authors report no biomedical financial interests or potential conflicts of interest.

We thank Emily Allen for critical reading of this commentary.

Article Information

From the Institute of Biomedical Research (YC), Yunnan University, Kunming, China, and the Department of Human Genetics (PJ), Emory University School of Medicine, Atlanta, Georgia.

Address correspondence to Ying Cheng, Ph.D., Institute of Biomedical Research, Yunnan University, Kunming, 650091, China; E-mail: ycheng@ynu.edu.cn; or Peng Jin, Ph.D., Department of Human Genetics, Emory University School of Medicine, Atlanta, GA 30322; E-mail: peng.jin@emory.edu.

Received Jun 16, 2019; accepted Jun 17, 2019.

References

1. Vissers LE, Gilissen C, Veltman JA (2016): Genetic studies in intellectual disability and related disorders. Nat Rev Genet 17:9–18.
2. Sandin S, Lichtenstein P, Kuja-Halkola R, Hultman C, Larsson H, Reichenberg A (2017): The heritability of autism spectrum disorder. JAMA 318:1182–1184.
3. Belmonte MK, Bourgeron T (2006): Fragile X syndrome and autism at the intersection of genetic and neural networks. Nat Neurosci 9:1221–1225.
4. Fombonne E (2002): Epidemiological trends in rates of autism. Mol Psychiatry 7(suppl 2):S4–S6.
5. Kleinhans NM, Johnson LC, Richards T, Mahurin R, Greenson J, Dawson G, *et al.* (2009): Reduced neural habituation in the amygdala and social impairments in autism spectrum disorders. Am J Psychiatry 166:467–475.
6. Fenckova M, Blok LER, Asztalos L, Goodman DP, Cizek P, Singgih EL, *et al.* (2019): Habituation learning is a widely affected mechanism in *Drosophila* models of intellectual disability and autism spectrum disorders. Biol Psychiatry 86:294–305.
7. Yizhar O, Fenno LE, Prigge M, Schneider F, Davidson TJ, O'Shea DJ, *et al.* (2011): Neocortical excitation/inhibition balance in information processing and social dysfunction. Nature 477:171–178.
8. Kelleher RJ 3rd, Bear MF (2008): The autistic neuron: Troubled translation? Cell 135:401–406.
9. Borrie SC, Brems H, Legius E, Bagni C (2017): Cognitive dysfunctions in intellectual disabilities: The contributions of the Ras-MAPK and PI3K-AKT-mTOR pathways. Annu Rev Genomics Hum Genet 18:115–142.
10. Chang S, Bray SM, Li Z, Zarnescu DC, He C, Jin P, *et al.* (2008): Identification of small molecules rescuing fragile X syndrome phenotypes in *Drosophila*. Nat Chem Biol 4:256–263.