BIOPHYSICS

Unfolding the brain

The folded surface of the human brain, although striking, continues to evade understanding. Experiments with swelling gels now fuel the notion that brain folding is modulated by physical forces, and not by genetic, biological or chemical events alone.

Ellen Kuhl

Exactly four decades ago, a group from Harvard proposed a physical model of differential growth to explain the formation of folds during human brain development[1]. The model challenged the conventional wisdom that surface morphogenesis, pattern selection and evolution of shape are purely biological phenomena. To no surprise, this rather hypothetical approach was perceived as highly controversial. Now, writing in *Nature Physics*, Tuomas Tallinen and colleagues[2], again from Harvard, have provided the first experimental evidence of the theory of differential growth and demonstrated that physical forces — not just biochemical processes alone — play a critical role in neurodevelopment. Their findings could have far-reaching clinical consequences for diagnosing, treating and preventing a wide variety of neurological disorders[3].

The average adult human brain has a volume of 1,200 cm^3, a surface area of 2,000 cm^2 and a cortical thickness of 2.5 mm. It contains 100 billion neurons, of which 20 billion are located in the outermost layer, the cerebral cortex[4]. Each cortical neuron is connected to 7,000 other neurons, resulting in 0.15 quadrillion connections and more than 150,000 km of nerve fibres[5]. Gyrification, the process of cortical folding, is recognized as a fundamental mechanism to maximize the number of cortical neurons and minimize the distance between them[6]. In humans, gyrification begins at week 23 of gestation and our brain continues to grow until adulthood — about 20-fold in volume and 30-fold in surface area — while the cortical thickness remains virtually unchanged. But folding can go awry: severe under- or over-folding, clinically known as lissencephaly and polymicrogyria, respectively, are typically associated with serious developmental disorders such as seizures, motor dysfunction, mental retardation and developmental delay[7]. In an attempt to unravel the biology of neurodevelopment, researchers are contemplating phylogenic, neurogenetic and biochemical theories[8]; yet, to date, the precise mechanisms of cortical folding have remained elusive.

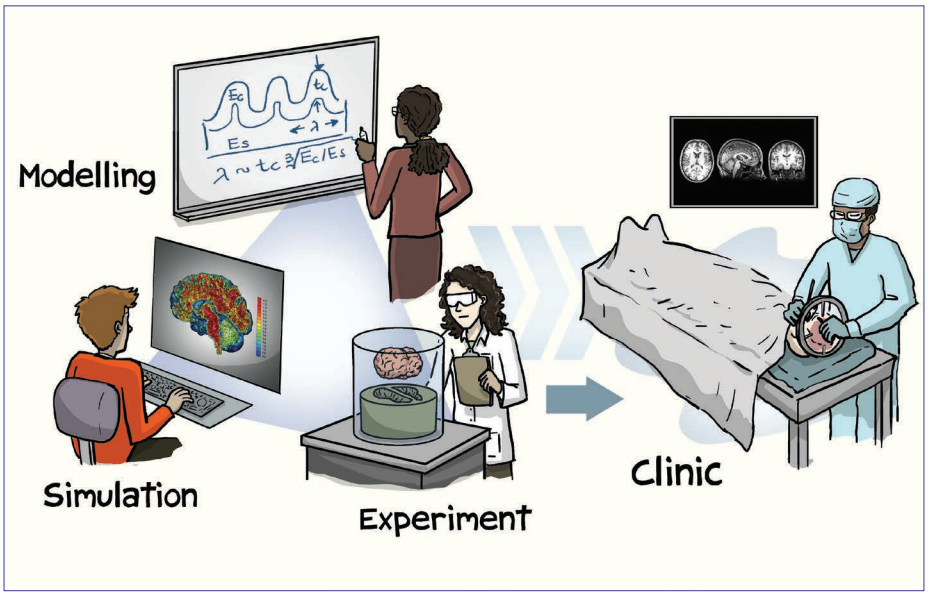

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Figure 1 | The work of Tallinen *et al.*[2] provides insight into the growth and form of cortical convolutions through physics-based modelling, simulation and experiment. Experiments with swelling-gel brain models, created from clinical magnetic resonance images, now provide evidence for a forty-year-old analytical model of differential growth[1]. In this model, the gyral wavelength λ, the distance between two folds, is proportional to the cortical thickness t_c and to the cube root of the stiffness contrast between the outer layer and the inner core, E_c/E_s. Simulations with finite element models, created from the same clinical images, support these findings and suggest that cortical folding is a mechanism to release residual stresses by surface buckling.

Inspired by soft-matter physics, the forty-year-old model of differential growth is now moving back into the spotlight[1]. The model represents neurodevelopment as a physical instability problem of constrained growth and explains cortical folding as a mechanism to release residual stresses by surface buckling. It predicts that the gyral wavelength λ, the distance between two folds, is proportional to the cortical thickness t_c and also to the cube root of the stiffness contrast between the outer layer and the inner core, E_c/E_s (Fig. 1, modelling)[9]. Because the cortical layer is extremely thin and ultra-soft, even tiny alterations in any of these parameters can induce tremendous changes in convolution and shape. Although this concept is immediately intuitive to physicists — in fact, it is a universal law in geophysics, smart materials and flexible electronics — the lack of experimental evidence has been the major point of criticism from the neuroscience community. This concern is legitimate, yet difficult to address[9]: by their very nature, experiments with human brains are ethically questionable; experiments with small animals can be misleading as most rodent brains do not fold; and experiments with brain-like surrogates are challenging because man-made materials do not grow. Undoubtedly, without sound experimental evidence, the theory of differential growth remains speculative and incomplete.

In a cleverly designed series of experiments with swelling gels, Tallinen *et al.*[2] have now demonstrated how expansion-induced physical instabilities can provoke cortical folding. Starting with

magnetic resonance images of a 22-week-old unfolded fetal human brain, the researchers 3D-printed a polymer cast, produced a negative mould of silicone and created a white-matter core of the brain from a soft elastomer (Fig. 1, experiment). Using layer-by-layer drop casting, they coated the structure with a grey-matter layer in the form of a swelling gel. When immersed in a solvent, the outer layer swelled relative to the inner core, building up residual stresses and ultimately folding into a structure that displayed striking similarities with a real human brain.

The results of the swelling-gel experiment are in excellent agreement with the early mathematical model; yet the model can only predict the behaviour of simple, regular structures at the very onset of folding. Finite element analysis is a powerful tool for predicting growth-induced folding in real biological structures beyond the first instability point[7]. The underlying principle is to divide the brain into thousands of simple, regular structures — the finite elements — and allow each element to grow. Inner elements grow in volume and outer elements grow in surface area but not in thickness. Surface growth induces cortical stress, and once this stress reaches a critical level — similar to the experiment — the brain begins to fold (Fig. 1, simulation). Simulating the complex dynamic process of multiple emerging folds is technically challenging: the governing equations are highly nonlinear; the brain undergoes large deformations; the folds form contact and interact with one another; and the solution has numerous critical points. Tallinen *et al.*[2] proposed an innovative and elegant solution to circumvent these difficulties by running the simulation backwards and unfolding the brain. Their simulations explain why folding always begins in weakly curved regions and why gyri and sulci align perpendicular to the direction of maximum compressive stress.

Experiments with swelling brain models provide the essential missing link between modelling, experiment and simulation. However, a few limitations remain: the model is beautiful and simple, but it is limited to the initial folding of idealized structures; the experiment is useful for exploring instabilities beyond the onset of folding, but it is limited to moderate changes in volume. The simulation addresses both limitations, but it fails to address the role of the skull. Including the skull, the meninges and the ventricles would be straightforward to advance the predictive capabilities of the model.

Now that we have a physical theory with rigorous modelling, experiment and simulation to identify the regulators of pattern selection, what do we do with it? A logical next step would be to functionally correlate surface and volume growth to the molecular and cellular drivers of neuronal division, migration and connectivity, and replace the phenomenological growth rates of the model by the time constants of neurodevelopmental events. Remarkably, the current theory can already predict the shape, size, location and orientation of gyri and sulci. It can also explain the gyrification index, which is an important metric towards translating this knowledge to the clinic. For example, functional magnetic resonance imaging now allows us to correlate regional specificity to function, thereby allowing us to trace the learning of specific language or motor skills back to changes in brain surface morphology. Making these connections can help us identify topological markers for the early diagnosis of autism, schizophrenia or Alzheimer's disease, and, ultimately, design more effective treatment strategies. ❐

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