Mammalian neocortex has a highly organized, six-layered structure that contains hundreds of different neuronal cell types. The correct differentiation of neurons during corticogenesis is responsible for higher brain functions. Previous discoveries have focused on the transcriptional factors expressed by neural progenitor cells (NPCs) and neuronal subtype specification has been assumed to happen in NPCs. Increasing evidence, however, suggests another model, in which microenvironment surrounding the immature neurons is also critical for neuronal subtype differentiation. Based on the observations of mutant mice in which virtually all thalamocortical axons (TCAs) were absent in the neocortex, our group reported that TCAs, which mainly terminate in layer IV (L4), are likely to provide some extrinsic signal that is important for the subtype specification of L4 neurons (Oishi et al., eLife, 2016; Oishi et al., PNAS, 2016).

It is still unclear whether thalamocortical inputs are sufficient to induce L4 subtype identity. In order to show directly that TCAs play a role for L4 subtype specification, we tried to establish a co-culture system, in which organotypic slices of the isolated cortex and thalamus were cultured together. In this co-culture assay, however, many wild-type cortices from postnatal day 0 cultured without thalamus had already expressed Rorb, suggesting that the critical contact between TCAs and cortical neurons may occur at earlier stages than previously thought. We are going to do the same experiment with cortices from mutant mice that lack dorsal thalamus, in which its contact is not supposed to occur. These experiments give insights into the regulation of subtype specification during later periods of neuronal differentiation.

Moreover, the molecular mechanisms underlying this presumptive function of TCAs remain to be clarified. We analyzed proteome and RNA-seq data in layer II/III and layer IV in primary somatosensory cortex at postnatal day 5. Combining them with public whole-brain single-cell RNA-seq database and in situ hybridization database, we identified potential candidate transmembrane proteins that are highly expressed in L4 neurons while those mRNA levels are low in L4 neurons but high in the thalamus. We will discuss their contributions on the L4 subtype specification.