PERSPECTIVE

Fine-tuning of cortical progenitor proliferation by thalamic afferents

During cerebral cortical cortex neurogenesis two major types of progenitors generate a variety of morphologically and functionally diverse projection neurons destined for the different cortical layers in non-gyrified mice. Radial glia cells (RGCs) undergo mitosis in the cortical ventricular zone and exhibit an apical-basal cell polarity, whereas non-polar intermediate progenitor cells (IPCs) divide basally in the subventricular zone (Franco and Muller, 2013; Taverna et al., 2014). The contribution of these cortical progenitor subtypes to particular cortical layers remains debated. Several lines of evidence suggest that IPCs give rise to the majority of cortical neurons, while only 10–20 % are directly generated by apical progenitors (Franco and Muller, 2013). The generation of excitatory cortical neurons follows a temporal order with deep layer neurons born first and upper layers are generated successively during later stages of neurogenesis. Furthermore, the symmetry of mitotic division is instructive for the prospective fate of daughter cells and thus for their laminar destination (Taverna et al., 2014). Various intrinsic and extrinsic cues have been identified that regulate the division mode and cell fate of cortical precursors. In addition to intra-cortical control of cortical neurogenesis such as paracrine interactions between progenitor cells (North et al., 2009) and feedback signaling from post-mitotic neurons of the cortical plate (Seuntjens et al., 2009), extra-cortical signals exerting control over cortical proliferation are increasingly attracting attention. These include factors from the cortico-spinal fluid (Lehtinen and Walsh, 2011) or invading glutamatergic neurons (Teissier et al., 2012). However, the impact of invading thalamo-cortical axons on cortical neurogenesis and layer formation remains largely unknown. Previous *in vitro* studies suggested that thalamic fibres regulate cell cycle properties and division mode by releasing soluble factors (Dehay et al., 2001). In turn, our data show a contact-mediated extra-cortical regulation of cortical neurogenesis by invading thalamic afferents (**Figure 1**) (Gerstmann et al., 2015).

Interactions of transmembranal Eph-receptor tyrosine kinases with their ligands, the ephrins, modulate a variety of developmental processes including the regulation of cortical progenitors (North et al., 2009). We found, that the depletion of either the ephrin type-A 4 (EphA4) receptor or its ligand ephrin-A5 leads to a shift of the laminar organization of the neocortex reflected by expanded deep and reduced upper cortical layers. This phenotype emerges from alterations during neurogenesis, since mice deficient for the ephrin-A5 ligand revealed variations in the number of progenitor subtypes and neuronal output at distinct developmental stages. At the onset of neurogenesis, we observed higher numbers of IPCs associated with increased neuronal production in ephrin-A5 deficient mice. In contrast, the pool of IPCs, as well as the generation of cortical neurons, was reduced during the generation of the upper layer neurons at later developmental stages. These results support studies outlining a contribution of IPCs to all cortical layers (Franco and Muller, 2013) and propose a modulation of cortical neurogenesis by ephrin-A5/EphA4 interactions.

The EphA4 receptor is expressed by radial glia cells in the transient proliferative zones of the developing cortex. The receptor protein is located in the membrane of the soma and along the radial processes that provide an excellent structural scaffold for local interactions between glial precursors and signaling molecules outside the proliferative zones. We next aimed to identify the cell type that expresses ephrin-A5 at embryonic stages, and thus interacts with EphA4 expressing progenitors. Therefore, we chose a sensitive polymerase chain reaction (PCR)-based strategy for single cell transcriptome profiling of cortical cells, as this approach even detects transcripts of low abundance in individual cells. According to the expression of specific marker genes, we discriminated the transcriptomes of progenitors versus post-mitotic neurons. The presence of ephrin-A5 transcripts was investigated in particular in these different cellular subsets. In agreement with *in situ* hybridization expression studies, ephrin-A5 was neither detected in cortical precursors nor in cortical post-mitotic neurons. Thus, ephrin-A5 induced EphA4-activation by paracrine interactions of neighboring progenitors or feedback signaling from the cortical plate seems rather unlikely. Interestingly, ephrin-A5 ligand expression was observed in the mantel zone of the thalamus from where first thalamo-cortical afferents grow into the developing neocortex. Binding studies confirmed the location of potential EphA4-affine ephrin ligands along thalamic axons. Thus, we determined whether invading thalamic afferents import membrane bound molecules such as ephrin-A5 into the developing cortex, thereby affecting the proliferation and differentiation of cortical precursors. As axonal mRNA localization and protein synthesis have been described, we investigated whether ephrin-A5 transcripts can be detected in thalamic axons. Axonal compartments of cultured thalamic explants were manually dissected and representative cDNA-libraries from axonal mRNAs were generated. Indeed, ephrin-A5 transcripts were found in cDNA-libraries of thalamic axons, indicating that the ligand is present in thalamic afferents. This result pointed to a sub-cortical regulation of cortical proliferation by thalamic fibers importing ephrin-A5, which directly interacts with its cognate receptor expressed along glial processes. To confirm whether invading thalamic axons indeed influence cortical precursors, we determined the output of progenitor division depend on direct contact with ephrin-A-positive and negative thalamic axons *in vitro*, combining the pair-cell assay with thalamic explant cultures. We revealed reduced rates of neurogenic cell pairs when progenitor cells were in contact with ephrin-A-expressing thalamic axons, which was reminiscent of decreased neurogenic division induced by stimulation with purified ephrin-A5 protein. This is further in agreement with the increased neuronal production detected in ephrin-A5 deficient mice at the onset of neurogenesis.

Taken together, our data provide evidence for an extra-cortical control of cortical neurogenic events by thalamic axons providing instructional information *via* membrane-bound cues. Thereby, direct cell-contact-mediated interactions enable precise modulations with presumably greater accuracy than diffusible thalamic factors, a broader range of cells, depending on diffusion properties and half-life of the secreted molecule. This suggests that membrane-bound thalamic signals might execute fine adjustments of cortical neurogenesis, which is consistent with the mild defects observed in the ephrin-A5 or EphA4 deficient mice. Yet, in the light of evolution, fine-tuning of cortical neurogenesis and alterations in the timing of neurogenesis are suggested as a key mechanism for cortical surface expansion (Poluch and Juliano, 2015). It is notable that gyrencephalic primates exhibit

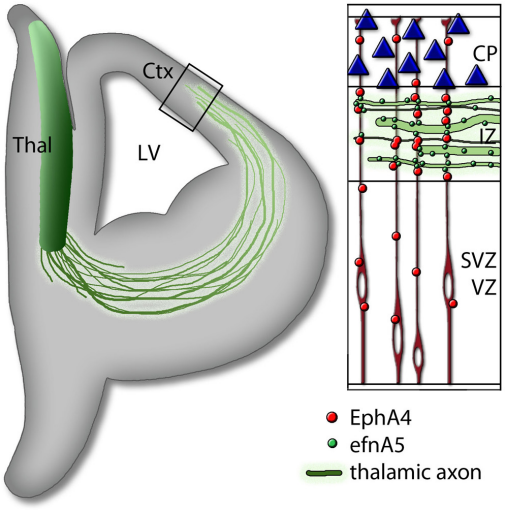

Figure 1 The model depicts how thalamic afferents regulate the mitotic activity and cell fate decision of cortical progenitor cells *via* contact-mediated or contact-independent mechanisms.
During early neurogenesis, thalamic axons grow into the developing neocortex releasing diffusible factors (Dehay et al., 2001) and importing membrane-bound molecules that locally interact with factors along the glial processes. Ctx: Cortex; Thal: thalamus; LV: lateral ventricle; CP: cortical plate; IZ: intermediate zone; SVZ: subventricular zone; VZ: ventricular zone; EphA4: ephrin type-A receptor 4.

more abundant thalamo-cortical axons and a longer waiting period in the intermediate zone than lissencephalic rodents, suggesting that IPC proliferation in the SVZ is more strongly affected by thalamo-cortical afferents in gyrencephalic species. Binocular enucleation experiments in ferrets, disrupting thalamo-cortical axons in the visual cortex, support this hypothesis, as they result in reduced proliferation of basal RGCs in the outer SVZ inducing a smaller visual cortex (Reillo and Borrell, 2012). Interestingly, invading thalamo-cortical projections enter the lateral cortex and then grow to dorso-medial regions, thus following the latero-dorsal neurogenic gradient of murine corticogenesis. This raises the issue of a thalamic contribution to these progressive neurogenic events. This question is of further relevance, as the elimination of the neurogenic gradient is suggested to be a prerequisite for an expansion of the parietal cortex in an evolutionary context (Poluch and Juliano, 2015). The impact of the thalamus on the neurogenic gradient could be addressed by detailed and comprehensive analysis of mice lacking thalamo-cortical projections, such as Celsr3/Dlx-mutant mice ("neocortex isolé"). Indeed, these mice exhibit cortical atrophy accompanied by diminished tangential extension and reduced cortical thickness (Zhou et al., 2010). The decreased cortical thickness in Celsr3/Dlx-mutant mice results from reduced numbers of deep layer neurons indicating that the thalamus already exerts influence during early stages of neurogenesis, when deep layer neurons are born. This is supported by our data, as we observed alterations in neurogenesis as early as E13.5 in ephrin-A5-deficient mice, when deep layer neurons are generated. These findings are in further agreement with another study showing pioneer thalamic axons in the dorso-lateral telencephalon already at E12 (Auladell et al., 2000).

In conclusion, our results suggest an extra-cortical contribution of thalamic afferents to the regulation of proliferation and cell fate decision of cortical progenitor cells, thereby affecting the formation of the cortical layers. This fine-tuning by thalamic surface molecules is crucial for the accurate development and might have had an impact for the evolutionary expansion of the neocortex. Decoding regulatory mechanisms and mediators of cortical proliferation and cell fate determination are moreover crucial to develop stem cell-based neuroregenerative therapy strategies facing the implications of brain injury or neurodegenerative diseases.

Katrin Gerstmann, Geraldine Zimmer*

Institute of Human Genetics, Jena University Hospital, Friedrich-Schiller-University Jena, 07743 Jena, Germany (Gerstmann K, Zimmer G)
CGphiMC UMR CNRS 5534, University Claude Bernard Lyon1, 69001 Villeurbanne, France (Gerstmann K)

***Correspondence to:** Geraldine Zimmer, Ph.D., geraldine.zimmer@med.uni-jena.de.*
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References

Auladell C, Perez-Sust P, Super H, Soriano E (2000) The early development of thalamocortical and corticothalamic projections in the mouse. Anat Embryol 201:169-179.
Dehay C, Savatier P, Cortay V, Kennedy H (2001) Cell-cycle kinetics of neocortical precursors are influenced by embryonic thalamic axons. J Neurosci 21:201-214.
Franco SJ, Muller U (2013) Shaping our minds: stem and progenitor cell diversity in the mammalian neocortex. Neuron 77:19-34.
Gerstmann K, Pensold D, Symmank J, Khundadze M, Hubner CA, Bolz J, Zimmer G (2015) Thalamic afferents influence cortical progenitors via ephrin A5-EphA4 interactions. Development 142:140-150.
Lehtinen MK, Walsh CA (2011) Neurogenesis at the brain-cerebrospinal fluid interface. Ann Rev Cell Dev Biol 27:653-679.
North HA, Zhao X, Kolk SM, Clifford MA, Ziskind DM, Donoghue MJ (2009) Promotion of proliferation in the developing cerebral cortex by EphA4 forward signaling. Development 136:2467-2476.
Poluch S, Juliano SL (2015) Fine-tuning of neurogenesis is essential for the evolutionary expansion of the cerebral cortex. Cereb Cortex 25:346-364.
Reillo I, Borrell V (2012) Germinal zones in the developing cerebral cortex of ferret: ontogeny, cell cycle kinetics, and diversity of progenitors. Cereb Cortex 22:2039-2054.
Seuntjens E, Nityanandam A, Miquelajauregui A, Debruyn J, Stryjewska A, Goebbels S, Nave KA, Huylebroeck D, Tarabykin V (2009) Sip1 regulates sequential fate decisions by feedback signaling from postmitotic neurons to progenitors. Nat Neurosci 12:1373-1380.
Taverna E, Gotz M, Huttner WB (2014) The cell biology of neurogenesis: toward an understanding of the development and evolution of the neocortex. Ann Rev Cell Dev Biol 30:465-502.
Teissier A, Waclaw RR, Griveau A, Campbell K, Pierani A (2012) Tangentially migrating transient glutamatergic neurons control neurogenesis and maintenance of cerebral cortical progenitor pools. Cereb Cortex 22:403-416.
Zhou L, Gall D, Qu Y, Prigogine C, Cheron G, Tissir F, Schiffmann SN, Goffinet AM (2010) Maturation of "neocortex isole" in vivo in mice. J Neurosci 30:7928-7939.