Understanding our brain

Using mouse models, Dr Michèle Studer aims to elucidate the mechanisms of neurogenesis in the developing cerebral cortex. She describes her captivation with this intricate region of the brain and highlights the difficulties in pinpointing the function of essential genes in the organ during its construction.

*In utero* electroporation of EGFP (green) at embryological age 14.5 and taken at post-natal age 8 showing the soma and dendrites of upper layer neurons of the cortex. Red is a marker (Satb2) for callosal projection neurons. Blue stains nuclei (DAPI).

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**How did your interest in brain development arise? What motivates your work in this complex area of research?**

The cerebral cortex is a highly elaborate organ, but it is also fascinating, since it is the seat of high brain functions, such as consciousness, abstract thinking, language and perceptual awareness: everything that makes us human. To obtain such complexity, the cortex needs to be highly coordinated during its development; stem cells must first be committed to becoming neurons, and neurons need to know where to go and project. All these features, established during development, dictate neuronal function.

These early events have always fascinated me; it’s like watching a baby discovering the world and making its first steps in life. It’s not surprising that any mistake arising during these early events can lead to cognitive deficiencies. As a consequence, I strongly believe that understanding how the brain develops will contribute to helping children who are born with neurodevelopmental cognitive disorders, such as intellectual disabilities, epilepsy and autism.

**Can you explain the term ‘arealisation’?**

Arealisation, or areal patterning, describes the mechanisms that control the tangential subdivision of the neocortex (the evolutionarily most recent part of the cerebral cortex) into distinct functional areas. It is a crucial developmental event because neocortical areas form the basis for sensory perception and control voluntary movements. Many key processes must be established during arealisation, such as cell identity, neuronal migration (so that neurons reach their final position) and layer formation. The proper regulation of transcription factors, expressed in gradients, is crucial during the early process of areal patterning. In addition, extrinsic stimuli, originating from the periphery and relayed then to the cortex by the thalamus in an area-specific manner, are fundamental for the correct functioning of the entire cerebral cortex.

**Which key transcription factors are involved in this process?**

Several transcription factors are involved, but the most studied are Emx2, Pax6, Sp8 and COUP-TFI, present in distinct and complementary expression gradients along the different cortical axes. At least two independent pathways are involved in early arealisation: one controlled by Emx2 and Pax6, and the other regulated by Sp8 and COUP-TFI. In both cases, members have complementary expression patterns and co-repress each other such that precise levels of expression can be produced in single cells.

**How have your studies on COUP-TFI altered understanding of the role it plays in brain development?**

Initial evidence for the role of COUP-TFI in arealisation came from studies of constitutive null mice, but since analyses were limited (the mice die within a few days after birth) and the majority of projections from the thalamus failed to reach the cortex, its role in areal patterning remained debated. Thanks to the Cre-lox...
Subdivision of the neocortex into tangentially distinct domains – each with a different function, cellular composition, input and output, and gene expression pattern – means its structure is exceptionally complicated.

An insight into the cerebral cortex

A group at the Institute of Biology Valrose of the University of Nice Sophia Antipolis, France, is gradually revealing the mechanisms at play in the organisation of the developing cerebral cortex, insights which could explain some behavioural impairments in children.

The Cerebral Cortex is the brain’s outer layer of neural tissue. In the most part, it is composed of the neocortex – the largest and most complex structure in the brain – accounting for 76 per cent of the organ’s volume. It commands sensory perception, the generation of motor commands, spatial reasoning, conscious thought and language.

A large and folded neocortex is what distinguishes mammals from all other animals and, essentially, what makes us human. The accurate development of this structure during embryogenesis is critical for human health.

As the neocortex forms, a vast array of different neuronal cell types are produced from a collection of multipotent stem and progenitor cells. Their differentiation follows a precise spatial and temporal pattern before assembling into specific maps and circuits. Scientific understanding of the details of this process, unfortunately, remains incomplete.

Dr Michèle Studer aims to close these knowledge gaps. To this end, she leads a group of scientists at the Institute of Biology Valrose, an international research centre that is part of the University of Nice Sophia Antipolis. Together with her team, Studer is working to understand – at the molecular and cellular levels – how different neuronal cell types in the cortex are temporally and spatially regulated and how, ultimately, they reach their final targets.

Areal and laminar identity are specified not only in cortical progenitor cells, but also in early differentiating neurons, as recently discovered by Studer, which eventually undergo final maturation into more specialised neurons. Once areal identity has been established during embryogenesis, very little is known about how it is maintained after birth. How are particular neuronal subpopulations allocated to certain functional areas?

What are the mechanisms that shape and maintain the cellular composition, circuitry and layering of the neocortical areas?

Studer aims to answer these questions by unravelling the mechanisms underlying cell-type specific differentiation during the formation of distinct areas and layers in the developing neocortex, before and after birth, when it is still plastic. To do this, her team combines molecular and cellular approaches, including in vitro and biochemical assays, neural stem cell cultures, developmental neuroanatomy, mouse genetics and behavioural studies. She hopes this interdisciplinary approach will elucidate how genetic factors interact with adaptive processes to promote mature cortical circuits.

Why is it so challenging to pin down the role of COUP-TFI in brain patterning?

COUP-TFI is expressed in several cell types in the brain from embryogenesis to adulthood, and it is highly conserved during evolution. This reflects its importance during development, but also makes it harder to pin down its different functions. In null constitutive mutants, COUP-TFI function is abolished from its onset of expression, which leads to several complex defects. In fact, newborns die at birth. This is why we have to use a combination of different genetically engineered mouse models in which the gene is manipulated in distinct cells and at precise times.

What impact do you foresee your research having on the field of brain development?

Studying a mouse model in which the neocortical areas are so perturbed helps to link areal patterning to other key events during corticogenesis, such as cell-type specification and circuit formation. People tend to focus on the somatosensory mouse neocortical area because this is most represented in the mouse cortex. However, many features change between areas. In humans, this is even more pronounced, and it is not uncommon to find area-specific abnormalities in patients with neurodevelopmental impairments.

Technology, we were able to delete COUP-TFI only in the cortex or the thalamus, and unravel its specific functions independently in the two tissues.

We found that COUP-TFI is crucial in imposing a sensory caudal identity in the developing neocortex and in controlling thalamic inputs into distinct cortical areas. Among the several mouse mutants dealing with defects in arealisation, the COUP-TFI mouse model is the more severe one. This allows us to go further and probe how areal organisation is established and maintained in the brain.

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A NEUROLOGICAL COUP

There are many different patterning genes expressed as gradients in progenitor and neuronal cells, required to establish a ‘map’ of the neocortex. ‘Gradients are important for the differentiation of specific cell types in a distinct spatial and temporal order,’ Studer explains. ‘Multiple gradients overlap and give rise to several cell types and thus increase neuronal diversity.’

Of these, the nuclear receptor and transcription factor COUP-TFI stands out, as its expression is also maintained in post-mitotic cells that are no longer dividing. COUP-TFI is clearly important, as changes to its expression result in the impairment of several processes, including neuronal migration, axonal growth and the proper positioning of somatosensory areas. Indeed, results obtained by Studer show that the absence of COUP-TFI leads to disturbed areal size, neuronal specification and behavioural impairments, such as voluntary skilled movements.

Unfortunately, it is not really known why. Tackling this deficiency, Studer’s team started a series of studies, culminating in some astounding discoveries regarding COUP-TFI and other post-mitotically expressed genes in controlling the areal and laminar architecture of the cerebral cortex.

MOUSE MANIPULATION

The researchers developed two novel mouse lines. In the first, COUP-TFI is specifically deleted in post-mitotic cortical neurons. In the second, it is activated only in these neurons. Using these models, the group found that the post-mitotic activation of COUP-TFI leads to a change in cellular identity (re-specification) of sensory and motor neurons, which also occurs following the deletion of COUP-TFI in all cortical cells.

The novelty of the finding was that COUP-TFI does not regulate sensory area patterning exclusively in progenitor cells, as was previously thought, but does so in early neurons – as soon as they leave the cell cycle. ‘This revealed an unexpected plasticity in arealisation, which may account for the complexity of the mammalian neocortex,’ Studer comments. The researchers, published recently in Nature Communications, bring to light the previously hidden importance of post-mitotic genes in both areal and laminar specification.

As well as focusing on COUP-TFI, Studer has taken an interest in other mechanisms underlying neuronal diversity, particularly within layer V cortical projection neurons – the major output population of the cerebral cortex. Investigating this, Studer found a higher-than-expected level of subtype diversity. To this regard, her team is studying a novel epigenetic mechanism that enables the simultaneous expression of cell type-determining genes, previously thought to regulate only individual classes of projection neurons. This revelation will accelerate our understanding of how neuronal cell-type diversity is generated between distinct functional areas during cortical development.

LOOKING TO THE CLINIC

Studer has identified a regulatory gene that plays a major role in controlling areal and laminar identity – in a temporally defined manner – in the developing cerebral cortex. Her team has demonstrated that COUP-TFI is important in multiple steps of corticogenesis: dividing the neocortex into motor and sensory areas, and controlling the generation and targeting of subcortical projection neurons, such as corticospinal motor neurons, which degenerate in patients affected with amyotrophic lateral sclerosis, a major motor neuron disease.

Despite this, Studer still has many unanswered questions – perhaps to be expected when a subject matter is complex. ‘We would like to understand how neuronal electrical activity shapes the developing cortical circuits, and how genetic programmes and neuronal activity interact to this end,’ she comments. This is uncharted territory, although many have attempted to identify the transcription factors regulating the differentiation of specific cortical subclasses, little is known about how intrinsic genetic cortical regulation is linked to spontaneous activity (present in the brain before birth) or experience-driven activity (which arises after birth). This could ultimately lead her to identify a key mechanism responsible for the area-specific architecture of the cortex.

Through COUP-TFI, Studer is contributing to clarify the vast complexity of the neocortex. Importantly, beyond the field of neuroscience, this work could also have a major clinical impact. ‘We envisage that our studies will help in understanding the aetiology of neurodevelopmental disorders,’ Studer enthuses. Indeed, altered cellular organisation and neuronal activity in the cortex are hallmarks of many cognitive disorders. As understanding of human genetics has advanced, we now know that there are links between the networks that control activity-dependent transcription and neurological disorders, including autism. The potential for Studer’s work to explain this, at the molecular level, could lead to new therapeutic targets and biomarkers, advancing diagnosis and treatment.