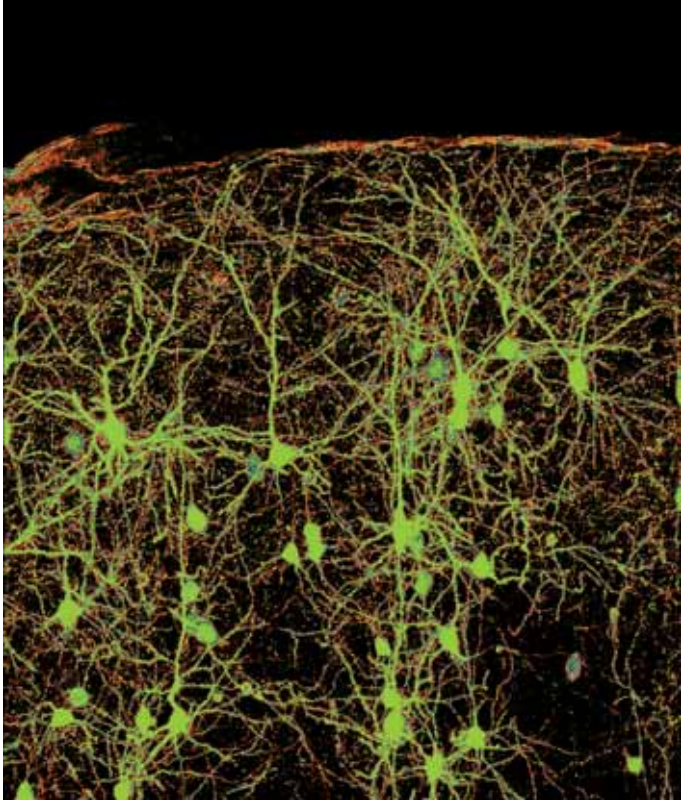
Cortical denrite development in Madrid

Cux: The Crux of Cognition



The human cortex confers man with the exceptional quality of cognizance. While research staggered for years in identifying the 'cell-intrinsic' molecular basis of cognition, Marta Nieto and colleagues have been pioneers in illustrating a novel mechanism in cortical development. And that adds another piece to the puzzle...

One of the most remarkable aspects of human behaviour is the autonomous learning of basic skills in infanthood. Even within the first few months of birth, the newborn begins to entertain us with his antics. He learns to recognise faces and voices, turns his head in the direction of sound, frowns and laughs at gimmicks, babbles and rolls, all this without a teacher! Behind the chiming cries of the newborn is a clamour of developmental events in the brain. This 'perinatal' period that lasts until to two three years of age, is a very crucial time in brain development when a large repertoire of proteins dictates the end structure of the organ and enables the child to acquire intellectual capability.

The cortex: proteins translate into behaviour

Cognition or 'reasoning' is characteristic of human behaviour and is controlled by distinct regions of the cerebral cortex. Cell proliferation, differentiation and migration of neurons to their final destinations, formation of myelin sheets around axons, dendritic development and formation of dendritic spines viz. the regions of synapse, are hallmarks of cortical development. These key events contribute to the formation of the six-layered adult cortex.

Early in the 19th century, drawings by the famous Spanish histologist and physiologist Santiago Ramón y Cajal* illustrated the elaborate dendritic morphology of the cortical upper layer neurons, which enable the latter to integrate numerous intra-cortical inputs. Much later, it was reported that these neurons are the last to appear during development and evolution, and that they have a more profound architecture in higher primates. These observations steered research to resolve the molecular pathways that control human intellect, now that there were two clues to getting closer: i) superficial neurons of the cortex and ii) their striking morphology viz. higher orders of dendritic arborisation.

A rush of papers that followed examined the effect of several extrinsic or extracellular factors that control dendritic development in the mammalian brain. Neurotrophins, semaphorins, slits and cadherins became mighty names as extracellular molecules that provide growing dendrites appropriate, attractive and repulsive cues in the formation of dendritic trees. However, not until recently, did scientists unveil the cell-autonomous or 'intrinsic' commanders that work independent of the environment driving dendritic development.

Unravelling the molecular basis of cognition: **Marta Nieto** (ctr.) ***et al.***

Homologues provide valuable clues

Marta Nieto founded an independent research group about five years ago at the CNB in the Consejo Superior de Investigaciones Científicas (CSIC) in Madrid, after having identified her primary focus: to obtain a comprehensive insight into cortical development by addressing the molecular mechanisms of cell-type specification in the different cortical layers. The superficial layers specially grabbed her attention owing to their potential role in cognition. She was convinced that for the study of the upper layer neurons one must include at least two phases, namely the early embryonic development for early directive cues and early-late (early postnatal) development, to trace the early phases of synapse formation. Since it was suggested then that the specification of upper layers is different from that of deeper layers, Marta had to look in new places for prospective candidates.

In an occurrence that she calls 'serendipity', Marta stumbled upon the *Cux* genes. The *Cux* genes, *Cux1* and *Cux2* are the vertebrate homologues of the *Drosophila* homeobox transcription factor *Cut,* whose loss-of-function was shown to reduce dendritic growth and terminal branching in postmitotic neurons of the peripheral nervous system (PNS) (Grueber *et al.*, *Cell*, 2003, vol. 112: 805-18).

The *Cux* genes were found to be abundant in the cortical upper layer postmitotic neurons but little

was known about their role in these cell populations. To dissect their function in the superficial layers, Marta and her group undertook a series of approaches and published their findings recently in *Neuron* (Cubelos *et al.*, 2010, vol. 66: 523-35). Their preliminary results were based on the single *Cux1* and *Cux2* knockouts, where they found not only a significantly low dendritic complexity viz. dendritic branching but also less than half the spine number in the upper layer neurons, compared to that in the wild-type. Moreover, the defective dendritic morphology of the knockouts could be reproduced by shRNA-induced gene-specific knockdown experiments. Since dendritic spines are the sites of synaptic contacts, the group also observed impaired electrical conductivity of the upper layer neurons from the knockouts in contrast to wild-type animals.

A whole picture came about

Marta did not leave it there but pounded deeper to elaborate on the downstream pathway that the Cux proteins triggered. "We discovered *Xlr* in a gene array with the *Cux2* knockouts", recollects Marta. "It was unbelievable when our ChIP assays revealed that Cux1 and Cux2 directly bind to the *Xlr3b* and *Xlr4* loci *in vivo*. It was a case of direct repression of the *Xlr* genes by the transcription factors and everything was so fitting," she adds. *Xlr3b* had been previously reported to be upregulated in the brain in a mouse model with Turner syndrome (a condition of monosomy of the X chromosome in females, which is associated with gonadal dysfunction and a specific pattern of cognitive defects). No role for the *Xlr* genes in the neurons had then been reported but because mental retardation is often associated with spine and dendritic defects, the *Xlr* genes looked like ideal candidates in the Cux pathway. Indeed, shRNA-induced knockdown of *Xlr3b* and *Xlr4,* not only in shCux1-RNA co-expressing but also in Cux2$^{-/-}$ neurons, significantly reverted the spine density to normal levels and also improved their morphology.

To wrap things up and make it a whole lucid mechanism, Marta and colleagues tested mouse behaviour in control and the *Cux2* knockouts. Their data revealed that the loss of *Cux* did significantly impair working memory in the knockouts, compared to the wild-type animals. In summary, their work holds potential implications for *Cux* and *Xlr* genes in human disorders associated with impaired cognition.

Long-term perspective

In a chat with Marta on what lies ahead with the Cux proteins, she elaborates, "We would like to address the Cux-mediated intrinsic mechanism of synapse regulation and functions in plasticity, on one hand. On the other, we are interested in studying how the expression of Cux in the different areas of the cortex may influence the integration of information and coordinated brain function." Before venturing into the Cux project, Marta had little support from literature on the Cux proteins, as they were only reported in the context of cell-cycle exit in kidney development. In her pioneering work, Marta started off by characterising the Cux proteins and elucidating their role in cell cycle proliferation of the immediate progenitors of the upper layers in the embryonic brain and in the specification of different sub-populations of the cortex such as the interneurons. "Our first studies revealed that the two Cux proteins have a redundant role in the specification of a subset of cortical interneurons, where their complete absence seems to affect Reelin expression. Failure of these neurons to develop may indicate early specification problems, proliferation defects or alterations to migration routes," explains Marta.

Rodents have been good models for their study, though the Nieto group would like to work with higher mammals such as ferrets. The reason for this, as Marta justifies, is that rodents have a lissencephalic cortex, whereas carnivores represent a model of cortical development that shows gyral formation.

The Nieto group hopes to have conditional knockouts for specific cortical areas as tools to answer their questions. Marta also emphasises on the need for improvised electrophysiological methods.

Besides her small but "enthusiastic" group, based at a Spanish setting, Marta has collaborators world-wide. She acknowledges experts in neuronal development, the group of Paola Bovolenta at the Cajal Institute, Spain, electrophysiologist Elisa Calcagnotto in Brazil, and her former advisor, Christopher Walsh in Boston, all of whom have been a great support in her career.

Marta concludes the conversation by stressing on how fascinating the cortex is for neurobiologists. "The cortex has been one of the best systems to study neuronal development. It is well described in terms of sequentially formed populations of cells and it holds a lot of scope for further research." Indeed, we would find ourselves nodding in agreement when she says, "The cortex is the site of intellectual capabilities, it is the one organ that has given us an advantage in evolution...it *is* what makes us human."

Madhuvanthi Kannan