Cortical folding is a highly regulated process involving amplification of neuroprogenitor cells, increased neurogenesis and migration of neurons along the tangential axis. A new paper in *Development* investigates the signalling processes behind cortical folding in mice, which have a smooth brain surface (lissencephaly) that evolved from the loss of folding present in gyrencephalic (folded cortex) mammals. To hear more about the story behind the paper, we caught up with the first author Matt Matrongolo and his supervisor Max Tischfield, Assistant Professor at Rutgers, The State University of New Jersey.

**Max, can you give us your scientific biography and the questions your lab is trying to answer?**

**MT:** I am a developmental biologist and neuroscientist by training, and we use human and mouse genetics to study craniofacial, neurovascular and neurodevelopmental disorders in mice. I have pursued many different types of questions in different model organisms over time, so there is quite a bit of breadth to the scientific questions we pursue. At the moment, major questions we are addressing pertain to how changes in the levels of intracranial pressure affect the development of functions of meningele lymphpatic vessels and brain-CSF (cerebrospinal fluid) perfusion, which is essential for brain waste clearance. We approach this using mouse models for craniosynostosis, a genetic condition that affects skull growth and intracranial pressure (and how this particular study happened to evolve). Another major focus of my lab is understanding the neuropathogenesis of Tourette syndrome using mice that have been engineered to express mutations in high-confidence Tourette genes. We are particularly interested in how these mutations affect both motor and cognitive functions in mice, especially how changes to striatal dopamine may affect habit formation.

**Matt, how did you come to work in Max’s lab and what drives your research today?**

**MM:** I began working in Max’s lab as an undergraduate student with interests in developmental biology, genetics and neuroscience, which Max’s previous work and current projects combined perfectly. Max had just started his lab at Rutgers and I had the opportunity to be one of his first students. Throughout my research in the Tischfield Lab my work has revolved around investigating the interactions between the brain and surrounding skull and dura, and the implications for overall brain health and development. As such, this topic has remained a driving force in my research, and I intend to continue studying the dynamic interactions between the brain, meninges, skull and cerebrospinal fluid in the future.

**What was known about the mechanisms maintaining secondary lissencephaly in mice before your work?**

**MT:** Most studies in mice have focused on ‘gain-of-function’ mechanisms, such as inserting genes present in humans and observing the effects on neurogenesis and folding. These models have been quite informative for our understanding of how cortical folding evolved. By contrast, there are fewer studies that focus on how mice maintain secondary lissencephaly. Work from Kathy Millen’s lab, for example, has shown that loss of BMP signalling from dorsal cranial mesenchyme alters WNT signalling and neurogenesis, leading to expansion of basal radial glia and gyri formation – which is a distinct process from what we show in our model. Changes to neurogenesis and expansion of basal radial glia are also noted in mice with shRNA knockdown of Trnp1, which also develop gyri. Other notable work involves the cellular adhesion proteins FLRT1 and FLRT3; knocking down expression of these proteins in newborn neurons alters radial migration, leading to regions with different cortical densities along the tangential axis and sulcus formation. By comparison, the types of folding we see in conditional Twist1 mice are more similar to FLRT1/FLRT3 mutants, as both models show folding and sulcus formation without localized expansion of basal radial glia in an expanded subventricular zone.

**Can you give us the key results of the paper in a paragraph?**

**MT:** We first show that Twist1 regulates cell proliferation in the meninges. Following conditional inactivation of Twist1, cell proliferation is reduced and the dura and arachnoid membranes that encase the brain become hypoplastic. Arachnoid fibroblasts are responsible for producing retinoic acid, an important signalling molecule that has been shown to influence both neurogenesis and radial migration of neurons. As the brain grows, our data suggest that the hypoplastic arachnoid membrane is stretched beyond its limits, and fewer arachnoid fibroblasts are present to produce retinoic acid (RA), leading to ‘pockets’ where expression is reduced. In this manner, our findings show that loss of balanced RA signalling causes forebrain lengthening plus regionalized changes to neurogenesis, such that we see greater numbers of intermediate progenitor cells at and near the cortical midline. This causes the midline to expand faster versus the dorsolateral cortex (which is much thinner by comparison and also in respect to wild-type mice). Maternal supplementation with all-trans RA helps restore and balance the levels of neurogenesis along the tangential axis. We no longer see folding, and radial growth at the cortical midline versus the dorsolateral cortex is more similar to wild-type mice.
The folding patterns we observe can be quite complex. As MT: transgenic models that develop folding? How do your Twist1 conditional mice compare with other instead more likely to be the causative factor for folding. Altering the levels of neurogenesis and/or inducing regionalized differences in neuronal density (whether it be by altering cell adhesion/migration or proliferation) is sufficient to cause folding in mice and may influence the types of folding patterns found in gyrencephalic mammals.

During the revision process, some very helpful insights from reviewers spurred us to revisit our findings and expand the study to additional timepoints, which revealed that regionalized differences in the levels of neurogenesis were instead more likely to be the causative factor for folding. Altering the levels of neurogenesis across the tangential axis causes some parts of the brain to expand faster than others, which likely causes cortical buckling and folding via mechanical instability. I should add that one of the many reasons that I enjoy publishing with Development is the excellent review process.

E18.5 cortical folding. Embryonic day 18.5 mouse brains corresponding to a wild-type mouse on the left and a conditional Twist1 knockout mouse with cortical folding on the right. The cortex is stained for DAPI (blue) plus layer markers Satb2 (green), Ctip2 (white) and Tbr1 (red).

Were you surprised that it is the balance of RA signalling that is required to maintain lissencephaly in mice? Any ideas about the mechanisms by which RA signalling affects cortical folding?

MT: As the study evolved, we began to suspect that loss of RA signalling was the major factor causing cortical folding, especially because an enzyme that helps produce RA (Raldh2) was decreased in the meninges overlying cortical folds. Our suspicions were confirmed by the RA rescue, but we were not exactly sure how loss of RA was influencing folding. In fact, in our preprint and first submission, our initial interpretations were inaccurate as we thought changes to neuronal migration affecting the tangential distribution of neurons was the primary mechanism. During the revision process, some very helpful insights from reviewers spurred us to revisit our findings and expand the study to additional time points, which revealed that regionalized differences in the levels of neurogenesis were instead more likely to be the causative factor for folding. Altering the levels of neurogenesis across the tangential axis causes some parts of the brain to expand faster than others, which likely causes cortical buckling and folding via mechanical instability. I should add that one of the many reasons that I enjoy publishing with Development is the excellent review process.

How about the flipside: any moments of frustration or despair?

MM: By far the biggest moment of frustration was learning that the EdU I had been injecting pregnant mothers with was not working. After spending several weeks monitoring mating cages, injecting pregnant mothers with EdU, collecting samples, paraffin embedding, sectioning and finally labelling, I learned that the new bottle of EdU I had been using was defective. I had lost several weeks of work, but we were able to ship a new bottle overnight and complete the required experiments in time.

Matt, what’s next for you after the paper?

MM: After 5 years in the Tischfield Lab, I will be pursuing a PhD in Neuroscience at Stanford University this fall.

Where will this story take your lab next, Max?

MT: Our results raise more questions about the role of RA for brain development. Before our findings, seminal work had shown that RA was required for neurogenesis. This work was done in Foxc1 loss-of-function mouse embryos with hypoplastic meninges, similar to conditional Twist1 mice. However, later studies modelling conditional knockout of RA-producing enzymes in the meninges or expressing dominant-negative RA receptors in neurons did not find effects on neurogenesis, but rather insults to radial migration that could alter the specification of cortical neurons. In these models, the meninges were intact versus Twist1 and Foxc1 mutant mice, suggesting that unidentified factor(s) in the meninges may be influencing how RA impacts brain development. Interestingly, we have reasons to believe that both processes may be affected in conditional Twist1 mice, but perhaps to different extents across the anterior-posterior axis of the developing brain.

Finally, let’s move outside the lab: what do you like to do in your spare time?

MM: Outside of the lab, I am a huge foodie and am always looking to try the newest taqueria, ramen or coffee shop in town. I am also a bit of a coffee nerd and spend way too much time ‘dialling-in’ my newest bag of espresso beans. Beyond that, I enjoy hiking, beach clean-ups and watching European soccer.
MT: I love listening to music, exploring the outdoors, exercising and spending time with family and friends. I used to travel a lot and visit many different cities, and I hope to do that more because I love seeing new places and learning about different cultures.

Reference