

Programme Club développement des Réseaux Neuronaux

Montpellier Mardi 22 Mai 2007

Cellules souches - Différenciation

09:00 - 09:30 - **Pierre Vanderhaeghen** – *ULB, Bruxelles.*

Corticopoiesis in a dish: coordinated specification of cortical neurons from embryonic stem cells through an intrinsic pathway

09:30-10:00 **Xavier Morin** – *IBDML, Campus de Luminy, Marseille*

LGN controls symmetric divisions of chick embryonic spinal cord neural progenitors

10:00 - 10:30- **Stéphane Baudouin/ Helène Boudin** - *INSERM U643, ITERT CHU Nantes*

The CD3 subunit CD3 is a negative regulator of dendrite development.

Electrophysiologie Réseaux

11:00 - 11:30 **Rustem Khazipov** - *Inmed/InsermU29 Luminy, Marseille*

Spindle-burst : the early pattern of the cortical activity

11:30 - 12:00 **Gilles Fortin** - *Neurobiologie Génétique et Intégrative, CNRS, Gif sur Yvette*

Réseaux respiratoires précoces imagerie calcique-

12:00 - 12:30 **Yehezkel Ben Ari** - *Inmed/InsermU29 Luminy, Marseille*

Le GABA et les activités périodiques dans la maturation cérébrale-

Nouvelles techniques

14:00 - 14:30 **Jean Livet**-*Harvard University, USA*

Brainbow: souris multicolores pour analyse connectomique

14:30 - 15:00 **Maxime Dahan**- *Ecole Normale Supérieure, Paris-*

Asymmetric redistribution of GABA receptors during GABA gradient sensing by nerve growth cones analyzed by single quantum dot imaging.

Guidage

15:00 - 15:30 **Sophie Chauvet** – *CNRS Luminy, Marseille*

Gating of semaphorin3e/plexind1 signalling by neuropilin-1 is required for establishment of the subiculo-mammillary tract.

16:00 - 16:30 **Cécile Lebrand**: *Université de Lausanne, Lausanne.*

Guidance of corpus callosum

16:30-17:00 **Bénédicte Durand** - : *Ecole Normale-Lyon*

Role de rfx3 dans la mise en place des structures issues de la ligne médiane dans le cerveau murin ?

17:00 - 17:30 **André Goffinet**- *University de Louvain, Bruxelles.*

Regional inactivation of Celsr3 to study the development of the anterior commissure and internal capsule

Modérateurs : [Valérie Castellani](#) et [Sonia Garel](#).

"CORTICOPOIESIS" IN A DISH: COORDINATED SPECIFICATION OF CORTICAL NEURONS FROM EMBRYONIC STEM CELLS THROUGH AN INTRINSIC PATHWAY.

Nicolas Gaspard¹, Raphael Hourez², Gilles Naeije¹, Lara Passante¹, Serge Schiffmann², and **Pierre Vanderhaeghen^{1*}**. University of Brussels (U.L.B.), ¹ Institute for Interdisciplinary Research (IRIBHM), ² Laboratory of Neurophysiology, 808 Route de Lennik, B-1070 Brussels, Belgium.

The cerebral cortex develops through the coordinated generation of a diverse repertoire of neuronal phenotypes, but the mechanisms involved remain poorly understood. Here we show that following an in vitro neurogenesis default pathway, embryonic stem (ES) cells display the intrinsic capacity to differentiate efficiently into neurons that display the molecular, cellular and functional landmarks of genuine neurons of the cerebral cortex. This pathway strikingly recapitulates the major milestones of cortical development observed in vivo, including the sequential generation of distinct subtypes of cortical neurons, from early-born Cajal-Retzius neurons to later-generated pyramidal neurons.

The demonstration of intrinsic 'corticopoiesis' from embryonic stem cells surprisingly suggests that cortical specification emerges from a primitive default programme of differentiation in mammals. It also provides novel tools for the genetic dissection of cortical development, and paves the way for the rational design of cellular therapies for diseases of the cerebral cortex.

LGN CONTROLS SYMMETRIC DIVISIONS OF CHICK EMBRYONIC SPINAL CORD NEURAL PROGENITORS

Xavier Morin, Florence Jaouen, Pascale Durbec
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Spatio-temporal regulation of symmetric versus asymmetric cell divisions directs the fate and location of cells within the developing central nervous system. G-protein regulators control spindle orientation in asymmetric divisions generating progeny with different identities in invertebrates. In vertebrate neural progenitors, precise orientation of the plane of division determines whether one or both daughter cells remain in the germinative zone. We investigated the role of the G-protein regulator LGN in the chick developing spinal cord. LGN, located at the cell cortex and spindle poles of neural progenitors, regulates spindle movements to orient their plane of division. Strikingly, expression of a dominant-negative LGN specifically perturbs proliferative symmetric divisions. Randomized spindle orientation leads one sister progenitor to exit the neuroepithelium prematurely and to proliferate in the mantle zone. Hence, symmetric divisions of vertebrate neural progenitors must be tightly controlled to maintain neural progenitors within the neuroepithelium, regulating proper development of the nervous system

THE CD3 SUBUNIT CD3 IS A NEGATIVE REGULATOR OF DENDRITE DEVELOPMENT

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A novel idea is emerging that a large molecular repertoire is common to immune and nervous systems, which might reflect the existence of novel neuronal functions for immune molecules in the brain, and vice versa. Here we show that CD3, a transmembrane signaling adaptor protein involved in T lymphocyte maturation and function, is widely expressed in the nervous system and plays a role in neuron development. Immunohistochemistry on adult rat brain sections evidenced that CD3 was exclusively expressed by neurons and was not detected in glial cells. Western blot experiments confirmed the expression of CD3 in the central nervous system and showed that the main molecular form of CD3 detected in the brain and in neuronal samples prepared from hippocampal cultures corresponded to a homodimer, a form similarly obtained in thymus and T cells. Measurements of intracellular Ca²⁺ on cultured neurons showed that application of a CD3 antibody, raised to an extracellular sequence of the protein, induced a rapid rise of intracellular Ca²⁺ concentration suggesting that CD3 is functionally coupled to intracellular signalling in neurons. Examination of CD3 cellular distribution at early developmental stages of hippocampal cultured neurons (< 6 days) showed a selective enrichment of CD3 at dendritic growth cones and filopodia, closely associated with actin cytoskeleton, suggesting a potential role of CD3 in neurite formation. Accordingly, neurons transfected with CD3 cDNA or treated with a CD3 antibody for 2 days induced an inhibition of dendrite length and branching, whereas the density of filopodia was massively increased. Our data demonstrated a novel role of CD3 in the nervous system as a negative regulator of dendrite development.

SPINDLE-BURST: THE EARLY PATTERN OF CORTICAL ACTIVITY

Khazipov Rustem

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Electrical activity in the developing cortex is organized in spatio-temporal patterns that are remarkably different from adult patterns. Early cortical activity is highly discontinuous (trace discontinu) with the bursts of activity separated by periods of silence. Bursts of activity are typically organized in oscillatory pattern which is called delta-brush and which is expressed during the fetal stage of human development. Homologous pattern, known as spindle-burst, is expressed in the neonatal rodents. Delta brushes/ spindle-bursts are characterized by local network oscillations at 5-25 Hz nested in slower delta waves. Delta brushes and spindle-bursts are self-organized oscillations synchronized by glutamatergic connections. GABAergic interneurons are important for their compartmentalization (Minlebaev et al. 2006). In the somatosensory cortex, delta brushes and spindle bursts are triggered in a somatotopic manner by sensory feedback resulting from spontaneous myoclonic twitches (Khazipov et al. 2004; Milh et al. 2006). In the visual cortex, spindle-bursts are driven by spontaneous waves of activity in retina before the retina starts to respond to light (Hanganu et al. 2006). Thus, early in development of sensory systems, cortical sensory stimulation is provided by endogenous mechanisms – spontaneous myoclonic twitches in the somatosensory system and retinal waves in the visual system. These endogenous mechanisms of cortical stimulation triggering specific cortical oscillatory pattern contribute to the activity-dependent plasticity in the developing cortex and formation of the cortical maps (Khazipov and Luhmann 2006).

1. Hanganu IL, Ben Ari Y and Khazipov R. Retinal waves trigger spindle bursts in the neonatal rat visual cortex. *J Neurosci* 26: 6728-6736, 2006.
2. Khazipov R and Luhmann HJ. Early patterns of electrical activity in the developing cerebral cortex of humans and rodents. *Trends Neurosci* 29: 414-418, 2006.
3. Khazipov R, Sirota A, Leinekugel X, Holmes GL, Ben Ari Y and Buzsaki G. Early motor activity drives spindle bursts in the developing somatosensory cortex. *Nature* 432: 758-761, 2004.
4. Milh M, Kaminska A, Huon C, Lapillonne A, Ben Ari Y and Khazipov R. Rapid Cortical Oscillations and Early Motor Activity in Premature Human Neonate. *Cerebral Cortex*, 2006.
5. Minlebaev M, Ben Ari Y and Khazipov R. Network Mechanisms of Spindle-Burst Oscillations in the Neonatal Rat Barrel Cortex in vivo. *Journal of Neurophysiology* 00759, 2006.

MULTIPLE RHYTHM GENERATORS IN THE EMBRYONIC MOUSE HINDBRAIN

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Recent advances in the neurobiology of breathing in mammals suggest that respiratory rhythm generation may depend on the coupling of multiple oscillators distinct in location, underlying rhythmogenic / neuromodulatory properties and in inspiratory or expiratory control ambitions. An ensuing feature of such an organisation is robustness and responsiveness of the resulting rhythm, a vital pre-requisite to comply with metabolic demands in response to environmental changes. The anatomical layout of reticular interneurons forming the oscillators and motor nuclei certainly results from coordinated anterior-posterior (AP) and dorsal-ventral patterning schemes specifying individual cell types within the hindbrain neural tube. Using reduced embryonic hindbrain preparations from wild type, vesicular glutamate transporter 2 and Krox20 (AP segmentation specifying gene) mutant mice, I will discuss the existence of a forerunning organisation of the respiratory rhythm generator in embryos, featuring a “para-facial” oscillator and a “para-vagal” oscillator that can be distinguished by their respective time of emergence, rhythmogenic properties, neuromodulatory control and upstream genetic specification.

BRAINBOW: SOURIS MULTICOLORES POUR ANALYSE CONNECTOMIQUE

Jean Livet, Tamily A. Weissman, Ju Lu, Robyn Bennis, Hyuno Kang, Joshua R. Sanes et Jeff W. Lichtman - *Harvard University-USA*

Je présente de nouveaux outils pour l'étude des circuits neuronaux basés sur le marquage de multiples neurones par des couleurs différentes. Deux configurations de transgène utilisant le système CRE/Lox, *Brainbow-1* et *-2*, ont été conçues pour exprimer aléatoirement plusieurs gènes dans une même population cellulaire. Les transgènes *Brainbow* offrent à la recombinaison CRE un choix entre plusieurs possibilités de recombinaisons (excisions ou inversions) qui déclenchent l'expression de protéines fluorescentes de différentes couleurs (XFPs). En utilisant un promoteur neuronal (Thy-1), nous avons généré des souris transgéniques *Brainbow* exprimant ces XFPs de manière mosaïque dans le système nerveux. Plusieurs des lignées obtenues présentent une expression combinatoire des XFPs. Les multiples couleurs ainsi créées permettent de distinguer de nombreux neurones adjacents dans un même échantillon. Nous avons ainsi reconstruit dans le cervelet plus de 300 axones (fibres myélinisées) et cellules granulaires à partir d'une unique série d'images confocales. Plusieurs contacts synaptiques entre neurones de couleurs différentes sont visibles dans cette reconstruction. Nous utilisons ce type de marquage multicolore pour étudier les remaniements de connectivité associés à des situations de polyinnervation, qui sont rencontrés dans plusieurs circuits en développement.

Brainbow est donc une technique permettant de visualiser et distinguer de multiples neurones composant un circuit, qui ouvre la voie à leur reconstruction « haut débit » et à l'analyse de leurs interactions. Cette technique est également adaptable à de nombreuses autres situations (analyse clonale, étude du *tiling* de cellules adjacentes). Elle pourra enfin être employée pour moduler l'expression de plusieurs gènes de manière mosaïque.

ASYMMETRIC REDISTRIBUTION OF GABA RECEPTORS DURING GABA GRADIENT SENSING BY NERVE GROWTH CONES ANALYZED BY SINGLE QUANTUM DOT IMAGING

Cédric Bouzigues¹, Antoine Triller² and **Maxime Dahan**¹

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During development of the nervous system, the tip of a growing axon – the growth cone (GC) – must respond accurately to stimuli that direct its growth. This axonal navigation depends on extracellular concentration gradients of numerous guidance cues, including gamma-aminobutyric acid (GABA). GCs can detect even weak directional signals, yet the mechanisms underlying this sensitivity remain unclear. Past studies in other eukaryotic chemotactic systems have pointed to the role of the spatial reorganization of the transduction pathway in their sensitive response. Here, we have developed a single-molecule assay to observe individual GABA_A receptors (GABA_A Rs) in the plasma membrane of nerve GCs subjected to directional stimuli. We report that in the presence of an external GABA gradient GABA_A Rs redistribute asymmetrically across the GC towards the gradient source. Single particle tracking of GABA_A Rs shows that the redistribution results from transient interactions between the receptors and the microtubules. Moreover, the relocalization is accompanied by an enhancement in the asymmetry of intracellular calcium concentration. Altogether, our results reveal a microtubule-dependent polarized reorganization of chemoreceptors at the cell surface and suggest that this polarization serves as an amplification step in GABA gradient sensing by nerve GCs.

GATING OF SEMAPHORIN3E/PLEXIND1 SIGNALLING BY NEUROPILIN-1 IS REQUIRED FOR ESTABLISHMENT OF THE SUBICULO-MAMMILLARY TRACT.

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Lying at the interface between the hippocampus and surrounding cortical areas, the subiculum represents the principal output structure of the hippocampal formation. Subicular neurons send dense projection via the fimbria/fornix to hypothalamic nuclei, including the medial mammillary bodies (MBs). The molecular mechanisms that guide the formation of this axon pathway remain unidentified. Here we show that the cell surface receptor PlexinD1 is a marker of the developing subiculum in mouse embryos. We provide in vitro and in vivo evidence that PlexinD1 and its ligand, the secreted Semaphorin 3E (Sema3E), are essential to promote the growth of subicular axons and control the establishment of the subiculo-mammillary tract. Adult mice deficient for Sema3E exhibit impaired stress responses and defective working memory, as would be expected from dysfunction of the MBs. We also identified a requirement of Sema3E and PlexinD1 function in controlling the development of subcortical projections from cortical layer 5 neurons. In this system, however, activation of PlexinD1 triggers a repulsive response of growing axons. As previously shown (Gu et al., *Science*, 307:265-8, 2005), repulsive Sema3E-PlexinD1 signalling is independent of Neuropilin (Npn) function. However, Npn1 appears to be cooperating with PlexinD1 to specify the stimulatory activity of Sema3E, since (1) Npn1 can physically interact with PlexinD1 to form a receptor complex for Sema3E, (2) loss of Npn1 function in subicular neurons converts Sema3E-triggered attraction to repulsion and (3) misexpression of Npn1 in cortical neurons converts Sema3E-induced axonal repulsion into attraction. This “gating” of PlexinD1 signalling by Npn1 reveals a novel biological function of Npn1 and provides the first example of a co-receptor-based mechanism by which a single Plexin mediates opposite effects in the developing nervous system.

ROLE DE RFX3 DANS LA MISE EN PLACE DES STRUCTURES ISSUES DE LA
LIGNE MEDIANE DANS LE CERVEAU MURIN ?

Bénédicte Durand:

GUIDANCE OF CORPUS CALLOSUM AXONS BY TRANSIENT NEURONAL POPULATIONS

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During development, subpopulations of glial cells have been shown to guide corpus callosum (CC) axons at the midline and control their navigation to the contralateral cerebral hemisphere. While it has been described that neuronal cells are also present in the developing CC, their role remains obscure. Here we show that the CC is transiently populated by numerous glutamatergic calretinin-positive and GABAergic GAD67/Mash1-GFP positive neurons. These two distinct neuronal populations, which were largely uncharacterized, converge towards the midline before callosal axons. Later on, GABAergic and glutamatergic neurons cooperate to form corridor and barriers that surround callosal axons and together form a superstructure delineating two different dorso-ventral paths within the CC. Using *Mash1* mutant mice, which lack CC GABAergic interneurons, and focal injections of domoic acid, which induce a selective ablation of CC glutamatergic cells, we show that these two neuronal subpopulations are essential to guide callosal axons at the midline. Taken together, our study reveals a novel role for transient neuronal populations in the development of a major cerebral commissure and brings a new perspective on the respective role of glial and neuronal cells in this process.

REGIONAL INACTIVATION OF CELSR3 TO STUDY THE DEVELOPMENT OF THE
ANTERIOR COMMISSURE AND INTERNAL CAPSULE

André Goffinet [Fadel et Libing](#)- *University of Louvain Bruxelles, Belgique* -