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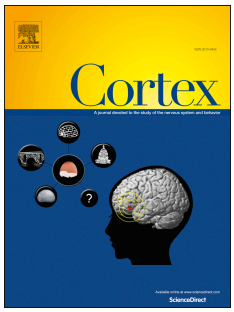

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Title:

Mechanical morphogenesis and the development of neocortical organisation

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Abstract

The development of complex neocortical organisations is thought to result from the interaction of genetic and activity-dependent processes. We propose that a third type of process – mechanical morphogenesis – may also play an important role. We review theoretical and experimental results in physics showing how even homogeneous growth can produce a variety of forms, in particular neocortical folding. The mechanical instabilities that produce these forms induce heterogeneous patterns of stress at the scale of the organ. We review the evidence showing how these stresses can influence cell proliferation, migration and apoptosis, cell differentiation and shape, migration and axonal guidance, and could thus be able to influence regional neocortical identity and connectivity.

1. Introduction

The neocortex is the most distinctive structure of the mammalian nervous system. Whereas its thickness and radial organisation vary little among species, its surface area can display >1000-fold changes, increasing the number of its specialised regions and the complexity of its connectivity (Sereno and Allman 1991, Braitenberg and Schüz 1998, Zhang and Sejnowski 2000, Rakic 2009, Krubitzer 2009, Van den Heuvel et al. 2016). This increase in complexity is the most likely biological substrate of the increased behavioural sophistication that leads to the emergence of cognitive functions such as consciousness, creativity and language. How do complex neocortical organisations develop and evolve?

Today, neocortical organisation is thought to result from the interaction of genetic and activity-dependent processes (see O'Leary et al. 2013 and Geschwind and Rakic 2013 for reviews). During development, morphogens and signalling molecules secreted from a reduced number of patterning centres are thought to produce gradients of expression of transcription factors, which together with neuronal activity driven by thalamo-cortical afferents establish and maintain areal identity (Krubitzer 2009, O'Leary et al. 2013). From the perspective of evolution, an increase in neocortical complexity should result from the increased sophistication of the genetic program that would control areal identity directly, or indirectly, by first establishing the connections that will later enable activity-dependent processes to operate.

Here we propose that a third type of process – mechanical morphogenesis – may also play an important role. We refer to mechanical morphogenesis as the process through which simple mechanical forces can lead to instabilities that conduct to the emergence of complex shapes. The general role of mechanical morphogenesis in biology is beautifully described in D'Arcy Thompson's work (Thompson 1917, see also Lecuit and Mahadevan 2017), and its role in embryogenesis was extensively studied in Wilhelm His and Wilhelm Roux's "developmental mechanics" (Dupont 2017). Recent theoretical and experimental results from the physics of soft tissues show that even homogeneous growth can produce a rich variety of forms (Wang and Zhao 2015). In particular, several biophysical models suggest, for example, that neocortical

folding could be caused by growth-induced mechanical instabilities. The underling mechanism is simple: growth generates residual stresses on the neocortical tissue, which eventually overcome a threshold value dependent on its material properties and geometry. Beyond this threshold, neocortical tissue cannot sustain this residual stress without folding, and folds are produced to minimise the stress. As a result of folding, the neocortical tissue develops complex, heterogeneous, patterns of mechanical stress at different scales. The developing tissue is very sensitive to these mechanical signals, which can influence cell proliferation, apoptosis, cell differentiation, cell shape, cell migration and axonal guidance. Through mechanical morphogenesis, a broad regulation of growth could then lead to an increased number of different areas, whereas the macroscopic changes in neocortical geometry could facilitate the formation of new connectivity patterns.

We will first briefly summarise the role of activity-driven and genetic processes in the production of neocortical arealisation and connectivity. We will then address the problem of explaining the relationship between neocortical arealisation and folding. To better understand neocortical folding and mechanical morphogenesis, we will briefly introduce the theory of mechanical elasticity and growth. We have developed a website with interactive mechanical simulations illustrating these principles, which can be accessed at http://neuroanatomy.github.io/growth. Finally, we review recent results highlighting the effect of mechanics on the developing brain tissue and discuss a mechanical morphogenesis hypothesis for the development and evolution of neocortical organisation, its predictions, and experimental approaches that should allow us to test them.

2. Development of neocortical arealisation

"It must remain an open question whether the refinement of the cortex through differentiation is always the result of external, physical causes, or whether many of the associated phenomena may be explained in other

ways, unrelated to external living conditions and unrelated to the struggle for existence, rather due to a property of the organism itself, an 'energy for refinement' (R. Hertwig) or, as Naegli expresses it, a 'principle of progression'" (Brodmann 1909, Brodmann and Gary 2006).

The neocortex is the outermost part of the cerebral hemispheres. In the radial direction it is commonly considered to be composed of 6 main layers containing neuronal cell bodies: the grey matter. The myelinated axons of these neurones constitute most of the cerebral white matter. Together, the neocortical grey matter and the white matter represent ~90% of the human brain (Toro et al. 2009). The thickness of the neocortex varies little across species. For example, the neocortex of mice has an average thickness of 1 mm, compared with 2.5 mm in humans (Fischl et al. 2000). By contrast, there are extreme differences in cortical surface area among mammals, and a 1000-fold difference between mice and men (Rakic 2009). The expansion of the neocortex correlates with the development of cortical folds and the formation of an increased number of specialised regions: the cortical areas (Changizi 2001). Although exact numbers of cortical areas are subject to debate, analyses of cytoarchitecture, chemoarchitecture and connectivity patterns, generally allow us to distinguish ~20 cortical areas in lissencephalic species such as mice (Herculano-Houzel et al. 2013), but ~50 in gyrencephalic species such as macaques, and probably more than 200 in humans (Sereno and Allman 1991, Kaas 2012). In gyrencephalic species, multiple studies have revealed a close relationship between cortical folding and the cytoarchitectonic, connective and functional organisation of the neocortex, suggesting that common biological processes shape them (Welker 1990). For example, in racoons the sulci of the somatosensory cortex precisely separate the functional fields of the palm and the fingers (Welker and Campos 1963). In primates, the central sulcus divides the primary motor cortex from the somatosensory cortex, and the calcarine sulcus divides the superior and inferior visual hemifields of the primary visual cortex in the occipital lobe (Brodmann 1909, Brodmann and Gary 2006, Fischl 2013, Fischl et al. 2008).

The major divisions of the nervous system develop during the early segmentation of the neural tube into neuromeres (Puelles 2009, Puelles 2013). The neocortex is a rather homogeneous structure – even called sometimes isocortex – which develops from the rostral-most neuromere. Only a few cortical areas, such as

the primary visual cortex, can be distinguished with the naked eye. For the most part, it was only after the development of staining and tracing techniques that neuroanatomists were able to describe them. A core group of areas is present in all mammals (Welker 1990, Krubitzer 2007, Krubitzer and Seelke, 2012). These areas, the primary sensory and motor cortices, receive preferential innervation from specific thalamic nuclei. In species with large cortices there is a progressive addition of multi-modal associative areas, connected mostly with other neocortical regions but also with thalamic nuclei (Krubitzer 2009, Krubitzer and Seelke 2012).

Despite their stability within a single species, neocortical areas are dynamic entities, whose organisation can be dramatically modified by changes in neuronal activity. For example, in animals with stereoscopic vision such as cats or monkeys, the primary visual cortex receives thalamic afferents associated with the left and the right eyes clustered into bands of ocular dominance. Early visual deprivation can change the balance between left and right innervation, or even completely suppress the formation of bands (Espinosa and Stryker 2012). The processes necessary to dynamically develop this type of cortical modules seem to be shared by all vertebrates, and ocular dominance bands can be made to develop even in species that do not have them naturally. For example, the primary visual cortex of mice, or the optic tectum of frogs, are innervated by axons related to the contra-lateral eye only, and do not develop ocular dominance bands. Bands can be made to develop, however, by driving connections from another eye, either by altering axonal guidance through gene knockout in mice (Merlin et al. 2013) or by grafting a 3rd eye in frogs (Constantine-Paton and Law 1978). The link between primary cortices and specific sensory modalities can be also modified by activity. In a series of experiments in ferrets, Sur and colleagues (Roe et al. 1990, Sharma et al. 2000, Sur et al. 1988) forced visual afferents to innervate what would normally be the auditory cortex, making it acquire many of the cytoarchitectonic and functional properties of the visual cortex(Sharma et al 2000). Despite using their auditory cortex for vision, rewired ferrets exhibited visual behaviour similar to that of normal ferrets (Von Melchner et al. 2000).

Because of this remarkable plasticity, neuronal activity induced by extrinsic stimuli was originally thought to

be the primary cause of neocortical arealisation. The existence of intrinsic processes, however, had been hypothesised since the first treatises on cortical cytoarchitecture (Brodmann 1909). The idea was much discussed at the end of the 80s with the introduction of the protomap (Rakic 1988) and the protocortex (O'Leary 1989) hypotheses, which emphasised differently the role of genetic and activity-dependent processes in neocortical arealisation. The first direct evidence for a genetic process came 10 years later, with the demonstration of the roles of Emx2 and Pax6 in the specification of cortical progenitor identity in mice (Bishop et al. 2000, Mallamaci et al. 2000, see O'Leary et al. 2013 for a review). Subsequent studies showed the existence of several patterning centres located at the periphery of the neocortex, secreting morphogens and signalling molecules such as members of the fibroblast growth factor family (FGFs) and bone morphogenetic proteins (BMPs). These molecules formed gradients over the ventricular zone – where most neocortical neurones are produced in mice – which controlled the expression of transcription factors such as Emx2, Pax6, COUP-TF1, or Sp8 in cortical progenitors. Neurones produced in the ventricular zone migrate radially to form the cortical plate, which later matures into the neocortex. Each position of the ventricular zone could be then characterised by a unique combination of transcription factors, encoding the neocortical areal identity that is propagated through radial migration. The experimental alteration of the morphogenic gradients, or the expression of transcription factors has been shown to result in the displacement or even the duplication of cortical areas (Fukuchi-Shimogori and Grove 2001), as well as marked changes in the proportions of others (Hamasaki et al. 2004).

Today, intrinsic genetic and extrinsic activity-dependent processes are thought to act in combination to produce neocortical arealisation. The initial gradients of gene expression in the ventricular zone and the cortical plate would be genetically encoded, defining in some cases areal identity directly, or defining first the connectivity patterns that would later allow neuronal activity to refine the basic area map, producing the discrete areas and modules of the adult neocortex(O'Leary et al 2013). The phylogenetic differences in neocortical complexity between mice and humans, for example, would result from differences in the sophistication of the genetic program that either prescribes areal identity directly, or indirectly by encoding first connectivity.

But how to explain the relationship between neocortical arealisation and brain folding in gyrencephalic species? One possibility is that the same genetic programme that encodes arealisation (directly, or indirectly through neuronal activity) would guide the formation of folds at specific locations (Ronan and Fletcher 2014, De Juan Romero et al. 2015, Borrell 2018). Species-specific folding patterns would then be a reflection of the genetic programs that produce the different neocortical area maps. For example, gyri could result from bulging due to a locally higher production of neurones (Welker 1990, Ronan and Fletcher 2014, De Juan Romero et al. 2015, Borrell and Götz 2014, Kriegstein et al. 2006, Nonaka-Kinoshita et al. 2013, Reillo et al. 2011, Smart and McSherry 1986a, Smart and McSherry 1986b, Stahl et al. 2013, Borrell 2018). Borrell and colleagues (De Juan Romero et al. 2015) have reported that in ferrets the density of dividing progenitors at P3 (3rd postnatal day, when the ferret cortex is still smooth) was higher under the region that will become later the splenial gyrus compared with the neighbouring regions, which will become sulci. Alternatively, folds could result from bending due to the active contraction of axonal fibres connecting different neocortical areas together (Van Essen 1997, Geng et al. 2009, Hilgetag and Barbas 2005, Hilgetag and Barbas 2006). Van Essen (1997) reported that neighbouring areas with strong interconnections were more often on facing sides of a gyrus, compared with weakly connected areas, which tended to be separated by a sulcus. In all cases, the same genetic program that encodes brain connectivity would produce neocortical folding as a by-product.

However, these explanations do not take into account the physics of growth and the geometric and mechanical properties of the developing brain. The mechanical stress patterns that would produce brain folding if it were due to bulging or bending are not compatible with those observed in real brains (Xu et al. 2009, Xu et al. 2010). Recent theoretical and experimental evidence suggest that brain folding is more likely the result of a buckling instability induced by global neocortical growth: submitted to homogeneous growth, physical systems similar to the developing brain produce folding patterns strikingly reminiscent to those of real brains. If this were the case, the intrinsic "energy for refinement" alluded by Brodmann could well be both biological and mechanical. We will now consider this possibility further.

3. The physics of mechanical morphogenesis

3.1. Elasticity

Brain tissue is composed in a large part of water, it is mostly incompressible (its volume is conserved despite deformation), elastic (after deformation it recovers its original shape), and to some extent plastic (strong or prolonged deformation produce a permanent change in shape) (Xu et al. 2009, 2010, Tallinen et al 2014, 2016). The elastic forces produced by deformation can be calculated relative to an idealised rest configuration, where the tissue is free of mechanical stress (see Fig. 1). Imagine that we subdivide the tissue at rest into small volume elements, and then that we look at how each of them is transformed in the deformed configuration (Fig. 1a). Each volume element can be described by a small box, and a corresponding stretched and skewed box in the deformed tissue (Fig. 1b). It is possible now, for each volume element, to compute a 3x3 transformation matrix F that changes the box at rest into the deformed box (Fig. 1c-e). This transformation matrix F is called the *deformation tensor*.

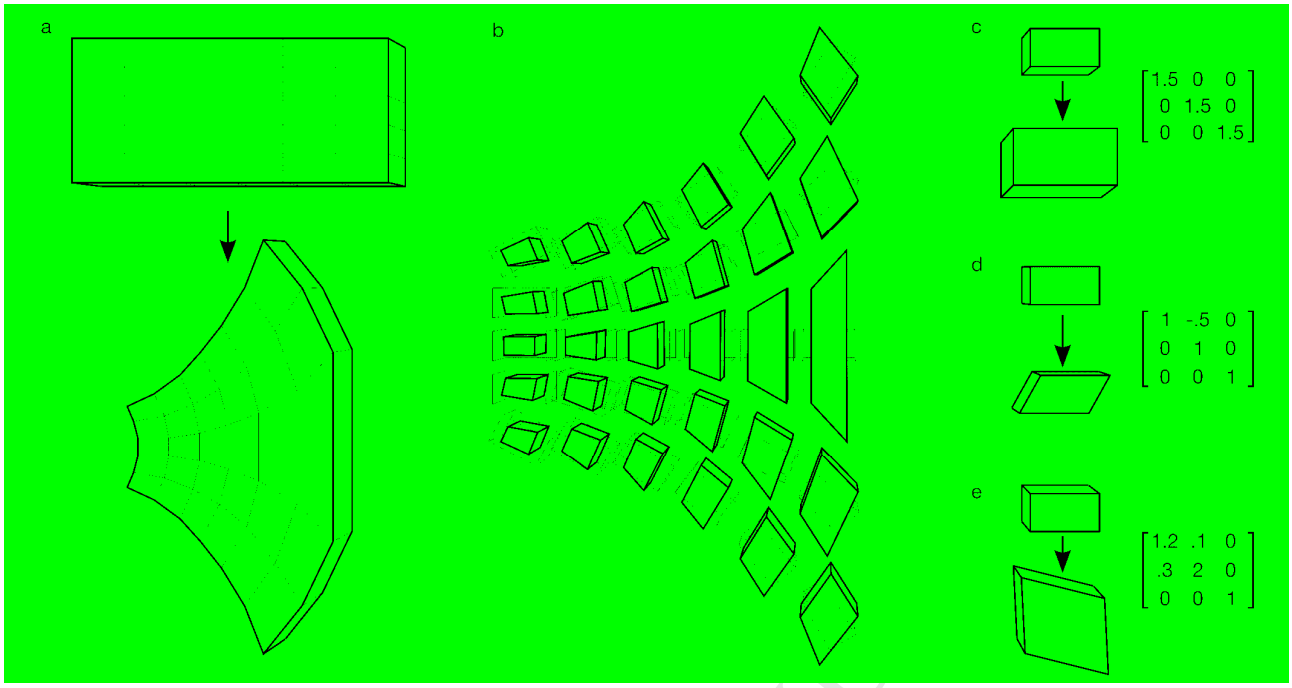

Figure 1. Deformation and elasticity. Tissues are decomposed into a continuous grid of volume elements. The elastic deformation of each volume element is measured relative to an idealised resting configuration. The deformation of each volume element is described by a matrix, the deformation tensor. (a) Deformation of a tissue. The tissue is decomposed into a continuous grid of volume elements. (b) The deformation of each volume element is measured with respect to the shape that it would ideally have at rest. (c) Matrix representation of a change in volume without change in shape. (d) Matrix representation of change in shape without change in volume. (e) Matrix representation of a combined change in shape and volume.

In order to compute the mechanical forces produced by deformation, we need a *constitutive model* that will describe how deformation and force are related. One of the simplest constitutive models is Hook's law, which states that force is proportional to deformation. In the case of a linear spring, for example, elastic force would be directly proportional to elongation. The proportionality constant is known as *Young's modulus* (E) whose unit of measurement is the Pascal (Pa). Brain tissue is very elastic, similar to jelly, with E~1.5 kPa. By contrast, Young's modulus for rubber is E~10-100 kPa, and for metal E>10 GPa.

In two or three dimensions, a new parameter is required to describe how much pulling in one direction will produce stretch in the orthogonal directions: *Poisson's ratio* (ν). Stretching a square of rubber to twice its length will make it shrink to half its width: a Poisson's ratio of 0.5. By contrast, compressing a piece of cork

in one direction produces almost no change in the others: the Poisson's ratio of cork is close to 0. A linear constitutive model will include these 2 constants E and ν. It is sometimes convenient to combine Young's modulus and Poisson's ratio into a bulk modulus K and a shear modulus μ. The bulk modulus represents the amount of mechanical stress produced by changes in volume, whereas the shear modulus represents the amount of stress produced by changes that do not affect the element's volume.

But biological tissues can be described using a linear constitutive model only for very small deformations. Most frequently, a hyperelastic constitutive model is required, where the relationship between deformation and force is non-linear. A widely used hyperelastic constitutive model is the Neo-Hookean model introduced by Rivlin (Rivlin 1948). The model is defined in terms of the elastic energy W that the deformation described by the tensor F will produce in a material of shear modulus μ and bulk modulus K:

$$W = \mu\left(Tr(FF^T)J^{-2/3} - 3\right) + K(J - log(J) - 1). \quad (1)$$

In this equation J measures the change in volume produced by the deformation F (mathematically, $J = det(F)$). If a volume element is shrunk by F then $0 < J < 1$, if it is expanded, then $J > 1$, and if volume is conserved as in the case of incompressible materials, then $J = 1$. The right-hand side of equation has 2 components. The first one, multiplied by μ, increases the energy W as the shear components of F increase (the off-diagonal elements of the matrix F). The second part, multiplied by K, increases W as F produces volume changes. In this second part, if there is no change in volume then $J = 1$, $log(J) = 0$, and there is no change in energy due to bulk volume changes. If F shrinks the volume element so that J is close to 0, then $-log(J)$ will be very large, increasing the total elastic energy W. W will also increase if $J > 1$, but the increase will not be proportional to the deformation because of the subtraction of $log(J)$: in the Neo-Hookean model disproportionately larger forces are required to keep increasing the deformation.

3.2. Growth

Growth introduces a different kind of deformation. In an influential article, Rodriguez et al. (1994) conceptualised the deformation induced by growth as the combination of a term related to elasticity and another related to growth itself:

$$F_T = F \cdot G. \tag{2}$$

Applying the deformation tensor F_T to a volume element is then equivalent to make it grow first, and introduce the deformation due to elasticity next. Importantly, only the elastic part of the deformation, however, changes the elastic energy W.

To illustrate how growth-induced mechanical instabilities works consider a first simple example: an initially flat ring of a hyperelastic material that grows only in circumference but not in thickness. If we cut the ring in two pieces before it grows, as shown in Fig. 2, the pieces could be stuck back without introducing any force. After growing, however, the length of the ring will have increased so that sticking the pieces back will introduce a gradient of residual stress all over it. After sticking the pieces, the ring will undergo compression in the circumferential direction, and depending on its Poisson's ratio, it will also undergo a force in the radial direction. Growth can be thought as a deformation of the resting configuration of the volume elements of an object, independently one from another. In this case, even if residual stress is not enough to produce large out-of-plane deformations, the requirement of continuity of the object will then introduce elastic forces, and a field of residual stress across the object (Fig. 2c).

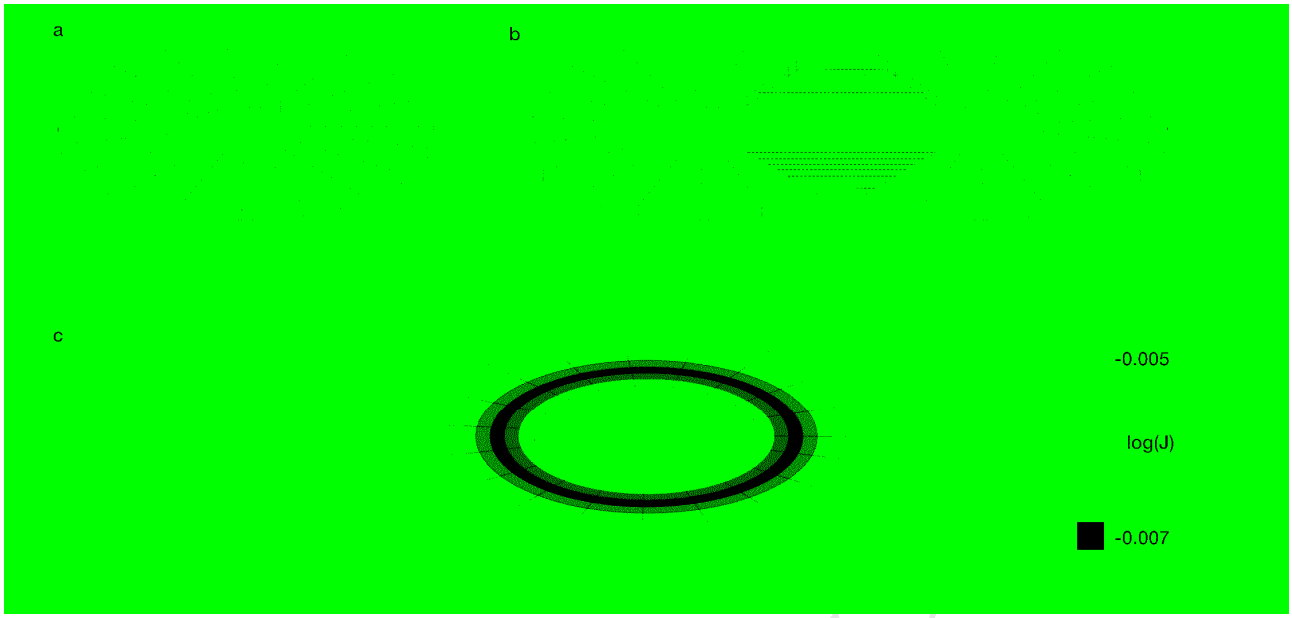

Figure 2. Residual stress induced by growth. Growth can lead to a configuration where stress cannot be eliminated, producing a gradient of residual stress. In this example, a ring originally at rest is made to grow such that its perimeter increases by 50% but without increasing neither the radial length of the ring nor its thickness. At equilibrium, the ring presents with a gradient of residual stress. (a) Original configuration of the ring at rest. (b) After growth, the ring could reach a zero stress configuration only if it were cut into two pieces. The dashed lines show the regions that should have to be stuck together to reconstruct the original ring. (c) The uncut ring after growth exhibits a gradient of residual stress, always bellow the critical value. The ring is contracted overall, but especially within a rim near its inner side. Grey level: logarithm of the deformation (Jacobian of the deformation tensor). Black: log(J)=-0.007, white: log(J)=-0.053, bulk modulus κ=50, shear modulus μ=1. An interactive simulation illustrating the formation of a residual stress gradient due to growth can be accessed at http://neuroanatomy.github.io/growth.

The morphogenetic effect of growth within the framework proposed by Rodriguez et al. (1994) has been addressed in several theoretical studies and confirmed experimentally. The elastic instabilities induced by growth and its geometrical constraints have successfully explained different aspects of the shape of algae, plant leaves and flowers (Dervaux and Ben Amar 2008, Liang and Mahadevan 2009, Lian and Mahadevan 2011), the shape of bacterial pellicles (Trejo et al. 2013), the morphologies of growing tumours (Ciarletta 2013), the vilification of gut and epithelial tissues (Shyer et al. 2013), mucosa wall patterning (Xie et al. 2014) and tubular tissues (Ciarletta et al. 2014) among many others. In particular, the effect of growth and elasticity in systems resembling the cerebral cortex has been observed to introduce different buckling

instabilities which are able to produce folds of a geometry and a scale similar to those of real brains (Tallinen et al. 2014, Tallinen et al. 2016).

3.3. Buckling instabilities and neocortical folding

By the end of neuronal migration the cerebral cortex of many gyrencephalic mammals is still geometrically smooth, cytoarchitectonic regions do not yet appear, cortical layering is immature, and cortico-cortical connectivity is absent (Welker 1990, Rakic 1988, Reillo et al. 2011, Rakic et al. 2009, Hansen et al. 2010). Once cell migration is finished, the neocortex starts a period of fast expansion, due to the increase of intracortical connectivity, dendrites and glia. At this early stage, the neocortex and subcortical structures (including radial glia and the developing white matter) can be viewed as a mechanical system of two elastic substrates, where one of the substrates undergoes much more growth than the other.

The deformation of a flat elastic layer whose surface area is much larger than its thickness (a *thin plate* in mechanics) is often described using Föppl-von Kármán equations. Although there is no general solution for these equations, they can be solved for particular cases or approximated through numerical simulation. When submitted to load, shrinkage or growth, Föppl-von Kármán equations predict different types of buckling instabilities. For small growth, stretching is energetically less costly than bending and the system grows without buckling (this is likely the reason why small brains are lissencephalic). As growth increases, however, there is a critical threshold after which bending becomes less costly than further stretching and the system buckles. Among these buckling instabilities, wrinkles (Bowden et al. 1998, Cerda and Mahadevan 2003, Davidovitch et al. 2011), folds (Pocivavsek et al. 2009) and creases (Tallinen et al. 2014, Tallinen et al. 2016, Hohlfeld and Mahadevan 2011) can produce patterns such as those observed in gyrencephalic brains. Wrinkles develop when a stiff, thin, elastic layer – a skin – grows over a softer one. Wrinkles are sinusoidal undulations and are the first instabilities observed after buckling (see Fig. 3 for an illustration). They can develop into folds, where deformations are more localised, with flat regions coexisting with valleys or depressions of the stiff layer. Creases (also called sulci) are similar to folds, but they do not require a

difference in stiffness between skin and substrate to develop. Whereas the depth of wrinkles and folds is smooth, similar to the first stages of gyrogenesis, the depth of creases is cusped, similar to the shape of adult brain sulci.

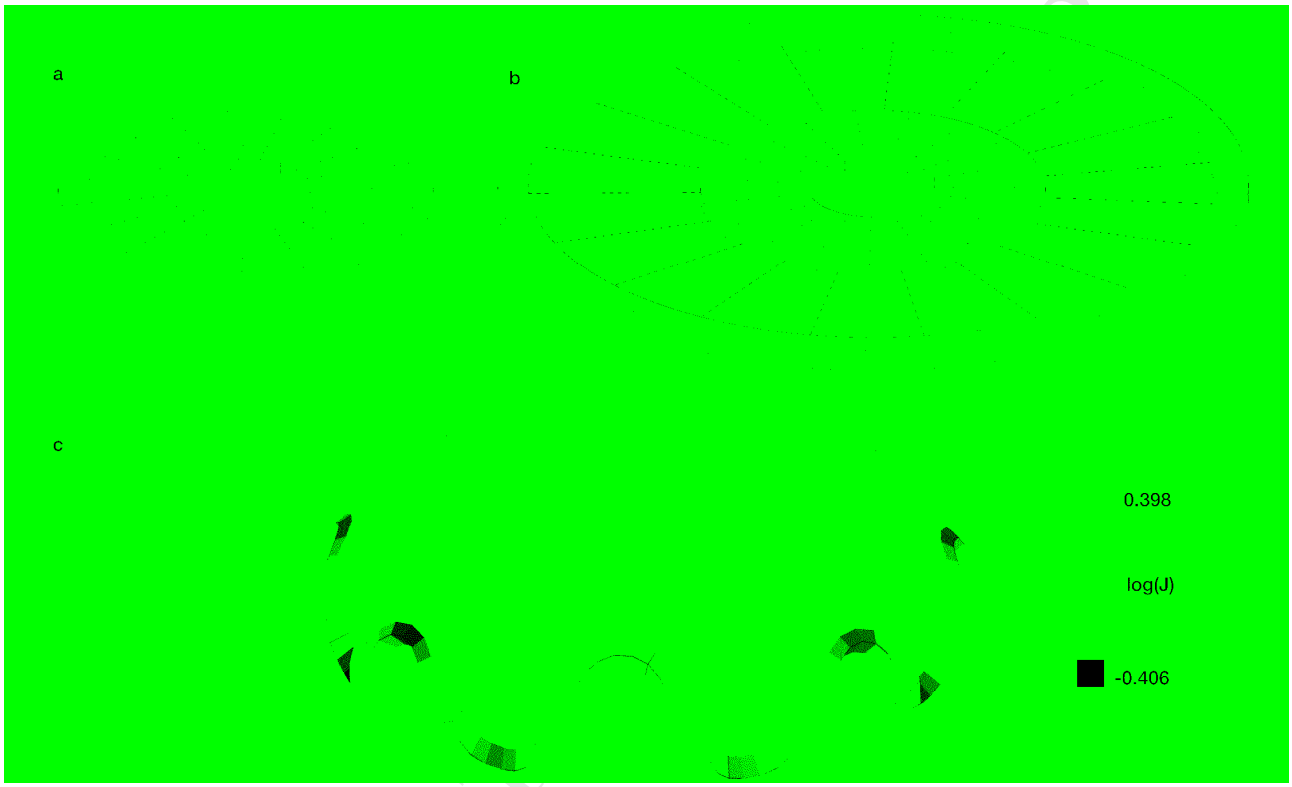

Figure 3. Buckling induced by growth. In this example, the outer rim of the ring grows to twice its original size. This leads to the formation of folds that minimise the elastic energy of the system. At equilibrium, a heterogeneous pattern of stress, appears with residual stress gradients from centre to periphery, in angular bands and through the thickness of the ring. Homogeneous growth leads then to a heterogeneous pattern of residual. (a) Configuration of the ring at rest. (b) After growth, the ring could reach a zero stress configuration only if its outer rim were cut from the inner core. The dashed lines show regions that should have to be stuck together to reconstruct the original ring. (c) The ring after growth. The outer rim is overall contracted, with gradients of residual stress from the inside to the outside of each fold. The inner core is overall dilated, with a deformation that decreases towards the centre of the ring. Grey level: logarithm of the deformation (Jacobian of the deformation tensor). Black: log(J)=-0.406, white: log(J)=-0.398, bulk modulus K=100, shear modulus μ=100. An interactive simulation illustrating buckling induced by growth can be accessed at http://neuroanatomy.github.io/growth.

The first model of brain folding based on the emergence of a buckling instability was that of Richman et al. (1975). This was a model of wrinkling where smooth, sinusoidal, folds developed because of a difference in growth and stiffness between the superior and inferior layers of the neocortex. The model proposed a process for producing folding through exclusively neocortical means (i.e., without requiring a cranial constraint). The model left unanswered, however, the questions of the constancy of species-specific folding patterns and the relationship between folding and neocortical organisation (cytoarchitecture, connectivity, function).

These questions were addressed by the model of Toro and Burnod (2005). In this 2D model, wrinkling was produced by the growth of a neocortical layer over an elastic and plastic substrate representing white matter and radial glia. In addition to elasticity, the tissues were also considered to be plastic: the heterogeneous mechanical stress produced by folding could induce local cortical growth and reabsorption, or the elongation and contraction of the white matter. Toro and Burnod (2005) observed that the formation and orientation of folds (wrinkles) could be modulated by cytoarchitectonic differences if they were already present, but also by the initial (unfolded) geometry of the cortical layer. This last mechanism could provide a purely geometric basis for the development and evolution of species-specific folding patterns (Toro 2012). Indeed, Tallinen et al. (2016) have shown that cortical growth in mechanical models (physical and computational) endowed with the geometry of the smooth foetal human brain, develop folds whose pattern resemble remarkably those observed in real folded human brains.

The models by Richman et al. (1975) and Toro and Burnod (2005) were purely theoretical, but more recent models based on mechanical parameters similar to those of real brains show that the morphology and stress patterns produced by buckling agree with those observed experimentally (Tallinen et al. 2014, 2016, Bayly et al. 2013, Budday et al. 2014a, Budday et al. 2014b, Kroenke and Bayly 2018).

These models suggest that mechanical instabilities induced by homogenous growth are sufficient to produce folds, without requiring specific, local, gyrogenetic processes such as a genetically determined, regionally higher rates of growth in pro-gyral regions (Welker 1990, De Juan Romero et al. 2014, Reillo et al. 2011,

Lefèvre and Mangin 2010, Borrell 2018), specific cortico-cortical connectivity between pro-gyral walls (Van Essen 1997, Hilgetag and Barbas 2005, Hilgetag and Barbas 2006), or a specific attachment of pro-sulcal regions (Smart and McSherry 1986a, Smart and McSherry 1986b, Régis et al. 2005).

Additionally, these models suggest that the pattern of folding could be determined by the initial geometry of the neocortex. The orientation of folds in many species has been observed to correspond with the lines of principal curvature of the surface (Todd 1982), which in many cases correspond with directions either concentric or orthogonal to the system formed by the corpus callosum and the Sylvian fissure (Régis et al. 2005, Toro and Burnod 2003). In the same way that the geometry of a dome rigidifies its structure, the initial geometry of the neocortex makes some regions easier to fold than others, facilitating the development of folds in specific positions, with preferential orientations.

Previous theories explained the relationship between neocortical organisation and brain folding by encoding both of them in the genome. But if folding and folding patterns result from mechanical growth-induced buckling instabilities, we have to consider the possibility that, at least in part, **brain folding may have a causal effect on neocortical organisation**.

4. Effect of mechanical forces on neocortical development

The macroscopic, heterogeneous, residual stress induced by brain folding could regionally modulate cell proliferation, cell fate and cell shape, influence axonal guidance and even synaptic activity, thus affecting the organisation of the neocortex (Franze 2013). Localised stress is known to modulate tissue volume, with tension facilitating tissue growth and compression promoting reabsorption (Rodriguez et al. 1994, Fung 1993). Compressing a tumour spheroid formed from carcinoma cells decreases cell proliferation in the regions of high mechanical stress, and cells could even undergo apoptosis if stress is sufficiently high (Cheng et al. 2009, Montel et al. 2012). In neural stem cells culture, the rate of cell division reaches a peak at 1-4 kPa, but decreases in stiffer or softer substrates. In gels softer than ~10 Pa, the spreading, self-renewal

and differentiation of neural stem cells is almost completely inhibited (Saha et al. 2008). Mechanical stress can affect progenitor cells directly, but also indirectly, by affecting the structure of the extra-cellular matrix. Mechanical stress could result in differential local concentrations of extra-cellular matrix components, which in turn could lead to differences in cell signalling and adhesion (Lutolf et al. 2009).

In addition to cell proliferation, cell fate and differentiation can be regulated by mechanical forces. Engler et al. (2006) showed that naive mesenchymal stem cells cultured on a substrate mimicking the elasticity of brain, muscle or bone, differentiated respectively into branched cells similar to primary neurones, spindle-shaped cells similar to myoblasts or polygonal cells similar to osteoblasts. Interestingly, whereas reprogramming these lineages by the addition of soluble induction factors was possible only during the initial week in culture, changes in cell fate through manipulation of matrix elasticity were possible even after several weeks. In neural stem cells culture, Saha et al. (2008) showed that soft gels (~100-500 Pa) produced mostly neurones, whereas increasingly harder gels (up to 1-10 kPa) produced progressively more glial cells. In vivo, neocortical neurones grow along glial cells, which may be due in part to the fact that glia are significantly softer than their neighbouring neurones (Lu et al. 2006).

The shape of differentiated cells can also respond to the mechanical properties of their environment. Neurones cultured on soft substrates (50-300 Pa) develop up to 3 times more branching than those cultured on stiffer gels (300-550 Pa, Flanagan et al. 2002). Neurones also seem to require a certain amount of mechanical tension to mature. In vitro, the axonal branches that attach strongly to the substrate are conserved and stabilised, whereas the remaining branches are retracted or eliminated (Anava et al. 2009). Pulling axons at different speeds not only increases their length accordingly, but also their calibre (Bray 1984). That is, nerve cell respond to tension applied along their axons by building more axon, increasing the length and number of its microtubules, neurofilaments and membrane components.

Buckling models of neocortical folding predict gradients of mechanical stress between gyri and sulci spanning the thickness of the cortex and close white matter (such as those in Fig. 3c). These gradients could

participate to the establishment of the differences in cell shape and layering observed in vertebrates (Bok, 1959, Welker 1990). Cell migration and axonal pathfinding respond to mechanical clues (Saez et al. 2007), and could be guided by the mechanical stiffness gradients produced by growth-driven buckling instabilities. This process is called durotaxis. In cases with stiffness gradient or transition in rigidity in the substrate, cells migrate preferentially along stiffer directions (Bollman et al. 2015, Lo et al. 2000). Finally, tension in neurones has been observed to produce an active accumulation of the synaptic vesicles involved in neurotransmitter releasing to postsynaptic cells Ahmed et al. 2012), which leads to a possible modulation of the synaptic activity by the mechanical properties of the surrounding tissue.

5. Conclusion

5.1. A hypothesis about neocortical development and evolution

From the previous considerations we propose a new model for the development and evolution of the neocortex, which result from the integration of 3 main processes: the classic genetic and activity-driven processes, plus mechanical morphogenesis. Brain development is the plastic deployment of a complex organisation, involving a massive production of cells, their migration, differentiation, specialisation and interconnection. Under the forces of biological growth, brain tissue undergoes major changes in geometry correlated with constant changes in the distribution of mechanical stress. This is the context within which patterning centres diffuse morphogens and signalling molecules. Through their effect on transcription factors and cell behaviour (proliferation, differentiation...), these molecules can affect local growth and the mechanical properties of the developing tissue, changing the global distribution of stress. In return, mechanical gradients can affect gene expression and cell behaviour back, in a continuous interplay between biological and physical forces. Neocortical organisation is laid out in this dynamic substrate, under the influence of gradients of morphogens, signalling molecules and mechanical stress.

New cortical areas could develop because of an increase in the complexity of the intrinsic response to transcription factors, as suggested by current models (O'Leary et al. 2013). This is conceptually the same as the "French flag" model of patterning proposed by Wolpert (1969). In this case, morphogenic gradients established by the diffusion of molecular signals from patterning centres could provide unique coordinates for each point in the cortical mantle. This could regulate the local expression of different transcription factors. In response to different combinations of transcription factor levels, cortical tissue could develop a restricted number of cell identities: the basis of the future cortical areas (this is how a continuous gradient plus thresholds could be used to generate the 3 sections of the pattern of the French flag). Through these morphogenic gradients, intrinsic genetic processes could also regulate the expression of axonal guidance molecules, and control the formation of specific connectivity patterns.

In addition to this genetic mechanism, we propose that new areas could also develop because of mechanical morphogenesis. In this case, homogeneous cortical growth could induce buckling instabilities that could produce cortical folding. Each fold would be characterised by a geometric deformation – a gyrus – and a mechanical stress pattern, with tension in gyral crowns, compression in sulcal fundi, and a gradual change in tension across cortical layers and close white matter. For comparable thickness, a large cerebral cortex should develop more folds, i.e., more of these discontinuous mechanical domains. The geometric and mechanical changes within each fold would introduce localised changes in cell identity and influence the formation of brain connectivity. Depending on their spatial situation and time of development, neurones within each fold would establish different sets of connections with the rest of the brain, in particular with sensory and motor thalamic nuclei.

The causes of the formation of a new area could be then genetic – a change in the complexity of the response to gradients of transcription factors – or mechanical – a change in the number of mechanical domains. In both cases, whether new cortical areas appear because of a change in the genetic program or because of the effect of mechanical morphogenesis, the organisation produced by intrinsic genetic or mechanical factors could be plastically modified by neuronal activity, which in turn could influence gene expression or change

the mechanical properties of the tissue.

5.1. Consequences of mechanical morphogenesis

Across species, the number of cortical areas and the complexity of the connectivity patterns increase with brain size (Sereno and Allman 1991, Krubitzer 2007, Bourgeois 1997, Krubitzer and Seelke 2012, Van den Heuvel et al 2016). In a small brain, the number of cortical areas should be determined by the discrete number of possible responses of the nervous tissue to different levels of transcription factors, by the response to monotonous gradients of mechanical stress (that should be present even in the absence of buckling and brain folding), or by the plastic response to activity driven by thalamic afferents. An evolutionary change in brain size sufficient to induce cortical folding should lead to the creation of new cortical areas and connectivity patterns even if the number of responses to transcription factor levels or to neuronal activity stays the same. Because large brains are progressively more folded than small ones (Prothero and Sundsten 1984), and develop an exponentially larger amount of connections (Zhang and Senjowski 2000, Horvát et al. 2016), mechanical morphogenesis could explain in part the augmentation in the number of cortical areas (Changizi 2001, Karbowski, 2003).

The effect of mechanical morphogenesis on neocortical organisation could also be studied within a single species, where genetic differences are much smaller, and restricted mostly to genetic polymorphism. Despite a high genetic similarity (Kaessmann et al. 2001), humans exhibit a remarkable diversity in total brain volume, mostly due to differences in total cortical surface area. Large human brains have disproportionately more cortical surface area and are significantly more folded than small ones (Germanaud et al. 2012, Toro et al. 2008). These differences are mainly due to tertiary folds (shallow, late developing and variable) and in some cases to secondary folds, but the deep, early developing folds, are very stable. Nevertheless, we should observe a larger number of neocortical areas in large, more folded brains, as well as differences in the connectivity patterns. Some reports suggest indeed that the presence of supplementary folds among humans, such as the paracingulate fold of the cingulate cortex, can be associated with differences in behaviour

(Fornito et al. 2004, Fornito et al. 2006) and cytoarchitecture (Vogt et al. 1995). In addition to natural inter-subject diversity, pathologies such as lissencephaly and microgyria should provide an opportunity to study the effect of mechanical morphogenesis on neocortical organisation. We should observe a change in the number of cortical areas and connectivity patterns associated with the changes in cortical folding, especially if the pattern of early developing primary folds is affected.

5.2. Experimental perturbations of mechanical morphogenesis

If our hypothesis were correct, mechanical perturbations of the developing cortex should be able to modify the area map, add new areas, or produce new connectivities. As with genetic perturbations (gene knockouts, knockins, etc.) or perturbations of neuronal activity (sensory deprivation, rewiring, etc.), perturbations of mechanical morphogenesis will require to develop appropriate controls to ensure that the effects observed are due to the intended perturbation and not to experimental artefacts.

The ferret has been often used to study brain folding (Smart and McSherry 1986a, Smart and McSherry 1986b, Barnette et al 2009, Reillo et al 2011, Knutsen et al 2013, De Juan Romero et al 2015). At birth, its neocortex is completely smooth, but after a first stage of growth without folding, somewhere between P4 and P8 it starts to fold. After one month, the ferret has a richly folded neocortex with deep sulci organised in a characteristic folding pattern. If brain folding were produced because of a buckling instability, the ferret neocortex should be at a maximally unstable state right before folding, probably by P4. A small mechanical perturbation introduced at this time should be enough to trigger the formation of an artificial fold. Mechanically, the most probable orientation of a fold should be along one of the 2 principal curvature directions of the surface before folding (Tallinen et al. 2014, 2016, Todd 1982, Toro and Burnod 2003). If a natural fold is formed along one principal curvature direction, it should be possible in theory to force the formation of an artificial fold along the other, orthogonal, principal curvature direction.

In wild-type ferrets, an increased rate of neurogenesis (measured by a higher density of *Tbr2+* progenitors)

has been observed in regions that will later underlie a gyrus (De Juan Romero et al. 2015, Reillo et al. 2011, reviewed in Borrell 2018). If neurogenesis is influenced by the mechanical stress that builds a fold, we should observe a new, ectopic increase of neurogenesis under the future position of artificial gyri, or a decrease under the future position of artificial sulci. As a result, the orientation of the cortical areas in the mechanically perturbed cortex should be different than in the wild-type ferret, and correspond with the orientation of the new folding pattern. Experimentally, a mechanical perturbation could be introduced non-invasively using, for example, acoustic radiation force (Fernández-Sánchez et al 2015), the folding pattern could be reconstructed from magnetic resonance images, and cytoarchitecture could be estimated using quantitative methods, such as those introduced by Schleicher et al (1999) and Spitzer et al (2017). Additionally, if connectivity is influenced by the geometry of folding, we should observe an altered pattern of cortico-cortical and cortico-subcortical connectivity. Neuronal activity should further influence the organisation of the new cortical areas, depending on the sensory modalities of the thalamic afferents they receive. Experimentally, changes in connectivity patterns could be studied using diffusion weighted magnetic resonance imaging and whole brain tractography methods such as those described by Jeurissen et al. (2014) or Smith et al. (2015); and functional ultrasound appears as a promising method for testing functional connectivity in ferrets (Demené et al. 2016). The behaviour of the animals should also be changed as a result of the new brain organisation. In ferrets, auditory fear conditioning is faster than visual fear conditioning, likely because of the more direct connection from the auditory thalamic nuclei to the amygdala than from the visual thalamic nuclei to the amygdala (Newton et al. 2004). In rewired ferrets, however, visual fear conditioning is mediated by auditory nuclei and is then faster than in control animals. The same phenomenon should be observed in ferrets with artificial folds: the different patterns of connections between the new cortical areas should produce similar behavioural alterations.

In conclusion, we have outlined a hypothesis on the role of mechanical morphogenetic processes in the definition of neocortical organisation. If this hypothesis is correct, processes such as brain folding should have a causal effect on brain development and play, together with genetic and activity-dependent processes, an important role on the evolution of the neocortex.

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RT, OF and MT wrote the manuscript. RT wrote the demonstration computer code.

Additional information

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