Final Report:
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1)Background to the Project

Neuronal migration is a fundamental process in brain development. All newborn neurons migrate from the progenitor zones where they are born to specific locations where they mature and contribute to circuit formation. However, the routes traveled by neurons can differ extensively (Valiente & Marin, 2010). Two modes of migration have been identified in the forebrain as in other regions of the neural primordium: radial and tangential. The first is performed by cortical projection neurons, born from progenitors of the dorsal telencephalon. They migrate radially following a glial route perpendicular to the ventricular surface to reach a particular layer of the cortical plate. In contrast, cortical interneurons, which are born in the ventral telencephalon, migrate tangentially to reach the cortex, integrating multiple guiding signaling during this journey. The neurogenic regions where the projection neurons and cortical interneurons are born share the expression of one transcriptional factor, the proneural protein Ascl. This factor coordinates the developmental programmes that drive not only the cell cycle exit of neural stem cells into neurons but also the migration of postmitotic neurons (Britz et al., 2006; Castro et al., 2011; Pacary et al., 2013; Pacary et al., 2011). However, the mechanisms underlying the migration-promoting activity of Ascl1 are still being elucidated and it is still unclear to what extent and it contributes to specify the different patterns of migration of telencephalic neurons.

2)Hypothesis

Many molecules have been implicated in the regulation of telencephalic neurons. Extracellular signals and receptors regulate cell motility and oriented movements through dramatic cascade of signaling mainly controlling the cytoskeleton organization. Most of these molecules are involved in both radial and tangential migration and little is known of the how same mechanisms are responsible for the different migratory behaviors of cortical projection neurons and interneurons (Marin et al., 2006). In particular, Ascl1 commonly regulate the microtubule-associated protein, Centromere protein J (Cenpj) in both neurogenic areas (Garcez et al.2015). Recent results from applicant suggest that Cenpj is involved in the regulation of radial migration and tangential migration. Moreover, these experiments indicate that Cenpj protein have divergent intrinsic activities in cortical neuronal morphology. Therefore, we would like to test the hypothesis if Cenpj protein may contribute to the specification of the diverse migratory behaviors of telencephalic neurons.

3)Specific aim

The main goal of this project is characterize and compare the cellular activities of Cenpj in migrating telencephalic neurons. The methodology consists in achieving a gene knockdown in specific populations of the ventral and dorsal telencephalon.

4)Described work

In order to investigate the mechanisms that underline neuronal migration, we depleted Cenpj in radial and tangential neurogenic area, respectively. Aberrant morphologies often reflect alterations in the cytoskeleton. It has been shown that Cenpj is required for microtubule cytoskeleton dynamics and cilia formation in neurons (Garcez et al., 2015, Bazzi & Anderson, 2014). Both processes are involved in signaling convergence required for cytoskeleton regulation during neuronal migration. After ex vivo electroporation, we compared these processes using immunohistochemistry for microtubule stability markers such as acetylated tubulin (Sigma) and tyrosinated tubulin (Sigma) and cilia markers like ACIII (Santa Cruz) together with gamma tubulin (Abcam).

5) Outcomes and significance

Our results suggest that Cenpj is expressed in ventral telencephalon neurons, and its depletion cause morphological defects (Figure 1). Similar to the radial migration cells, the GABergic interneurons fail to enter into the cortical plate. However, the Cenpj depleted interneurons displayed a different morphology in comparison to the radial neurons. Their growth cones are enlarged and the leading processes are longer. Further investigations will shed a light by which mechanisms is Cenpj promotes migration in the cortical interneuronal populations. Our group is working to answer those questions.

Meanwhile, during 2015 and 2016, there was a sudden rise in microcephaly cases in Brazil associated with Zika virus. I had been studying microcephaly during my postdoc and decided to investigate if the Zika virus was indeed the cause of microcephaly. Therefore, I started collaboration with virologist and developmental biologist to test in vitro and in utero the effect of Zika virus. Interestingly, found that the Zika virus is a new TORCH factor and causes congenital infection. Zika virus has multiple effects of in the developing brain and the subtle phenotypes are yet to be described. There is evidence from human brain scans and histology of autopsy displaying heterotopia and indicating a possible neuronal migration defect caused by Zika virus. The background in neuronal migration will help in to elucidate this question in the near future.

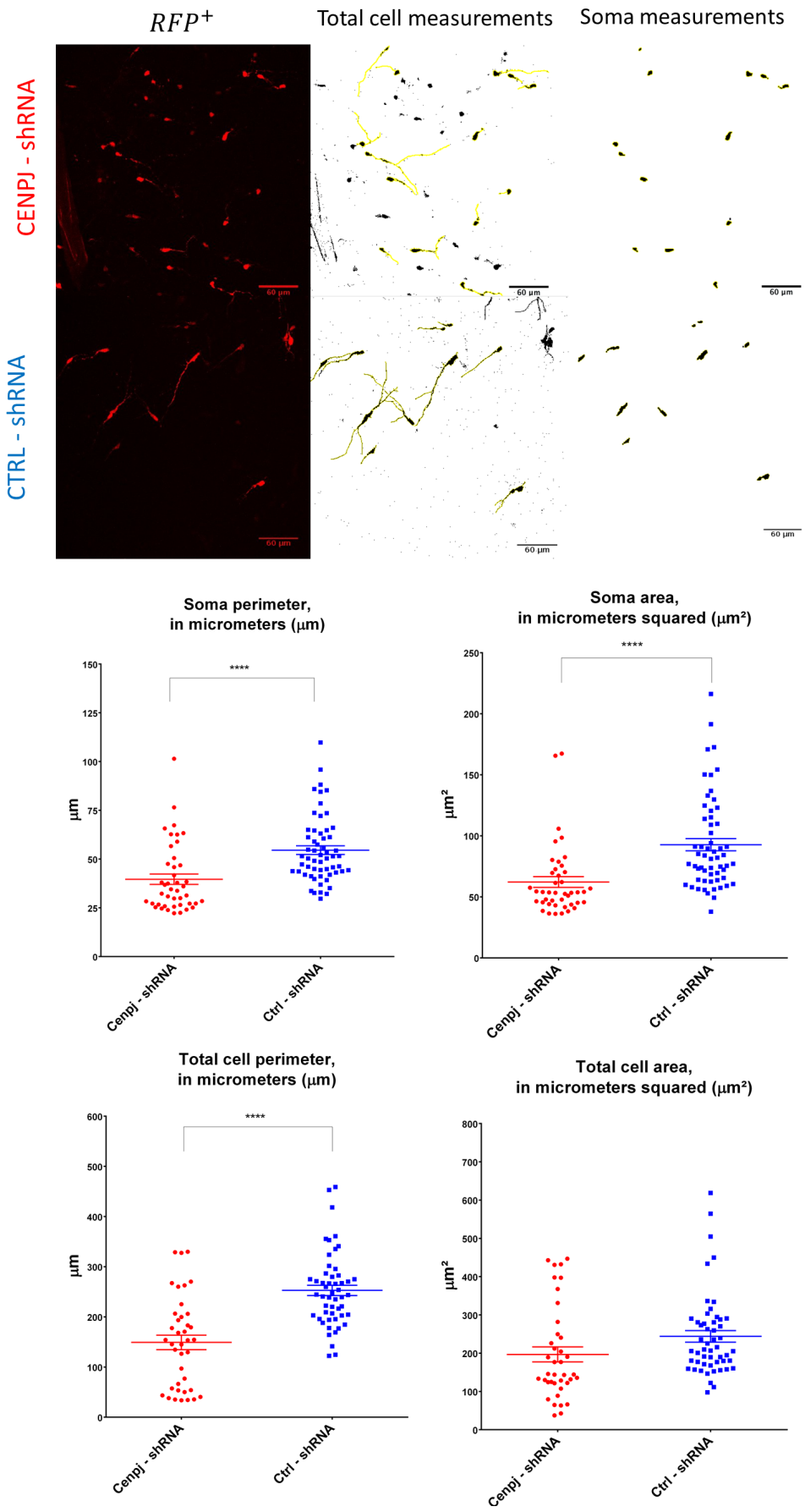

Figure 1: Comparison between Control shRNA and Cenpj shRNA electroporated GABAergic interneurons. Total cell measurements refer to area and perimeter calculated from the soma and cell process. ****= $p < 0.0001$

6) Detailed Expenses:

Laboratory Computer Software and subscriptions:

1) Microsoft Office 365 1 year subscription: USD$ 119.50
2) Adobe Creative Cloud 1 year subscription: USD$ 500.00
3) GraphPad 1 year subscription: USD$ 150.00
4) Online protocol book tool "Lab Guru" one year subscription: USD $240.00
5) Prezi (presentation software) subscription: USD $300.00
6) AAAS 1 year subscription: USD $164.00
7) Society for Neuroschemistry membership: USD $100.00

Laboratory Technical support (12 months): USD $3000.00

Subtotal: USD$ 4573