# ELUCIDATING NEURONAL CELL POPULATIONS UNDERLYING CIC HAPLOINSUFFICIENCY SYNDROME

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**Background:** Capicua (CIC) is a transcriptional repressor that reduces target gene expression by recruiting histone deacetylase complexes. Our lab discovered that heterozygous de-novo loss-of-function mutations in CIC lead to CIC Haploinsufficiency Syndrome (CHS), a neurodevelopmental disorder characterized by intellectual disability, ADHD, autism and seizures. To understand how CIC loss leads to these phenotypes, we deleted Cic conditionally in different brain regions in mice. Loss of Cic from the developing neocortex and hippocampus leads to a robust ADHD-like hyperactivity phenotype and impaired learning and memory. Additionally, ablation of Cic from inhibitory neurons recapitulated seizures observed in patients. The overlap in phenotypes between mice and humans suggests we can utilize these models to identify the biological processes driving CHS. Although Cic is ubiquitously expressed in neurons, its loss leads to a post-mitotic reduction in the number of upper layer cortical neurons but does not affect lower layers. Thus, it is possible that a population of upper layer cortical neurons is implicated in the behavioral phenotypes in Cic mutant mice, but a direct link is not yet known. We seek to determine if there is a subpopulation of upper layer cortical neurons responsible for hyperactivity and elucidate the role of Cic in this cell population.

**Materials/Methods:** To identify changes in cellular heterogeneity upon loss of Cic in the developing cortex we performed single-cell RNA sequencing (scRNA-seq) on postnatal day 5 cortices of Emx1-Cre Cic KO and control mice. We used CUT&RUN to identify CIC binding sites to distinguish primary vs secondary transcriptional changes. After identifying a Cic dependent cortical cell population, we used conditional mouse genetics to evaluate whether deletion of Cic in this population was sufficient to drive hyperactivity.

**Results:** scRNA-seq showed a 60% reduction in layer IV cortical neurons compared to other layers. Integration of CUT&RUN and scRNA-seq suggested that layer IV cortical neurons are preferentially vulnerable to Cic loss, rather than Cic having a unique function in this population. Finally, removal of Cic from layer IV cortical neurons caused a slight increase in hyperactivity.

**Conclusions:** Layer IV cortical neurons are preferentially affected upon loss of Cic from the developing cortex and likely contribute to hyperactivity in Cic mutant mice. Further investigation of the vulnerability of Layer IV neurons will help explain the processes underlying CHS.

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