Unfolding the Folding Problem of the Cerebral Cortex: Movin' and Groovin'

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FLRT adhesion molecules regulate cerebral cortex folding by controlling neuron migration

Unfolding the Folding Problem of the Cerebral Cortex: Movin' and Groovin'

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Abstract: The development of reproducible folding in the gyrencephalic cerebral cortex is a topic of great interest to neuroscientists. In a recent paper in Cell, del Toro et al. (2017) show that changing the adhesive properties of neurons in the normally lissencephalic mouse cortex leads to the formation of stereotyped folding.

How the cerebral cortex evolves and expands from just a few square centimeters of flat cortex in rodents to approximately 2½ ft.² in the adult human – roughly the area of a face towel or a large dinner napkin – and how that cortex is successfully and reproducibly compacted into a sensibly-sized cranium that is able to safely passage through relatively limited-diameter birth canals, is a major question in brain science. The horizontal brain expansion that occurs in humans, with its familiar surface peaks (gyri) and tucked-away valleys (sulci) is quite remarkable, especially considering that the thickness of mature human cortex is only about twice that of rodent cortex (Defelipe, 2011). It speaks to the dramatic expansion of sensory, motor, and cognitive-integrative abilities that occurred during evolution, from the flat, or lissencephalic, cortex of rodents to the remarkably similarly organized but highly folded cortex of carnivores like ferrets and cats, nonhuman primates, humans, and other gyrencephalic (folded cortex) mammals. In gyrenecephalic animals, approximately 2/3 of the entire cortex is "hidden" from the surface in sulcal folds. Aristotle, who saw function in structure, canonized

gyrification as the most prominent of brain features when he postulated that the brain, due to its convoluted vascularized surface, functioned as a type of radiator, a cooling unit for the vital heat pumped by the heart (Gross, 1995). The quest to explain this striking folding of the brain has been enchanting scientists ever since, and work published this month in *Cell* by del Toro, Klein, and colleagues uncovers an exciting new twist to this convoluted phenomenon (del Toro et al., 2017).

The reproducibility of cerebral cortex folding, placing function-specific areas in discrete and reproducible sulcal-gyral locations across members of a species, and evolutionarily across species, argues that this is in large part genetically predetermined. Several events underlie the dramatic expansion of the mammalian neocortex. These include the emergence of an expanded diversity of progenitor types, distinct and differential controls over the proliferation and positioning of new neurons, the location and both pial and ventricular tethering of subsets of radial glia, which act both as radial migration substrates and progenitors themselves, and the arc-like expansion from a relatively small and less expanded early developmental germinal ventricular zone to the vastly expanded surface of the fully formed cerebral cortex. Since several of these might exert cellular and/or mechanical organizational influences and forces, there is much interest in identifying which of these elements contribute to this core feature of evolutionary advancement of cognition, sensorimotor control, and high-level behavior.

Though most experiments have focused on proliferation and radial substrates of migration, or biophysical mechanics and forces folding the surface via material properties and constraints (Fernández et al., 2016), recent investigation has included molecular indicators of the folded positions themselves (de Juan Romero et al., 2015), biophysical and tension-based models (Budday et al., 2015, Tallinen et al., 2014), and examination of differential and competitive migration rates (Gertz and Kriegstein, 2015). Many experiments have investigated whether simple enhancement of proliferation might be a core evolutionary mechanism, but results from these studies have largely revealed non-stereotyped, disorganized folding that is not very reminiscent of normal gyrencephalic cortex. For example, Anjenn Chenn and Chris Walsh genetically dysregulated β-catenin in the mouse cortex and observed dramatically expanded cortical tissue, with invariably penetrant and non-reproducible compaction folding within the

skull (Chenn and Walsh, 2002). Other experiments have revealed similarly non-specific fold locations with regard to functional areas, arguing against this proliferation-based mechanism as the core or only organizational principle, and indicating that it might not be sufficient or specific. Directionality and variable rates of migration, linked to biophysical folding mechanisms, are an attractive set of factors for sculpting the three-dimensional organization of an evolutionarily expanding cerebral cortex. It is this set of linked mechanisms that are investigated by del Toro, et al. (del Toro et al., 2017).

The authors report that deletion of the adhesion molecule genes FLRT1 and FLRT3 in the normally lissencephalic mouse brain causes ectopic cortical folding, a striking result with much to interpret and unfold in terms of cortical evolution and development. The authors show that FLRT1/3 double gene deletion produces sulci-like folds with partial (~30%) penetrance, induces clustering of neurons in the cortical plate, and accelerates radial neuronal migration. Unlike several previous mouse models with experimentally-induced gyrified cortices, FLRT1/3 double knockout mice appear to be the first genetic model to induce cortical folds without altered progenitor proliferation. Using computational models to simulate adhesive and repulsive interactions in mixed populations of FLRT-positive and -negative neurons, the authors propose that reduced intercellular adhesion and faster migration in FLRT1/3 double knockout neurons underlies cortical sulcus formation through uneven dispersal and clustering of neurons as they populate the cortical plate. Looking at gyencephalic ferret cortices, the authors find lower levels of FLRT1/3 expression specifically in areas destined to fold into sulci, suggesting an endogenous role for reduction of FLRT-mediated adhesion in neurons migrating between nascent sulci. Together with the body of previous work on the role of uneven proliferative expansion of basal progenitors associated with gyri, this new work prompts the emergence of a composite model in which local acceleration of migrating neuron clusters complements local progenitor expansion to promote the sulci and gyri, respectively, of gyrencephalic animals (Figure 1).

As striking as these results are, the intricacies of such a mechanism remain to be revealed, as do the regulating mechanisms that coordinate this complex sequence of events in a manner that enables cortical folding to be reproducibly executed between individuals of a species. Intriguingly, Klein and colleagues previously reported that

FLRTs are able to mediate both adhesion and repulsion (Seiradake et al., 2014), an intriguing functional pleiotropism that might be central to their ability to coordinate the push and pull of migrational paths toward structuring the developing cortical plate.

Beyond the specific results of this elegant paper, the new range of potential intercellular differential adhesion and interaction mechanisms unveiled by del Toro et al. (2017) will be deeply influential, and this work is likely to become a landmark study toward understanding how gyrencephalic brain structure is physically built during development. One future step might be to ask whether the findings in this study are generalizable and applicable across different types of folding in brains– more widely in the cerebellum, e.g., or at recognizable folds and flexures. It also would be interesting to identify upstream regulators of FLRT1/3 expression, such as transcriptional regulators and promoter/enhancer regulatory elements, and investigate their changes during evolution and at the transitions between lissencephalic and gyrencephalic species. Merging multiple models of gyrification, it will be interesting to explore whether even quite small physical effects of such differential adhesion might sufficiently bias “hinge regions” for reproducible folding from mechanical material-biophysical forces due to differential proliferation and radial tethering, e.g.. Combinations of molecular “hinge” regions plus proliferative regional expansion plus biophysical forces from tethering constraints might add up to elegant brain folding. Aristotle was right– cerebral folding is a very hot topic.

Figure 1: A two-stage model of cortical gyrification: Apical progenitors (blue cells) produce basal progenitors (green cells) that expand unevenly. Mixed populations of neurons (purple and red cells) with varying FLRT expression levels emanate from the proliferative zones with different migration speeds (purple low-FLRT neurons migrating faster; red high-FLRT neurons migrating slower). The resulting heterogeneities 1) of cell densities due to proliferation, and 2) of migration speeds and clustering due to differential adhesion synergize to delineate areas where gyral peaks and sulcal ridges will form, respectively. Thus two distinct developmental mechanisms, one mitotic and one post-mitotic, combine to sculpt the two main features of cortical gyrification.

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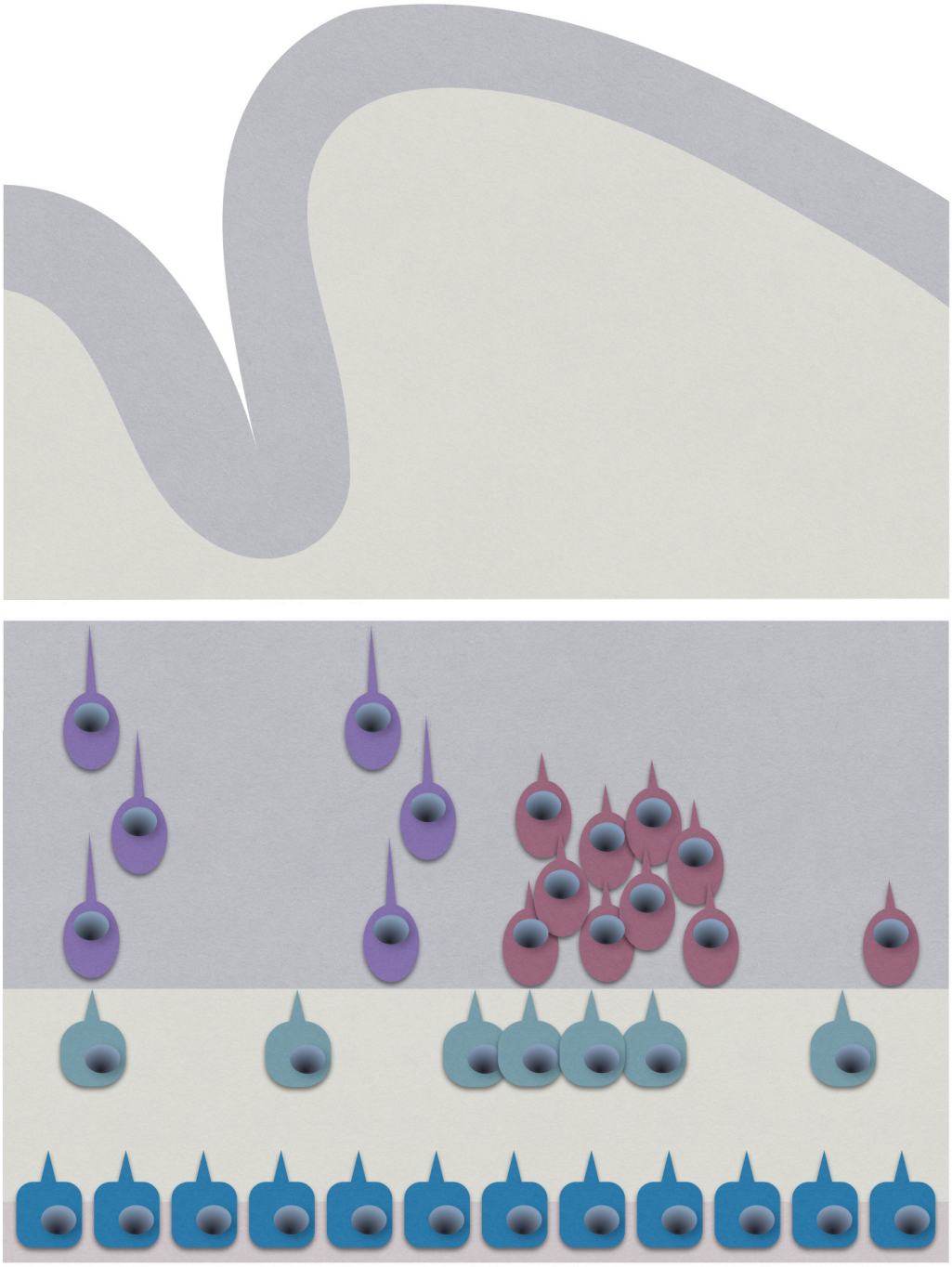
Basal progenitors
Fast-migrating low-FLRT neurons
Apical progenitors
Slow-migrating high-FLRT neurons