Final report of the research group of

Dr. P. Vanderhaeghen

Université Libre de Bruxelles (ULB)

Dr. Pierre Vanderhaeghen

Institute of Interdisciplinary Research (IRIBHN)
ULB, Campus Erasme
808, Route de Lennik
B-1070 Brussels
Tel.: +32 2 555 41 86
Fax: +32 2 555 46 55
pvdhaegh@ulb.ac.be

Molecular mechanisms controlling the development and evolution of the cerebral cortex.

The cerebral cortex is one of the most complex and important structures in our brain. The mechanisms of formation of cortical networks have direct relevance to several diseases, such as epilepsy and mental disorders, as well as for the development of rationally designed cell therapies for neurological conditions. The major research goal in our laboratory is to understand the mechanisms controlling the development of the cerebral cortex, from stem cells to neuronal networks and from mouse to man, by combining molecular and cellular approaches, both *in vivo* (using mouse transgenesis and in utero electroporation) and *in vitro* (using organotypic assays and embryonic stem cells).

We have summarized below the work completed in in this programme in 2008-2010 thanks to the Funding of the FMRE/GSKE, providing a link with recently published work and in preparation, as well as its perspectives in the future.

1. An intrinsic mechanism of corticogenesis from embryonic stem cells.

The cerebral cortex consists of several hundreds of different types of neurons, organized into specific cortical layers and areas, that display specific profiles of gene expression, morphology, excitability and connectivity. The molecular mechanisms underlying the generation of such a cellular diversity remain largely unknown, in particular due to the lack of appropriate reductionist models of cortical development. Recently we have developed an in vitro model of neural differentiation of embryonic stem (ES) cells to study the specification of cortical neurons (Gaspard et al., 2008). Using a chemically defined medium devoid of any exogenous morphogen factors, we found that mouse ES cells cultured as monolayers spontaneously and efficiently (>80%) give rise to a population of neural precursors expressing regional markers indicative of a forebrain identity. When exposed to appropriate morphogen antagonists during their differentiation, in particular inhibitors of the *Sonic-Hedgehog* pathway, the fate of the ES cell-derived forebrain-like neural progenitors can be efficiently (>75%) directed to an identity corresponding to the cortical lineag. ES cell-derived cortical-like progenitors subsequently differentiate into a stereotyped population of neurons, most of which display landmarks of cortical pyramidal neurons, including a glutamatergic phenotype and a pyramidal morphology. Most strikingly, ES cell-derived neurons correspond to distinct subtypes of cortical neurons that expressed layer-specific markers and are generated sequentially, in a manner strikingly similar to the in vivo situation. Most importantly, when grafted into neonatal mouse brain, they can connect with the rest of the brain like genuine cortical projection neurons (Gaspard et al., 2008, 2009).
This model of in vitro “corticopoiesis” recapitulates all milestones of cortical development observed in vivo, including regional and temporal patterning, and therefore constitutes an attractive and robust system, which we currently use for the genetic dissection of the mechanisms of cortical neuron specification (Gaspard and Vanderhaeghen, 2010). We have started to implement a gain of function screen by overexpression of transcription factors that can later the identity of the generated neurons. We thus identified several candidates (including zBTB20, Bcl6 and Tbr2) for which we have started to define transcriptional targets through microarray and CHIP analyses. In parallel, we have started to explore the relevance of our model for cell replacement following cortical lesions in the adult, using a combination of anatomy, physiology and functional imaging. Our first sets of data already indicate that ES-derived cortical neurons can efficiently integrate in lesioned adult cortex, with signficant and specific

axonal outgrowth to cortical and subcortical targets , providing a first prrof of principle of their potential use for brain repair (Michelsen et al., in preparation). On the other hand have succesfully started to to implement the system to human ES cells. Using a similar default prototol, we have been able to generate forebrain progenitors and cortical neurons from hES cells, following a temporal sequence similar to the in vivo situation (Espuny et al., unpublished data). The ability to differentiate in vitro cortical neurons from hES cells would constitute a primary tool to study human cortical neuron development. Finally we have started to generate novel models of neurodevelopmental diseases, by generating specific iPS cell lines from patients displaying some of these rare diseases (Takahashi and Yamanaka, 2006). We have obtained the first candidate iPS cell lines (Hasche et al., unpublished data), which are now characterized in depth in vitro and in vivo as we have done previously for hES cells (Deleu et al., 2009).

2. Multiple roles for ephrin/Eph guidance genes in the development of the forebrain.

We previously demonstrated that ephrin/Eph genes are involved in several aspects of the development of the connectivity of the forebrain, including the patterning of cortical sensory areas and development of area-specific thalamo-cortical projections (Vanderhaeghen and Polleux, 2004; Dufour et al., 2003; Seibt et al., 2003; Egea et al., 2005; Dufour et al., 2006). In parallel we also showed an important role for ephrins in the control of forebrain size, through the unexpected regulation of apoptosis of neural progenitors (Depaepe et al., 2005; Depaepe and Vanderhaeghen, 2005). These findings suggest that ephrins, like neurotrophins, have evolved as pleiotropic factors that can control very different functions depending on the cellular context (Vanderhaeghen and Cheng, 2010). We have now pursued these findings by looking at the potential involvement of ephrin/Eph genes in the neuronal migration in the forebrain. This has led to the demonstration that ephrins are required for the propoer patterning of the striatum, through a novel mechanism of temporal control of striatal neuron guidance cues (Passante et al., 2008).
To gain insight into the mechanisms involved in these processes, we have set up in utero electroporation to dissect the molecular and cellular mechanisms that control the migration of distinct populations of neurons to dorsal vs ventral domains of the telencephalon. Using these in vitro assays, we have identified several candidate guidance factors, including ephrins-B1-2, in the patterning of the migratory streams in the basal forebrain and cortex (Dimidschstein and PV, unpublished data). We followed up on these observations using appropriate mouse transgenic models (in particular ephrin-B1-2 conditional kos, available in the laboratory), in order to test for the consequences of the early disruption of migration patterns on cortical and striatal function in mature animals. Using in vivo clonal analyses we have been able to demonstrate that ephrin-B1 is required for the proper migration of cortical neurons and their arrangements into radial columns.

3. Developmental basis of human-specific features in the cerebral cortex.

Although many aspects of brain development seem to be remarkably conserved throughout evolution, a number of neural features have undergone a considerable divergence in mammals, in particular in the forebrain. We therefore started a project focusing on the developing human brain, trying to reveal what are the specific developmental programmes underlying the emergence of human-specific features in our brain.
We previoudly showed that HAR1 (*Human accelerated Region 1*), a novel non coding RNA gene that

is highly conserved throughout amniotes but contains among the most highly divergent sequences in the human lineage, is strongly expressed in the human embryonic neocortex (Pollard et al., 2006). Given its potential involvement in the development and evolution of the cerebral cortex, we study the function of HAR1 in the mouse brain. To this end we are undertaking a gain-of-function approach, using electroporation of human and mouse HAR1 expression constructs, as well as a knock-in line where human HAR1 is conditionally expressed in the cortex, for which the first mice are now being analyzed, with special emphasis on potential impact on the reelin pathway. In parallel we have generated knock-out mice for the mouse HAR1 gene for which the firstt mice are now available.

Finally, we recently completed a microarray analysis that led to the identification of several hundreds of candidate genes differentially expressed between a subset of presumptive cortical areas in the human fetal cortex, using a novel approach combining three-dimensiona reconstruction of sectioned tissue (Lambot et al., 2009). Most strikingly we identified a small (around 50) subset of genes that display differential expression between presumptive language and association areas of the developing cortex in humans, which also display strong evidence of aceelerated evolution of their promoter regions in the human lineage (Lambert et al., PLOSone , in press).

Reference List (bold from our laboratory)

- **Depaepe,V., Suarez-Gonzalez,N., Dufour,A., Passante,L., Gorski,J.A., Jones,K.R., Ledent,C., and Vanderhaeghen,P. (2005). Ephrin signalling controls brain size by regulating apoptosis of neural progenitors. Nature *435*, 1244-1250.**
- **Depaepe,V. and Vanderhaeghen,P. (2005). [Lethal signals controlling brain size]. Med. Sci. (Paris) *21*, 795-797.**
- **Dufour,A., Egea,J., Kullander,K., Klein,R., and Vanderhaeghen,P. (2006). Genetic analysis of EphA-dependent signaling mechanisms controlling topographic mapping in vivo. Development *133*, 4415-4420.**
- **Dufour,A., Seibt,J., Passante,L., Depaepe,V., Ciossek,T., Frisen,J., Kullander,K., Flanagan,J.G., Polleux,F., and Vanderhaeghen,P. (2003). Area specificity and topography of thalamocortical projections are controlled by ephrin/Eph genes. Neuron *39*, 453-465.**
- **Egea,J., Nissen,U.V., Dufour,A., Sahin,M., Greer,P., Kullander,K., Mrsic-Flogel,T.D., Greenberg,M.E., Kiehn,O., Vanderhaeghen,P., and Klein,R. (2005). Regulation of EphA4 Kinase Activity Is Required for a Subset of Axon Guidance Decisions Suggesting a Key Role for Receptor Clustering in Eph Function. Neuron *47*, 515-528.**
- **Gaspard,N., Bouschet,T., Hourez,R., Dimidschstein,J., Naeije,G., van den Ameele .J., Espuny-Camacho,I., Herpoel,A., Passante,L., Schiffmann,S.N., Gaillard,A., and Vanderhaeghen,P. (2008). An intrinsic mechanism of corticogenesis from embryonic stem cells. Nature *455*, 351-357.**
- **Gaspard N, Bouschet T, Herpoel A, Naeije G, vandenAmeele J, and Vanderhaeghen P. Generation of Cortical Neurons from Embryonic Stem Cells. *Nature Protocols* 4 (2009), 1454-63.**
- **Gaspard N, Gaillard A, and Vanderhaeghen P Making Cortex in a Dish: corticopoiesis from embryonic stem cells.. *Cell Cycle* 8 (2009), 2491-6.**
- **Gaspard, N., and Vanderhaeghen, P. (2010). Mechanisms of neural specification from embryonic stem cells. Curr Opin Neurobiol *20*, 37-43.**
- Gerfen,C.R. (1989). The neostriatal mosaic: striatal patch-matrix organization is related to cortical lamination. Science *246*, 385-388.
- **Lambert N, Lambot MA, Bilheu A, Albert A, Englert Y, Libert F, Noel JC, Sotiriou C, Holloway AK, Pollard K, Detours V, and Vanderhaeghen P. Genes Expressed in Specific Areas of the Human Fetal Cerebral Cortex Display Distinct Patterns of Evolution. PLOS*ONE*, in press.**
- **Lambot MA, Mendive F, Laurent P, Van Schoore G, Noël JC, Vanderhaeghen P, and Vassart G.Three-dimensional reconstruction of efferent ducts in wild-type and Lgr4 knock-out mice.*Anat Rec* 292 (2009), 595-603.**
- Marin,O. and Rubenstein,J.L. (2003). Cell migration in the forebrain. Annu. Rev. Neurosci. *26*, 441-483.
- **Passante,L., Gaspard,N., Degraeve,M., Frisen,J., Kullander,K., De,M., V, and Vanderhaeghen,P. (2008). Temporal regulation of ephrin/Eph signalling is required for the spatial patterning of the mammalian striatum. Development *135*, 3281-3290.**
- **Pollard,K.S., Salama,S.R., Lambert,N., Lambot,M.A., Coppens,S., Pedersen,J.S., Katzman,S., King,B., Onodera,C., Siepel,A., Kern,A.D., Dehay,C., Igel,H., Ares,M., Jr., Vanderhaeghen,P., and Haussler,D. (2006). An RNA gene expressed during cortical development evolved rapidly in humans. Nature *443*, 167-172.**

- **Rosso,L., Marques,A.C., Weier,M., Lambert,N., Lambot,M.A., Vanderhaeghen,P., and Kaessmann,H. (2008). Birth and rapid subcellular adaptation of a hominoid-specific CDC14 protein. PLoS. Biol. *6*, e140.**
- **Seibt,J., Schuurmans,C., Gradwhol,G., Dehay,C., Vanderhaeghen,P., Guillemot,F., and Polleux,F. (2003). Neurogenin2 specifies the connectivity of thalamic neurons by controlling axon responsiveness to intermediate target cues. Neuron *39*, 439-452.**
- Takahashi,K. and Yamanaka,S. (2006). Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell *126*, 663-676.
- van der Kooy,D. and Fishell,G. (1987). Neuronal birthdate underlies the development of striatal compartments. Brain Res. *401*, 155-161.
- **Vanderhaeghen,P. and Polleux,F. (2004). Developmental mechanisms patterning thalamocortical projections: intrinsic, extrinsic and in between. Trends Neurosci. *27*, 384-391.**
- **Vanderhaeghen, P., and Cheng, H.J. (2010). Guidance molecules in axon pruning and cell death. Cold Spring Harb Perspect Biol *2*, a001859.**

Publications of the laboratory in the frame of the FMRE/GSKE grant 2008-2010:

- Genes Expressed in Specific Areas of the Human Fetal Cerebral Cortex Display Distinct Patterns of Evolution.
  Lambert N, Lambot MA, Bilheu A, Albert A, Englert Y, Libert F, Noel JC, Sotiriou C, Holloway AK, Pollard K, Detours V, and **Vanderhaeghen P**.
  PLoS ONE (2011), in press.
- From stem cells to neural networks: recent advances and perspectives for neurodevelopmental disorders.
  Gaspard N and **Vanderhaeghen P**.
  Dev Med Child Neurol 53 (2011): 13-7.
- Axon Guidance Molecules in Cell Death and Axon Pruning.
  Vanderhaeghen P, and Cheng, H-J.
  Cold Spring Harb Perspect Biol. 2010 Jun 1;2(6):a001859
- Mechanisms of neural specification from embryonic stem cells.
  Gaspard N, and **Vanderhaeghen P.**
  Curr. Op. Neurobiol. 20 (2010), 37-43.
- Mechanism of primitive duct formation in the pancreas and submandibular glands: a role for SDF-1.
  Hick AC, van Eyll JM, Cordi S, Forez C, Passante L, Kohara H, Nagasawa T, **Vanderhaeghen P**, Courtoy PJ, Rousseau GG, Lemaigre FP, Pierreux CE.
  BMC Dev Biol. 14 (2009) e66.
- Generation of Cortical Neurons from Embryonic Stem Cells.
  Gaspard N, Bouschet T, Herpoel A, Naeije G, vandenAmeele J, and **Vanderhaeghen P**.
  Nature Protocols 4 (2009), 1454-63.
- Wnts blow on NeuroD to promote adult neuron production and diversity.
  Vanderhaeghen P.
  Nature Neurosci. 9 (2009), 1079-1081.
- Making Cortex in a Dish: corticopoiesis from embryonic stem cells.
  Gaspard N, Gaillard A, and **Vanderhaeghen P.**
  Cell Cycle 8 (2009), 2491-6.
- GPR3 receptor, a novel actor in the emotional-like responses.
  Valverde O, Célérier E, Baranyi M, **Vanderhaeghen P**, Maldonado R, Sperlagh B, Vassart G, and Ledent C.
  PLoS ONE 4 (2009) e4704.
- Human cystic fibrosis embryonic stem cell lines derived on placental mesenchymal stromal cells.
  Deleu S, Gonzalez-Merino E, Gaspard N, Nguyen TM, **Vanderhaeghen P**, Lagneaux L, Toungouz M, Englert Y, and Devreker F.
  Reprod. Biomed. 18 (2009), 704–716.
- Three-dimensional reconstruction of efferent ducts in wild-type and Lgr4 knock-out mice.
  Lambot MA, Mendive F, Laurent P, Van Schoore G, Noël JC, **Vanderhaeghen P**, and Vassart G.
  Anat Rec 292 (2009), 595-603.
- An intrinsic mechanism of corticogenesis from embryonic stem cells.
  Gaspard N, Bouschet T, Hourez R, Dimidschstein J, Naeije G, vandenAmeele J, Espuny-Camacho I, Herpoel A, Passante L, Schiffmann S, Gaillard A, and **Vanderhaeghen P**.
  Nature 455 (2008), 351-357.

– Temporal regulation of ephrin/Eph signalling is required for the spatial patterning of the mammalian striatum.
Passante L, Gaspard N, Degraeve M, Frisen J, Kullander K, Demartelaer V, and **Vanderhaeghen P**.
Development 135 (2008), 3281-3290.
– Birth and rapid subcellular adaptation of a hominoid-specific CDC14 protein.
Rosso L, Marques AC, Weier M, Lambert N, Lambot MA, **Vanderhaeghen P**, and Kaessmann H.
PLoS Biol. 6 (2008), e140.