Epilepsy Currents
2021, Vol. 21(4) 287–289

DOI: 10.1177/1535759721012963
journals.sagepub.com/home/epi
SAGE

The Course of Inhibition Never Did Run Smooth: Parvalbumin Interneuron Dysfunction in a Mouse Model of Lissencephaly

Emergence of Non-Canonical Parvalbumin-Containing Interneurons in Hippocampus of a Murine Model of Type I Lissencephaly

Ekins TG, Mahadevan V, Zhang Y, et al. *eLife*. 2020;9:e62373.

Type I lissencephaly is a neuronal migration disorder caused by haploinsufficiency of the *PAFAH1B1* (mouse: *Pafah1b1*) gene and is characterized by brain malformation, developmental delays, and epilepsy. Here, we investigate the impact of *Pafah1b1* mutation on the cellular migration, morphophysiology, microcircuitry, and transcriptomics of mouse hippocampal CA1 parvalbumin-containing inhibitory interneurons (PV + INTs). We find that WT PV + INTs consist of 2 physiological subtypes (80% fast-spiking, 20% non-fast-spiking [NFS]) and 4 morphological subtypes. We find that cell-autonomous mutations within interneurons disrupts morphophysiological development of PV + INTs and results in the emergence of a noncanonical "intermediate spiking (IS)" subset of PV + INTs. We also find that now dominant IS/NFS cells are prone to entering depolarization block, causing them to temporarily lose the ability to initiate action potentials and control network excitation, potentially promoting seizures. Finally, single-cell nuclear RNAsequencing of PV + INTs revealed several misregulated genes related to morphogenesis, cellular excitability, and synapse formation.

Commentary

Malformations of cortical development (MCD) are associated with a high incidence of epilepsy and are a major cause of treatment-resistant pediatric epilepsy,[1-3] yet how MCD causes epilepsy is not understood.[4] Lissencephaly is a form of MCD defined by a "smooth brain" due to impaired gyrification of the cerebral cortex; this condition is largely due to genetic causes, the most common of which being heterozygous deletion of or pathogenic variant in *PAFAH1B1* (also known as *LIS1*) which encodes an enzyme that regulates microtubule dynamics of migrating neurons during cerebral cortex development.[5,6] Mice with heterozygous deletion of *Pafah1b1* display abnormal structural organization of the brain (particularly of the hippocampus), lowered seizure threshold, and learning impairment. Although the immediately obvious structural brain abnormalities characteristic of lissencephaly in humans arise from abnormal migration of the excitatory principal neurons, deficits in inhibition may also contribute to hyperexcitability and seizures. Previous work in animal models also demonstrated abnormal migration of hippocampal GABAergic inhibitory interneurons and deficits in synaptic inhibition onto both interneurons and excitatory neurons in *Pafah1b1* mutant mice.[7,8] However, there is a broad diversity of interneurons of the hippocampus.[9] Furthermore, it remains unclear if observed changes in inhibition are due to cell autonomous effects of *Pafah1b1* loss in interneurons or are the consequence of migration into and development within an abnormal circuit scaffold.

Parvalbumin-expressing interneurons (PV-INs) are a key cell type implicated in the pathophysiology of multiple epilepsy syndromes and other neurodevelopmental disorders. PV-INs have distinct properties such as high-frequency spiking, fast inhibitory control over target neuron action potential generation, and potent feedforward inhibition essential to their role as critical regulators of neuronal network function,[10] including synchronization of large-scale oscillations.[9] In this study, Ekins et al[11] examined hippocampal PV-INs across multiple levels of analysis in the *Pafah1b1* heterozygous deletion mouse model of lissencephaly to determine the effect of loss of *Paha1b1* on the migration, integration, cellular anatomy, and synaptic function of these cells, and whether any alterations occurred via cell autonomous or noncell autonomous mechanisms.

The authors used cell type–specific Cre driver mouse lines to create mice with heterozygous loss of *Pafah1b1* in all cells ("GlobalLis"), in only pyramidal neurons ("EmxLis," using Emx1-Cre mice), or only in interneurons derived from the

medial ganglionic eminence (NkxLis, using Nkx2.1-Cre mice, which includes PV-INs). Both the global and pyramidal neuron-specific *Pafah1b1* mutant mice exhibited abnormal lamination in the hippocampus, while cytoarchitecture was preserved in the interneuron-specific mutant. However, that the laminar organization of PV-INs was similarly disrupted in all 3 mouse lines indicated that loss of *Pafah1b1* has both cell autonomous and nonautonomous effects on the migration of PV-INs. The observation in the EmxLis mice is consistent with the serial migration of first pyramidal cells followed by interneurons at later developmental time points, with late migration and integration of interneurons being dependent on signaling cues from pyramidal cells or upon the earlier formation of a normal pyramidal cell scaffold.

Next, the authors examined the morphology and physiology of PV-INs in the GlobalLis mice. They found that approximately half of PV-INs in the GlobalLis mice had properties characteristic of normal PV-INs observed in wild type (WT) animals; however, the other half exhibited abnormal properties including loss of high-frequency action potential firing and progression to depolarization block during repetitive firing in response to current injection. These PV-INs also had disorganized axonal branching, extending outside of the pattern typical for these cells, but normal dendritic morphology. In comparison to the effects on neuronal migration, these physiologic and morphologic differences occurred only with loss of *Pafah1b1* in interneurons. PV-INs had normal morphology and physiology in the EmxLis mice.

Disruption in axonal morphology as well as physiologic properties of PV-INs can result in downstream effects on the inhibition of excitatory neurons. Accordingly, PV:pyramidal cell synapses in hipoocampus were less reliable in the GlobalLis mouse only when the presynaptic PV cell had impaired physiology. The affected PV-INs also received less excitatory input themselves, suggesting that, in addition to less reliable inhibition of pyramidal cells, PV-INs will be recruited less effectively for feedforward inhibition.

Finally, single-cell RNA sequencing (scRNAseq) was used to assess the large-scale transcriptomic impact of the *Pafah1b1* mutation. Affected transcripts identified as altered include genes involved in interneuron migration such as cell adhesion molecules as well as elements of intracellular signaling pathways. Although genes for several ion channels that could alter PV-IN excitability were dysregulated, the potassium and sodium channels which underlie the high-frequency firing of PV-INs were not affected. However, the scRNAseq profiles were derived from all hippocampal PV interneurons in the GlobalLis mouse, and hence an effect specific to the abnormal PV-INs could have been washed out.

Overall, this work highlights the cellular- and microcircuit-level abnormalities in lissencephaly in an experimental model system in impressive detail. Although the *Pafah1b1* mutant mice have neuronal migration defects and recapitulate some albeit nonspecific phenotypes of human lissencephaly such as impaired performance on various learning paradigms, this disease model has several limitations. First, the mouse brain is lissencephalic to begin with. Additionally, these mice do not have epilepsy per se; while the mutant mice do have lowered seizure threshold, the need to use evoked seizure models complicates the interpretation of the applicability of these findings toward the question of how interneuron pathology in lissencephaly might contribute to epilepsy. Despite these limitations, it would be interesting to compare the propensity for seizures and learning deficits in GlobalLis, interneuron specific, and pyramidal cell-specific mutant mice, with the hypothesis that disruptions to PV-IN mediated inhibition in the interneuron-specific mutant mice would be sufficient to lower seizure threshold.

Another question relates to what *Pafah1b1* is doing in interneurons to disrupt their migration, post-migratory integration, and structural and physiological development and function, and when expression of *Pafah1b1* is required. It would be of interest to disrupt *Pafah1b1* in PV-INs under temporal control (such as with a tetracycline-responsive element or a tamoxifen-inducible Cre). This experiment might allow for separation of a role for Pafah1b1 in interneuron migration versus post-migratory integration versus electrophysiologic and synaptic function.

An intriguing question is why some PV-INs continue to develop typical morphology, physiology and synaptic properties, while others are dramatically impaired. The authors hypothesize that differences from cell to cell in birth date, Pafah1b1 protein levels during development, or excitatory input to interneurons post-migration could underlie this variability. Understanding what makes certain cells resilient versus vulnerable to the deleterious effects of the *Pafah1b1* mutation could lead to identification of potential therapeutic targets for manipulating interneuron function, which could have broad impact in developmental epilepsies beyond lissencephaly.

The inciting events in lissencephaly occur prenatally and lead to what may be irreversible brain malformation. Preventative therapies would require detection of a known pathogenic or predicted pathogenic variant and intervention prior to formation of the cerebral cortex. The study by Ekins et al highlights the possibility that postnatal interventions directed at interneuron function could remain efficacious, perhaps by compensation or rebalancing abnormal circuits to improve epilepsy or cognition. This approach may be particularly relevant to PV-INs, which have a delayed and prolonged developmental trajectory compared to other cell types including significant postnatal development. However, it is not known whether there may be critical periods for such intervention and to what extent cellular, circuit and ultimately behavioral function could be modulated. Stated simply, epilepsy in lissencephaly could be due to interneuron dysfunction in addition to or rather than the structural brain malformation itself, which would have important implications for therapy. Tools for cell type–specific or global rescue of genetic perturbations (such as using CRISPR/Cas9), replacement of dysfunctional interneurons via cell transplantation,[12] or augmenting function of existing interneurons (such as with optogenetics, chemogenetics or cell

type–specific small molecules), could be viable potential therapeutic approaches.

By Laura M. McGarry and Ethan M. Goldberg

ORCID iD

Ethan M. Goldberg https://orcid.org/0000-0002-7404-735X

References

1. Guerrini R, Dobyns WB. Malformations of cortical development: clinical features and genetic causes. *Lancet Neurol*. 2014;3(7): 710-726.
2. Leventer RJ, Guerrini R, Dobyns WB. Malformations of cortical development and epilepsy. *Dialogues Clin Neurosci*. 2008;10(1): 47-62.
3. Shain C, Ramgopal S, Fallil Z. Polymicrogyria-associated epilepsy: a multicenter phenotypic study from the epilepsy phenome/genome project. *Epilepsia*. 2013;54(8):1368-1375.
4. Represa A. Why malformations of cortical development cause epilepsy. *Front Neurosci*. 2019;13:250.
5. Cardoso C, Leventer RJ, Dowling JJ, et al. Clinical and molecular basis of classical lissencephaly: mutations in the LIS1 gene (PAFAH1B1). *Hum Mutat*. 2002;19(1):4-15.
6. Pilz DT, Matsumoto N, Minnerath S, et al. LIS1 and XLIS (DCX) mutations cause most classical lissencephaly, but different patterns of malformation. *Hum Mol Genet*. 1998;(13):2029-2037.
7. Jones DL, Baraban SC. Inhibitory inputs to hippocampal interneurons are reorganized in Lis1 mutant mice. *J Neurophysiol*. 2009;102(2):648-658.
8. Dinday MT, Girskis KM, Lee S, Baraban SC, Hunt RF. PAFAH1B1 haploinsufficiency disrupts GABA neurons and synaptic E/I balance in the dentate gyrus. *Sci Rep*. 2017;7(1): 8269.
9. Pelkey KA, Chittajallu R, Craig MT, Tricoire L, Wester JC, McBain CJ. Hippocampal GABAergic inhibitory interneurons. *Physiol Rev*. 2017;97(4):1619-1747.
10. Hu H, Gan J, Jonas P. Interneurons. fast-spiking, parvalbumin^{+} GABAergic interneurons: from cellular design to microcircuit function. *Science*. 2014;345(6196):1255263.
11. Ekins TG, Mahadevan V, Zhang Y, et al. Emergence of non-canonical parvalbumin-containing interneurons in hippocampus of a murine model of type I lissencephaly. *Elife*. 2020;9: e62373.
12. Tyson JA, Anderson SA. GABAergic interneuron transplants to study development and treat disease. *Trends Neurosci*. 2014; 37(3):169-177.