*LIS*sen up!

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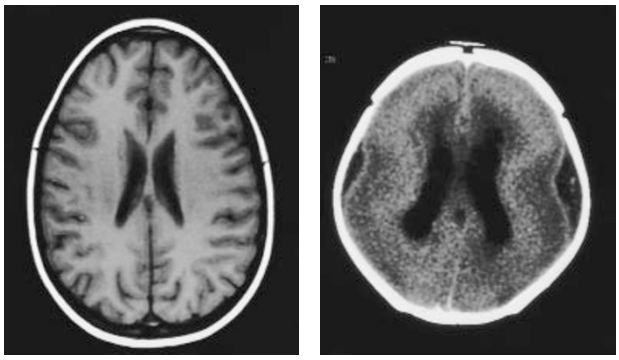
Trying to understand how the cerebral cortex develops really represents a sneaky search for what defines us as human. All of our most deeply cherished human features reside squarely in our cortical grey matter: from our drive to figure out everything else in the world, to our elusive 'consciousness' —that controversial notion that otherwise sober scientists argue about after cortical function has been disinhibited by a few cocktails. Yet, while some philosophers may fret about the dialectical circularity of a human cortex figuring out itself, like a Max Escher image, cortical development is increasingly viewed as just another biological problem in search of a 'saturation mutagenesis' solution. On page 333, Shinji Hirotsune and colleagues show how mouse knockouts can be used to exquisitely elucidate the biological function of genes required for making the human cortex[1].

Hirotsune *et al.* have made several mice that contain specific mutations (in different combinations) of *Pafah1b1*, the mouse orthologue of a gene that is mutated in one of the most striking genetic anomalies of the human brain, lissencephaly. Lissencephaly (literally, 'smooth brain') represents a syndrome in which the normal folds of the cortex (which allow more grey matter to be crammed into the confined box that is the skull) are lost (see figure). The resulting cortex is not only smooth but thickened, and devoid of the usual crisp six layers that contain neurons of diverse form and function[2]. Traditionally, lissencephaly has been classified as a 'neuronal migration disorder', in which neurons are specified and formed normally, but then migrate improperly. Neurons in the cortex are formed deep in the brain, in specialized proliferative zones, and receive all the information they need about the sort of cell they will become and their ultimate destination before or during their final mitosis. Postmitotic neurons then migrate, like salmon upstream, for distances that approximate 100 cell body-lengths in the mouse brain, and more than 1000 cell body-lengths in the human brain, before finding their pre-specified location in the cortex. As they reach the cortex, each new cohort of neurons is faced with the final challenge of having to squeeze past all of the already-present cortical neurons to reach the top of the pile, producing a cortex that is generated in an 'inside-out' fashion; the last neurons generated end up in the uppermost layers. At a cellular level, lissencephaly has been thought to represent an arrest of the ordered migration to the cortex—the study by Hirotsune *et al.* now proves that this is so.



The rough and the smooth. The figure shows magnetic resonance images taken through a normal brain (left) and the brain of an individual with lissencephaly (right). The normal brain shows a thin rim of grey matter composed of neuronal cell bodies, atop the deeper white matter which contains interconnecting fibers; deep folds called gyri indent the grey matter. The six cellular layers within the grey matter are not visible at this magnification. In lissencephaly the brain is smaller and virtually all gyri are absent, producing a smooth surface. In addition, the grey matter is thicker and the white matter indistinct.

Two genes cause similar types of lissencephaly in humans, although additional human lissencephaly genes are probably out there. Hirotsune *et al.* have focussed on *Pafah1b1*, the mouse orthologue of *PAFAH1B1*, also known as *LIS1*. Heterozygous loss of *PAFAH1B1,* commonly in the form of gross gene deletions that involve chromosome 17p13, causes an estimated 40% of human lissencephaly[3]. A second X-linked gene, *DCX,* encoding doublecortin, causes lissencephaly in affected males that is virtually identical to *PAFAH1B1* heterozygous mutations[4,5]. While *PAFAH1B1* was identified five years ago[6], its precise function has remained obscure; while it was independently co-purified as a noncatalytic subunit of platelet activating factor (PAF) acetylhydrolase[7], it has also been implicated in microtubule function[8]. Furthermore, *LIS1* homologues have been identified in many species, including *Aspergillus*, where one of them is necessary for nuclear translocation[9]. In establishing a mouse model of human lissencephaly, Hirotsune *et al.* have obtained not only a comprehensive description of the mutant defects, but have also provided an *in vitro* culture system in which cellular effects of *Pafah1b1* can be analysed.

They created several mutations in *Pafah1b1*, including a *lox*P insertion that appears to maintain some residual function, as well as a standard *neo* replacement and a Cre-*lox*P induced deletion that both appear to represent null alleles. Homozygous embryos carrying the strong alleles die very early, showing that *Pafah1b1* is required in early embryogenesis. Heterozygotes carrying the *Pafah1b1* null allele show defects in neuronal migration and layering in the cerebral cortex, hippocampus and olfactory bulbs, but apparently normal neurogenesis. Considering how devastating heterozygous *Pafah1b1* mutations are in humans—profound mental retardation, seizures, and limb weakness are typical—the heterozygous mice seem remarkably hale and hearty, and the cortical phenotype is certainly more subtle than is seen in humans. Perhaps the species difference in the phenotype reflects the tenfold greater distance that neurons need to migrate in humans *versus* mice.

Hirotsune *et al.* also studied cultured cerebellar cells from the heterozygous mutant mice and showed that there is a cell-autonomous defect in the migration of neurons. In another elegant stroke, they created compound heterozygotes of the stronger and weaker alleles, and these embryos survived until birth, showing a much more severe phenotype than heterozygotes—never encountered in humans—in cortex, hippocampus, and in the cerebellum as well. The more severe compound heterozygote phenotype implicates *Pafah1b1* more broadly in many types of neuronal migrations, and at earlier stages of neuron formation, perhaps during neurogenesis.

An especially pleasing aspect of these studies is that they permit comparison between the *Pafah1b1* phenotypes and several other intensively studied mouse mutants in which neuronal migration is affected. The reeler mouse, first identified 50 years ago, shows a remarkably 'pure' neuronal migration disorder in the cortex: neurons are formed and specified normally, but migrate in an aberrant, almost systematically aberrant, fashion, so that the six layers of the cortex are turned almost exactly upside down[10]. A mutant identified more recently, scrambler[11], shows essentially the same phenotype[12]. While the 'reeler' gene encodes a large secreted protein[13,14], the 'scrambler' gene encodes a cytoplasmic adapter protein that is thought to represent part of the downstream signalling mechanism for reelin[15,16]. Mutations in two additional genes that encode cyclin-dependent kinase 5 (CDK5; which, unlike other CDKs, is expressed predominantly in postmitotic neurons and is not involved in cell-cycle regulation; ref. 17) and Cdk5's major regulatory protein, P35 (ref. 18), also result in systematic misrouting of cortical neurons, although with subtle differences when compared with the reeler and scrambler phenotypes. Perhaps disappointingly, none of the '*Pafah1b1*' phenotypes fit neatly into any of the boxes defined by the previously characterized mouse mutants. The *Pafah1b1* heterozygous phenotype in the cerebral cortex is subtle, and milder than that of any of the other mouse mutants. It lacks the inversion of cortical layers that characterizes reeler, scrambler, P35 and Cdk5. The primary aberration of the '*Pafah1b1*' heterozygote cortex is a relative slowing of neuronal migration that appears to catch up later in development. In contrast, the *Pafah1b1* compound heterozygote phenotype appears to have a more severe phenotype than any of the other mutants and probably has unique defects in neurogenesis. It will be interesting to characterize mouse mutants that contain inducible mutations in *Pafah1b1*, which should allow its roles in proliferation and migration to be separated out from one another. The current availability of multiple mouse mutants now allows genetic epistasis studies that are routine in invertebrate genetic systems. These, together with the continued identification of genes that affect cortical development in humans and generation of mouse models of these disorders, promise to be a rich source of information that may allow our brains to figure out how they got to be the way they are. □

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