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Supersize me—new insights into cortical expansion and gyration of the mammalian brain

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During evolution, the mammalian brain massively expanded its size. However, the exact roles of distinct neural precursors, identified in the developing cortex during embryogenesis, for size expansion and surface folding (i.e., gyration) remain largely unknown. New findings by Nonaka-Kinoshita *et al* advance our understanding of embryonic neural precursor function by identifying cell type-selective functions for size expansion and folding, and challenge previously held concepts of mammalian brain development.

Over the course of evolution, the mammalian brain massively expanded its size and complexity, which is believed to be responsible for an increase in cognitive functions and intellectual skills. The increase in brain size and number of cortical neurons is primarily due to an increased surface area by generating folds (gyrations) while the cortical thickness remained relatively constant (Lui *et al*, 2011). In the last decade, substantial progress has been made in identifying the cellular sources of cortex development. Using genetic lineage tracing of individual cell populations and time-lapse imaging of rodent and human slices of the embryonic cortex, radial glial cells (RGCs) were identified as the primary progenitors or neural stem cells (NSCs) in the developing

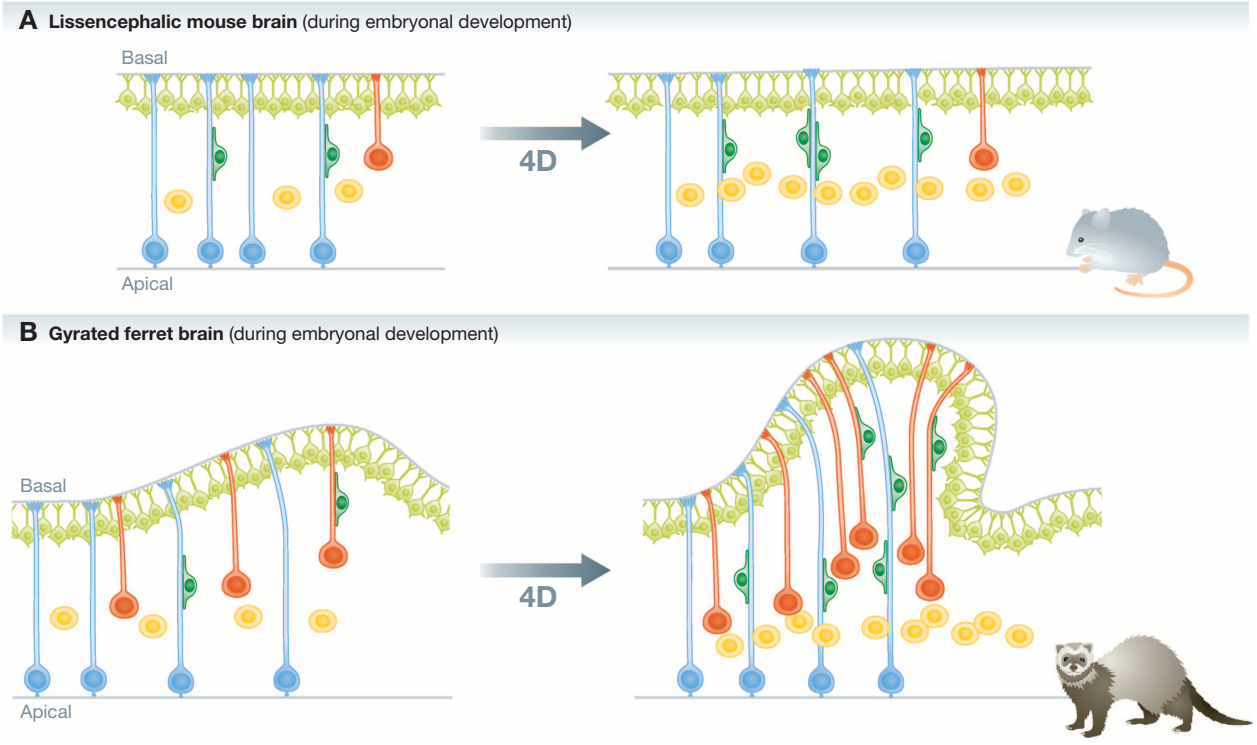

Figure 1 How different neural precursors appear to regulate size expansion and folding during mammalian brain development. (**A**) Shown are the main cellular components of the cortex of the lissencephalic mouse brain during embryonic development with RGCs (blue) lining the lateral ventricles in the VZ that generate BPs (yellow) in the SVZ and provide a scaffold for migrating neurons (left; green). Note that the mouse developing brain contains only a few bRG in the OSVZ (red). Notably, expansion of BPs using the 4D strategy developed in the Calegari laboratory increases surface area of the murine cortex without inducing the folding of the smooth mouse brain surface (right panel). (**B**) In contrast to lissencephalic animals, the developing cortices of species with gyrated brains (e.g., humans and ferrets) contain a substantial number of bRG located in the OSVZ (left panel). 4D-based, virus-mediated expansion of bRG in the ferret cortex leads to the induction of additional folds in the ferret cortex, indicating that the proliferative activity of bRG is critically involved in the extent of folding in physiologically gyrated brains (right panel).

cortex (Gotz and Huttner, 2005). Simplified, RG in the ventricular zone (VZ) line the ventricular surface and self-renew through symmetric divisions or give rise to basal progenitors (BPs; also called intermediate progenitors) in the subventricular zone (SVZ) that typically divide symmetrically and generate neurons. In contrast to the lissencephalic rodent brain, the developing cortex of gyrated mammals (e.g., humans and ferrets) contains a large number of basal radial glial (bRG) cells that reside in the outer subventricular zone (OSVZ), retain a cellular process that is connected to the pial surface and that are, in contrast to BPs, multipotent, meaning that they have the potency to generate diverse neural cell types (Fietz et al, 2010; Hansen *et al*, 2010; Reillo *et al*, 2011).

Largely based on the anatomical differences between the developing cortex of lissencephalic and gyrencephalic brains, several hypotheses have been formulated aiming to explain the massive increase in size and induction of brain folding during mammalian evolution. One prominent hypothesis, called the radial unit hypothesis, suggests that the expansion of RGCs lining the ventricle leads to an increase of radial units that generate neurons and thus is responsible for the increase of surface area (Rakic, 1995). Others proposed that the increase in size and folding could be due to an increase in BP expansion in the SVZ compared to RGC numbers in the VZ, a hypothesis called the intermediate progenitor model (Kriegstein *et al*, 2006). These hypotheses were helpful to start explaining mammalian brain evolution, but testing the exact role of different neural precursors remained extremely challenging due to technical difficulties to selectively manipulating the proliferative activity of distinct precursor populations. Even though previous approaches were successful in enhancing brain size/neuron numbers in mouse models (e.g., by ectopically enhancing WNT signalling activity or manipulating the activity of the small RhoGTPase Cdc42 in neural precursors), these strategies had the drawback that the normal six-layered cortical topography was disrupted, making it difficult to draw definite conclusions (Chenn and Walsh, 2002; Cappello *et al*, 2006).

In a collaborative work from the Calegari and Borrell laboratories, Nonaka-Kinoshita *et al*, 2013 now used an elegant approach to selectively enhance proliferation of distinct precursor populations in the mouse and ferret developing cortex. They used a previously described approach manipulating cell cycle length and subsequently proliferation by overexpressing the cell cycle regulators cdk4 and cyclinD1 that is sufficient to enhance neurogenesis without affecting cortical layering (a system called 4D) (Lange *et al*, 2009). For their mouse experiments, Nonaka-Kinoshita *et al* used a transgenic strategy to transiently overexpress 4D in nestin-expressing precursors using a tetracycline-controlled gene expression system (nestin^{rtTA}/tetbi^{4D}). With this approach, they selectively enhanced proliferation of BPs in the SVZ without affecting the number or proliferation of RGCs in the VZ (Nonaka-Kinoshita *et al*, 2013). Strikingly, targeted expansion of BPs induced a substantial increase in surface area but was not sufficient to induce cortical folding in the otherwise smooth mouse cortex, challenging the radial unit hypothesis and the intermediate progenitor model with regard to their predictions on the effects on size and/or gyration of the cortex upon expansion of the BP pool. Complementing their findings of BP expansion in the lissencephalic mouse brain, Nonaka-Kinoshita *et al* used retroviral vectors and electroporation of 4D expression constructs to target 4D expression to neural precursors in the developing ferret cortex that is gyrated under physiological conditions. In the ferret, 4D expression induced proliferation of multipotent bRG located in the OSVZ, as outlined above, a cell type that is found predominantly in gyrated cortices compared to lissencephalic brains. Notably, enhanced proliferation of bRG triggered the formation of novel cortical folds, suggesting that indeed the expansion of bRG may represent a key event during evolution to induce gyration and subsequent surface expansion of the mammalian brain (Borrell and Reillo, 2012; Nonaka-Kinoshita *et al*, 2013) (Figure 1). This now experimentally supported hypothesis is strongly reinforced by two recent publications: one from (Tuoc *et al*, 2013)who found that deletion of the chromatin remodelling protein BAF170 increases the BP pool and subsequently enhances brain size ; and another one from the Götz laboratory where it was found that experimentally reduced expression levels of the DNA-associated protein Trnp1 substantially increased the expansion of bRG and BPs, inducing folding of the normally lissencephalic mouse brain (Stahl *et al*, 2013). Taken together, these studies suggest that bRG in the OSVZ play an important role in cortical folding by enhancing the generation of neurons and by providing a glial scaffold for newborn neurons to disperse more laterally and thus to form folds in the developing brain (Reillo *et al*, 2011).

Even though this new study challenges previously held concepts regarding size expansion and folding of the mammalian brain, future studies are required that even more selectively enhance the proliferation and expansion of distinct precursor subtypes with high temporal and spatial control. Thus, the combination of sophisticated genetic tools to enhance precursor activity with detailed molecular analyses (e.g., analysing gene expression in highly folded versus unfolded brain regions, an approach that already showed differential levels of Trnp1 expression; Stahl *et al*, 2013) and live-imaging studies in the developing mammalian cortex will further enhance the understanding how our brains developed during evolution.

Conflict of interest

The authors declare that they have no conflict of interest.

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