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2010

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Geneeskundige Stichting Koningin Elisabeth

Fondation Médicale Reine Elisabeth

Queen Elisabeth Medical Foundation

Geneeskundige Stichting Koningin Elisabeth 2010
Inleiding verslag activiteiten van de GSKE – FMRE

In 2010 eindigt de toewijzing van de onderzoekskredieten voor de 3 jaren 2008-2009-2010. De 16 begunstigde onderzoeksploegen hebben een afsluitend synthese verslag opgemaakt. Gedurende die 3 jaren hebben zij voor een totaal bedrag van 2.670.000 euro aan onderzoekskredieten ontvangen.

Deze verslagen zullen worden voorgelegd aan de leden van het Wetenschappelijke Comité, om een rangschikking op te maken, die zal voorgesteld worden aan de Raad van Bestuur voor het toekennen van wetenschappelijke prijzen van de Geneeskundige Stichting Koningin Elisabeth. Volgende prijzen worden toegekend: Solvay Prize, ING, Baron van Gysel de Meise, Janine en Jacques Delruelle, CBC Banque, en Cercle Gaulois-Thierry Speeckaert. Zij vormen een bijkomend krediet voor het onderzoek.

Gedurende dit jaar bezocht Prinses Astrid het laboratorium van Professor Emmanuel Hermans, een farmacoloog van de Université Catholique de Louvain, in Brussel op de site in Sint Pieters-Woluwe.
Nogmaals heeft Zij door haar interesse, haar vriendelijkheid en het stellen van pertinente vragen alle aandacht gevestigd op het onderzoek en de onderzoekers. Deze bezoeken zijn altijd een groot moment voor de stichting.

De uitreiking van prijzen van de Stichting 2010 vond uitzonderlijk plaats in het Kasteel te Laken, omdat het Koninklijk Paleis in Brussel werd gerenoveerd in het vooruitzicht van de ontvangst van de Eurazië top in het kader van het Belgische voorzitterschap van de Europese Unie. De talrijke aanwezigen (ongeveer 180 personen), waaronder ambassadeurs, politici en mecenassen en de vele leden van de onderzoeksteams, konden op een prachtige zonnige middag in mei, het Kasteel te Laken bewonderen en de winnaars van harte feliciteren. De Prinses overhandigde de prijs Solvay aan professor Gustave Moonen en zijn team (ULg), de prijs “Baron van Gysel de Meise”, aan de Professoren Vincent Timmerman en Peter De Jonghe (UA) en de prijs “Burggravin Valine de Spoelberch” aan Professor Yvette Michotte en haar team (VUB).
De Raad van Bestuur die vergaderde op 5 december, heeft op voorstel van het wetenschappelijk comité 13 nieuwe projecten van de 65 ingezonden dossiers, komende van alle Belgische universiteiten, weerhouden. Het totaal krediet voor de komende drie jaar, bedraagt 520.000 euro per jaar, gezien de financiële crisis ook in onze hoofdstad merkbaar is. De kredieten zullen tweemaal hernieuwd worden als de situatie het toelaat.

Dit jaarverslag is ook een gelegenheid om de leden van de Raad van Bestuur van de Geneeskundige Stichting Koningin Elisabeth te danken, voor hun vooruitziende blik, hun luisterbereidheid en hun vrijgevigheid ten voordele van het neurowetenschappelijk onderzoek in ons land, in de geest van de stichtster, Koningin Elizabeth.

Prof. em. dr. Baron de Barsy,
wetenschappelijk directeur
Brussel, december 2010

Fondation Médicale Reine Elisabeth
2010
Introduction rapport d'activités de la FMRE - GSKE

L'année 2010 termine l'attribution des crédits de recherche pour trois ans 2008-2009-2010. Les 16 équipes bénéficiaires ont rédigé un rapport final de synthèse des 3 dernières années durant lesquelles elles ont bénéficié de subsides pour une valeur totale de 2.670.000 euros.

Ces rapports seront soumis au conseil scientifique pour procéder à un classement qui sera proposé au conseil d'administration en vue de l'attribution des prix scientifiques de la Fondation Médicale Reine Elisabeth. Il s'agit des prix Solvay, ING, Baron van Gysel de Meise, Janine et Jacques Delruelle, CBC Banque, et Cercle Gaulois-Thierry Speeckaert. Ces prix constituent un supplément de crédit à la recherche.

Au cours de cette année la Princesse Astrid a rendu visite au laboratoire du Professeur Emmanuel Hermans, pharmacologue, sur le site de l'Université Catholique de Louvain à Bruxelles en Woluwe. Une fois de plus, elle a conquis toute l'assistance par son attention, l'intérêt évident qu'elle porte à la recherche et aux chercheurs, sa gentillesse et la pertinence de ses questions. Ces visites constituent toujours un grand moment dans la vie de la Fondation.

La remise des prix de la Fondation 2010 a eu lieu exceptionnellement au Château de Laeken, le Palais de Bruxelles étant en travaux pour l'accueil du sommet Eurasie dans le cadre de la Présidence Belge se l'Union Européenne. Une assistance nombreuse (environ 180 personnes), dont des ambassadeurs, des personnalités politiques, des mécènes et de nombreux membres des équipes de recherche, ont pu admirer le Château de Laeken et féliciter les lauréats par une superbe après-midi ensoleillée du mois de mai. La Princesse a remis le prix Solvay au Professeur Gustave Moonen et son équipe (ULg), le prix « Baron van Gysel de Meise », aux Professeurs Vincent Timmerman et Peter De Jonghe (UA) et le prix « Vicomtesse Valine de Spoelberch » au Professeur Yvette Michotte et son équipe (VUB).
Le conseil d'administration, réuni en date du 5 décembre, a retenu 13 nouveaux projets sur les 65 dossiers provenant de toutes les universités belges et soumis à l'examen du conseil scientifique. Le montant global des crédits, pour les trois années à venir, s'élèvera à 520.000 euros par an, compte tenu de la crise financière qui a aussi touché notre capital. Les crédits seront renouvelés deux fois si la situation le permet.

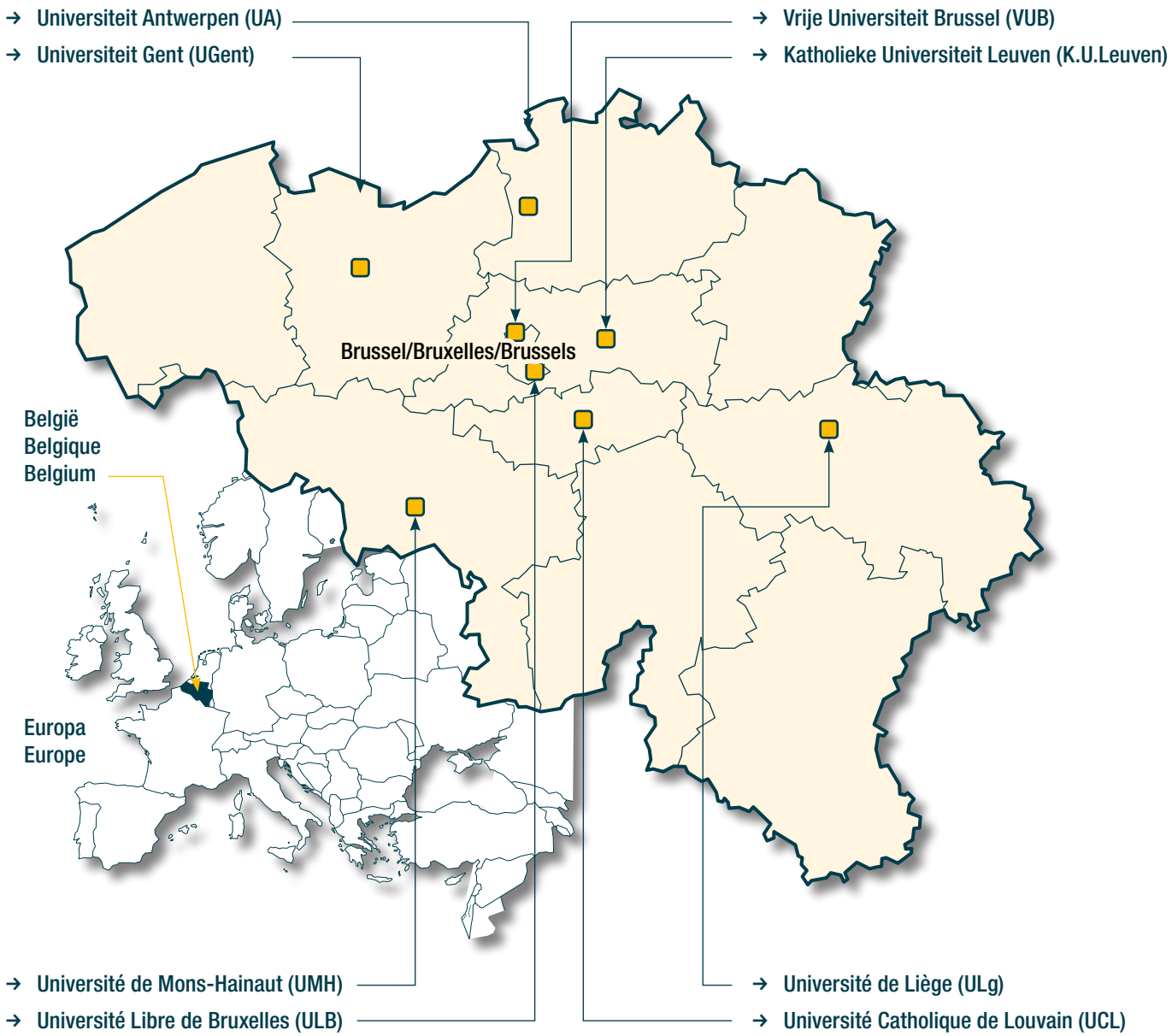
Ce rapport annuel est aussi l'occasion de remercier le conseil d'administration de la Fondation Médicale Reine Elisabeth, de sa clairvoyance, de son écoute et de sa générosité au profit de la recherche en neurosciences dans notre pays, dans l'esprit de la volonté de sa fondatrice la Reine Elisabeth.

Prof. em. dr. Baron de Barsy,
directeur scientifique
Bruxelles, décembre 2010

Universiteiten met onderzoeksprogramma's die gesteund worden door de G.S.K.E.

Universités ayant des programmes de recherche subventionnés par la F.M.R.E.

Universities having research programs supported by the Q.E.M.F.



Onderzoeksprogramma's gefinancierd door de G.S.K.E. - Programma 2008-2010

Programmes de recherche subventionnés par la F.M.R.E. - Programme 2008-2010

Q.E.M.F. funded research projects - Program 2008-2010

K.U.Leuven

- **Prof. dr. Peter Carmeliet**
  Unraveling the role and therapeutic potential of Flt1 receptor ligands in amyotrophic lateral sclerosis (ALS).

- **Prof. dr. Peter Janssen**
  The presentation of three-dimensional shape in posterior parietal and premotor cortex of the rhesus monkey.

- **Prof. dr. Wim Vanduffel**
  Large-scale causal functional interactions between cortical areas: from anatomy to neuro-pharmacology.

- **Prof. dr. Rufin Vogels**
  Coding of biological motion in macaque monkeys: relating perception and neuronal selectivity.

UA

- **Prof. dr. Vincent Timmerman**
  Molecular genetics and biology of Charcot-Marie-Tooth neuropathies.

- **Prof. dr. Christine Van Broeckhoven**
  Progranulin in neurodegenerative dementia: genetic, functional and neuropathological characterization.

UCL

- **Prof. dr. André Goffinet**
  Genetic, molecular and cellular mechanisms of cortical development.

- **Dr. Emmanuel Hermans**
  Cellular crosstalks in amyotrophic lateral sclerosis: influence of neuroinflammation on astrocyte function and stem cell differentiation.

UGent

- **Prof. dr. Frans Van Roy**
  Functional analysis of novel adhesive and signaling proteins in development and tumorigenesis of neural tissues.

ULB

- **Prof. dr. Marc Parmentier**
  Characterization of G protein-coupled receptors involved in drug addiction and motor diseases.

- **Prof. dr. S.N. Schiffmann**
  Roles of specific neuronal populations in functions and disorders of basal ganglia: a transgenic and molecular approach.

- **Dr. Pierre Vanderhaeghen**
  Mechanisms of the development and evolution of the cerebral cortex.

ULg

- **Prof. dr. Pierre Maquet**
  Characterization of spontaneous brain activity in unconscious participants by multimodal functional neuroimaging.

- **Prof. dr. Gustave Moonen**
  Characterization of new cellular and molecular mechanism underlying migration of interneurons in the telencephalon.

UMH

- **Dr. Laurence Ris**
  Role of protein synthesis in late long-term potentiation (L-LTP).

VUB

- **Prof. dr. Yvette Michotte**
  Exploration of the memory enhancing effects of angiotensin IV and unravelling its mechanism of action.

Final reports of the university research groups, supported by the Queen Elisabeth Medical Fondation in collaboration with the following professors and doctors (2010)

Final report of the research group of

Prof dr. P. Carmeliet

Katholieke Universiteit Leuven (K.U.Leuven)

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Carmeliet Peter (Main Applicant)

Applicant's Coordinator:

Lambrechts Diether (Co-Applicant)

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Unraveling the role and therapeutic potential of Flt1 receptor ligands in amyotrophic lateral sclerosis (ALS)

BRIEF SUMMARY OF ACTIVITIES

During the first year of funding, we investigated the neuroprotective role of VEGF-B in motoneuron degeneration and established that VEGF-B protects against mutant SOD1-mediated motor neuron degeneration (amyotrophic lateral sclerosis). These findings were published in the Journal of Neuroscience (Poesen et al., 2009). In the second year of funding, we investigated whether VEGF-B is also neuroprotective in other models of neurodegeneration. In particular, we investigated whether VEGF-B has therapeutic effects in models of sensory neuropathies. We were able to demonstrate that the neuroprotective activities of VEGF-B are not restricted to motor neurones but also extend to sensory neurons. During the third year of funding, we continued working on the role of VEGF-B in sensory neuropathies. Using transgenic mice overexpressing VEGF-B and its receptor Flt1 in neurons, we were able to demonstrate that VEGF-B selectively affects the sensory axon, and not the surrounding vasculature in the skin. The latter findings have been accepted for publication in the FASEB Journal (Dhondt et al., 2011). In the next pages of this report, we have summarized our data on the role of VEGF-B in motor neuron degeneration and sensory neuropathy more extensively. Additionally, we have provided an electronic copy of both publications at the end of the report.

1. Novel Role for Vascular Endothelial Growth Factor (VEGF) Receptor-1 and Its Ligand VEGF-B in Motor Neuron Degeneration

ALS IS AN INCURABLE DISEASE WITH HIGH MEDICAL IMPACT: 'Amyotrophic Lateral Sclerosis' or 'ALS' – the most common of all motoneuron disorders – is a devastating disease, in which the rapidly progressive loss of motoneurons paralyses patients, causing death within a few years. Precisely because the cause of this dreadful disease is not (yet) known, there is currently no cure available.

ANGIOGENIC FACTORS IN NEURODEGENERATION: Recently, a novel research avenue arose from the realisation that molecules known as angiogenic factors also exert important effects in the nervous system. Indeed, a few years ago, our laboratory discovered that VEGF-A, a prototype angiogenic factor, plays a major role in motoneuron degeneration (Nat Genet 28, 131-8, 2001, Nat Genet 34, 383-94, 2003) and when delivered as a gene or a protein drastically prolongs survival in rodent ALS models (Nature, 27:413-7, 2004; Nature Neuroscience, 8:85-92, 2005). Recently, missense mutations were also identified in another angiogenic factor, i.e. angiogenin (Nature Genetics, 38:411-3, 2006), raising the question whether other angiogenic genes will be identified as causative genes for neurodegenerative diseases.

VEGF-B, A NOVEL NEUROPROTECTIVE FACTOR IN THE NERVOUS SYSTEM: In this study, we analyzed the role of VEGF-B, a structural homologue of VEGF, which has been traditionally classified as an angiogenic factor. However, VEGF-B has only weak angiogenic activity, and only in a restricted set of conditions. We were able to show that VEGF-B, rather than acting as an angiogenic factor, exerts neuroprotective effects. Briefly, the 186kDa VEGF-B isoform, VEGF-B186 protected cultured primary motor neurons against degeneration. Mice lacking VEGF-B also developed a more severe form of motor neuron degeneration when intercrossed with mutant SOD1 mice: in particular, SOD1 mice expressing VEGF-B were able to stay on the rotarod until 125±3 days of age and died after 150±2 days of age. By contrast, SOD1 mice lacking VEGF-B performed significantly worse on the rotarod as they failed to stay on the rotarod beyond 111±5 days ($P=0.017$) and already died after 141±2 days ($P<0.05$). These

in vitro and in vivo effects of VEGF-B were strongly dependent on the tyrosine-kinase activities of its receptor Flt1 in motor neurons, as mice lacking a functional Flt1 receptor exhibited similar effects as mice deficient for VEGF-B. The observation that VEGF-B exerts direct neuroprotective effects is remarkable, since VEGF-B was originally considered to be an angiogenic factor. The in vitro neuroprotective effect of VEGF-B was also as strong as that of the classical neuroprotective factors (BDNF, CNTF). The neuroprotective effects of VEGF-B were also relevant in an in vivo setting, as VEGF-B protected SOD1 rats against motor neuron degeneration. Indeed, compared to SOD1 rats receiving control artificial cerebrospinal fluid (CSF), SOD1 rats treated with VEGFB tended to fall from the rotarod 11 days later than the CSF-treated rats (131±4 days for CSF-treated rats versus 142±4 days for VEGF-B-treated rats; P=0.073). VEGF-B-treated rats survived significantly longer (137±5 days for CSFtreated rats versus 152±3 days for VEGF-B186-treated rats; P=0.016). Compared to a similar dose of VEGF, VEGF-B was also safer and did not cause vessel growth or blood-brain barrier leakiness.

THERAPEUTICAL TRIALS WITH VEGF IN ALS: The therapeutic data with VEGF-B186 are particularly relevant, when considering that clinical trials to test the therapeutic potential of intracerebroventricular delivery of recombinant VEGF to ALS patients are being designed to commence before the end of 2009 (http://www.neuronova.com). In addition, a gene-based therapy using VEGF-expressing lentiviral vectors, MoNuDin, for human use is also being developed (http://www.oxfordbiomedica.co.uk/monudin.htm). However, the therapeutic window of intracerebroventricular VEGF delivery, which mainly affects neurons through the VEGF receptor-2, was rather limited due to side-effects: at low concentrations, VEGF exerted significant neuroprotective effects in SOD1 rats, but at higher levels it also triggered angiogenic side-effects and induced blood-brain barrier leakiness (Storkebaum et al., Nat Neuroscience, 2005). The therapeutic effects of VEGF were thus limited by its side-effects on the neurovasculature. Intracerebroventricular VEGF-B, by acting through the VEGF receptor-1, did however not affect the neurovasculature and could therefore be delivered at much higher concentrations than VEGF. Since the therapeutic effects of VEGF in SOD1 models are still among the largest reported in the field so far, we think that high levels of VEGF-B may be used to complement VEGF therapies in ALS patients, and further improve the therapeutic effects.

2. Novel Role for Vascular Endothelial Growth Factor (VEGF) Receptor-1 and Its Ligand VEGF-B in Motor Neuron Degeneration

RATIONALE TO STUDY VEGF-B IN SENSORY NEUROPATIES: A number of novel biological functions have recently been assigned to VEGF-B: Hagberg et al. showed that VEGF-B regulates the expression of fatty acid transporters in endothelial cells ('vascular effect') and thereby determines fatty acid accumulation in peripheral tissues (Hagberg et al., Nature 2010). Other studies have suggested that VEGF-B has neuroprotective effects in the central nervous system ('neuronal effect'): in a culture model of Parkinson's disease, VEGF-B was able to improve neuronal survival and in a rodent model of experimental nerve injury, VEGF-B was neuroprotective (Li et al., JCI, 2008). To more carefully investigate both the vascular and neuroprotective activities of VEGF-B, we also studied the role of VEGF-B in the sensory nervous system of the skin. Since the vascular and peripheral nervous systems are intimately associated and functionally intertwined in the skin, this system represents an ideal model for these studies. In particular, we assessed the protective effects of VEGF-B in a model characterized by the retrograde degeneration of sensory nerves in the skin, and studied whether the effects of VEGF-B were mediated through vessels or nerves. To carefully assess the neuronal effects of VEGF-B, mice overexpressing VEGF-B and its receptor Flt1 were also generated and studied.

VEGF-B IS AN IMPORTANT NEUROPROTECTIVE FACTOR FOR SENSORY NEURONS: We observed that VEGF-B exerts potent neuroprotective effects in the sensory nervous system. More specifically, we demonstrated that VEGF-B dose-dependently antagonizes paclitaxel-induced neuronal stress, mitochondrial membrane potential decreases, and cell death in cultured primary DRG neurons. Furthermore, by using mice lacking a functional Flt1 receptor or by using selective antibodies against Flt1, we found that this effect is dependent on the tyrosine-kinase activities of the Flt1 receptor (or VEGF receptor-1). By generating transgenic mice lacking VEGF-B and overexpressing VEGF-B specifically in neurons, we also demonstrated that these mice, respectively suffered from a more aggravated or less severe distal neuropathy. Likewise, by using transgenic mice lacking a functional receptor Flt1 or overexpressing Flt1 specifically in neurons, we demonstrated that VEGF-B through Flt1 significantly modulates the severity of the distal neuropathy. Additionally, we showed that the addition of VEGF-B, either by gene transfer or as a recombinant factor, can be used to prevent the retrograde degeneration of sensory neurons, without affecting the surrounding vasculature. Overall, these data convincingly indicate that VEGF-B, instead of acting as an angiogenic factor, exerts direct neuroprotective effects through its receptor Flt1.

THERAPEUTICAL POTENTIAL OF VEGF-B FOR SENSORY NEUROPATHIES: In this study, we have shown that VEGF-B protects against the retrograde degeneration of sensory neurons. The degeneration of sensory neurons in this study was induced by the local delivery of paclitaxel, leading to a painful distal neuropathy. Distal neuropathies induced by chemotherapeutic substances, such as paclitaxel, represent a major clinical problem as they occur with relatively high frequencies. The general lack of therapeutic measures to prevent or treat neuropathy-associated disabilities can be problematic, and often represents the dose-limiting factor in oncologic regimens. Our findings thus also suggest a clinically relevant role for VEGF-B in preventing distal neuropathies.

OVERALL CONCLUSION OF BOTH STUDIES: Even though VEGF-B has been considered as a putative angiogenic agent, it might have originated as a neuronal factor during evolution. Indeed, a genomic survey of the nematode Caenorhabditis elegans, which is devoid of a vascular system, predicts a single ancestral VEGF/PDGF ligand and 4 genes resembling the VEGF receptors, named ver-1 to ver-4. Intriguingly, these 4 receptors are expressed in specialized cells of neural origin. Vessels, which developed later in evolution than nerves, thus seem to have co-opted the ancestral VEGF/PDGF family as a critical regulator of their formation. Since VEGF family members originated from a single ancestral ligand, it is possible that VEGF-B has kept its original neuronal function, and still exerts neuroprotective -rather than angiogenic- activities in higher organisms. Our data support this hypothesis and lend support to the growing awareness that VEGF-B is a neuroprotective with interesting therapeutic potential for neurodegenerative diseases, rather than an angiogenic factor.

Publications in which funding by GSKE was mentioned:

1. Novel role for vascular endothelial growth factor (VEGF) receptor-1 and its ligand VEGF-B in motor neuron degeneration. Poesen K, Lambrechts D, Van Damme P, Dhondt J, Bender F, Frank N, Bogaert E, Claes B, Heylen L, Verheyen A, Raes K, Tjwa M, Eriksson U, Shibuya M, Nuydens R, Van Den Bosch L, Meert T, D'Hooge R, Sendtner M, Robberecht W, Carmeliet P. J Neurosci. 2008 Oct 15;28(42):10451-9. Impact Factor=7.178 An electronic copy is added below.
2. Meta-analysis of VEGF variations in ALS: increased susceptibility in male carriers of the -2578AA genotype. Lambrechts D, Poesen K, Fernandez Santiago R, Al-Chalabi A, Del Bo R, Van Vught PW, Khan S, Marklund S, Brockington A, Van Marion I, Anneser J, Shaw C, Ludolph A, Leigh N, Comi G, Gasser T, Shaw PJ, Morrison K, Andersen P, Van den Berg LH, Thijs V, Siddique T, Robberecht W, Carmeliet P. J Med Genet. 2008 Jul 17. Impact Factor=5.751
3. Neuronal Flt1 receptor and its selective ligand VEGF-B protect against retrograde degeneration of sensory neurons. FASEB Journal. Dhondt J, Peeraer E, Verheyen A, Nuydens R, Buysschaert I, Poesen K, Van Geyte K, Beerens M, Shibuya M, Haigh JJ, Meert T, Carmeliet P and Lambrechts D. Accepted for publication. Impact Factor=6.401 An electronic copy is added below.

Final report of the research group of

Prof. dr. A. Goffinet

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Genetic, molecular and cellular mechanisms of cortical development

Background

In 2007-2010, work supported by the FMRE in our laboratory has been mostly focused on the role of the seven pass cadherins Celsr1, Celsr2 and Celsr3. We have also completed a study of the action of oncogene p73 during brain development and in tumor progression. We also worked on the production and preliminary characterization of mice with invalidation of Lrrn1-3, three homologous neuronal receptors containing leucine-rich repeats.

1. Role of the seven pass cadherins Celsr1-3 in cortical development

Celsr1-3 are a family of three mammalian gene orthologs of Flamingo (Tissir et al., 2002a; Tissir et al., 2002b). Celsr1 is expressed in zones of neural precursor proliferation and plays probably a role in the neuroepithelium, possibly by controlling planar polarity. Celsr2 is expressed in both the neuroepithelium and postmigratory neurons, and remains expressed in the adult brain. Celsr3 expression is restricted to postmigratory neurons, and is particularly high in the forebrain, including the cortical plate. Previous studies have addressed the role of Celsr3 (Tissir et al., 2005; Zhou et al., 2008b). This has been extended with studies of the maturation of “cortex isolé”in vivo. Furthermore, in 2009-2010, we have studied ependymal development and the physiology of hydrocephalus in Celsr2 and Celsr3 mutants, development of facial branchiomotor neurons in all Celsr1-3 mutants, and skin development in Celsr1 mutant mice.

“Neocortex isolé” *in vivo*.

We previously showed that Celsr3|Dlx mice have no internal capsule. Yet, animals are able to survice up to P20, when cortical maturation is complete, in the absence of corticothalamic, thalamocortical and subcortical connections. We have analyzed that malformation in detail and shown that the protomap forms normally and that cortical lamination also proceeds normally. During maturation, absence of connections results in neuronal loss, predominantly in deep layers 5 and 6. Surviving neurons are atrophic but with consistent morphological features. In collaboration with the teams of S. Schiffmann (ULB) and G. Cheron (ULB), we showed that neurons in cortex isolé are hypo-excitable in vitro, but fire almost normally in vivo. Furthermore, some cortical oscillations occur in this cortex, albeit with a different basic frequency than in normal cortex. A manuscript has been published in 2010 (Zhou et al., 2010).

Ependymal and hydrocephalus in Celsr2 and double Celsr2+3 mutants.

Mice homozygous for a constitutive Celsr2 mutant allele survive and most of them are fertile. They develop progressive hydrocephalus, which is exacerbated in animals that are also mutated for Celsr3 in cortex (Emx1-Cre). We attribute this to defective planar cell polarity in the ependyma and have studied cilia and ependymal markers in Celsr2, Celsr2+3 and control mice with scanning EM as well as transmission EM, and in vitro systems to analyze cilia beats. Our results demonstrate that a signal generated by Celsr2 and Celsr3 is required for docking of ciliary basal bodies to the subapical domain of ependymal cells. That study, that sheds new light on the physiopathology of hydrocephalus, has been published in 2010 (Tissir et al., 2010).

Phenotype of Celsr1 mice.

We have produced a Celsr1 conditional mutant, from which we derived a constitutive mutant using crosses with PGK-Cre mice. Heterozygotes are normal, and homozygotes have a variable phenotype, probably strongly influenced by genetic background. Some animals die during embryonic development, from defective neural tube closure. Others survive to early postnatal period, and some to adult stages. Adult Celsr1 mutants have an intriguing hair whorl phenotype on head, feet and body. Interestingly, this is similar to the skin phenotype observed in homozygous Fzd6 mutants, indicating that, like Celsr3 and Fzd3, Celsr1 and Fzd6 act in a common genetic pathway. The study of hairs in Celsr1 mutant has been published (Ravni et al., 2009). We have also initiated studies of ependymal cilia. We also showed that a progressive atrophy of the olfactory bulb develops progressively with age in Celsr1 mutant animals, due to defective migration of neuroblasts in the rostral migratory stream. That observation shows that Celsr1 is a key regulator of adult neurogenesis in the subependymal zone, a theme that will be investigated further during the coming years.

Migration of facial branchiomotor neurons

We have studied in detail the migration of facial branchiomotoneurons (FBMN) in Celsr1-3 mutant mice. In wildtype animals, FBMN are generated in rhombomere 4 and migrate through rhombomere 5 to reach rh6 where they form the facial nucleus. This migration is defective in Celsr1 mutants, where the direction of migration is drastically perturbed, as well as in Celsr2 mutants, where the trajectory of caudal migration is disturbed. Migration is normal in Celsr3, but there is neuronal death in double Celsr2+3 mutants. By studying double Celsr1+2 mutants, we could show that Celsr2 is epistatic to Celsr1. Studies of Celsr1|Isl1 mice with conditional inactivation of Celsr1 using crosses with Islet1-Cre mice showed that the action of Celsr1 is not cell autonomous. This study was made in collaboration with the laboratory of Anand Chandrasekhar (U. Missouri, USA). A first manuscript is published (Qu et al., 2010). To study in depth the role of Celsr2, we have produced a new conditional Celsr2 allele that has been validated and used to show that the action of Celsr2 during FBMN migration is FBMN autonomous.

Reviews on planar cell polarity in brain development

Invited reviews have been published, giving us opportunity to present some new hypotheses on the role of PCP in brain development and cell sheet organization. In addition to reviewing data, our view makes an attempt to incorporate cell division in the PCP picture, in which this issue is mostly ignored (Zhou et al., 2008a; Zhou et al., 2009; Tissir and Goffinet, 2010).

2. Role of oncogene DNp73 in cortical development

p73 is a gene similar to p53 and p63. It is expressed in two mRNA and protein forms. The full length form is a transcription factor that, like p53, has tumor suppressing activity. The DN form is truncated in its N-terminal moiety, inactive as a transcription factor, but able to dimerize with normal p73, p53 and p63 and behaves as a dominant negative in vitro. DNp73 is highly expressed in Cajal-Retzius neurons, the main producers of Reelin during cortical development, and it has been proposed that it regulates their survival. In order to understand better the function of DNp73 in vivo, we produced and fully validated mutant mice in which the DNp73 isoform is selectively inactivated, leaving the full length p73 fully active, and in which the Cre and EGFP sequences are knocked in the p73 locus.This allowed us to show that DNp73 is expressed only in a subset of Cajal-Retzius neurons that originate from the paleoventricle area, whereas the others originate from the cortical hem. We have also shown that DNp73 is heavily

expressed in the accessory olfactory bulb and the organ of Jacobson, as well as in the embryonic thalamic eminence. By crossing our p73 allele with ROSA-DTA mice, we have genetically ablated p73 expressing cells and this confirmed expression in subsets of cells. We also showed that DeltaNp73 is a marker for the thalamic eminence, a neurogenic region that contributes neurons to basal telencephalon and was not widely known. Observations on the role of DeltaNp73 in brain development have been published (Tissir et al., 2009). We have also tested whether DNp73 acts as an oncogene in vivo by injecting wildtype and DNp73 -/- mice with methylcholanthrene. That study allowed us to show that DeltaNp73 influences tumor development as predicted, but the effect is modest and limited to female mice. These data have been published (Ravni et al., 2010). We have also undertaken to study brains of DeltaNp73 mutant mice aged more than one year, to test whether neurofibrillary tangles accumulate in such mutants. This is done in collaboration with Prof. JP Brion (ULB).

3. Lrrn1-3

Lrrn1-3 are a family of three closely related genes that encode membrane receptors with leucine-rich repeats that are heavily and preferentially expressed in the brain, whence the acronym that stands for “Leucine-rich repeat receptor neuronal” (Taguchi et al., 1996; Almeida et al., 1998; Hamano et al., 2004; Garcia-Calero et al., 2006; Andreae et al., 2007). This family attracted our attention after a role in development was demonstrated for two ortholog genes in Drosophila, named tartan and capricious (Milan et al., 2001; Krause et al., 2006; Mao et al., 2008). Last year, we reported production of conditional and constitutive mutants for Lrrn1 and Lrrn3. In 2009, we also produced double Lrrn1+3 constitutive mutants. Both Lrrn1, Lrrn3 as well as Lrrn1+3 mutant animals are viable and fertile. A preliminary examination of brains on paraffin, Nissl stained sections did not disclose any obvious pathology, indicating that the three genes have redundant actions, which is not unexpected given the very important similarity in sequence and genomic organization and the large overlap of expression patterns. We have successfully targeted ES cells with a conditional Lrrn2 construct, and performed several sequences of microinjection in blastocysts. We have obtained several chimeras, none of which was able to transmit the mutant allele in the germline thus far. Further injections are carried out and we hope to obtain that mutant in 2011.

References

- Almeida A, Zhu XX, Vogt N, Tyagi R, Muleris M, Dutrillaux AM, Dutrillaux B, Ross D, Malfoy B, Hanash S (1998) GAC1, a new member of the leucine-rich repeat superfamily on chromosome band 1q32.1, is amplified and overexpressed in malignant gliomas. Oncogene 16:2997-3002.
- Andreae LC, Peukert D, Lumsden A, Gilthorpe JD (2007) Analysis of Lrrn1 expression and its relationship to neuromeric boundaries during chick neural development. Neural Develop 2:22.
- Garcia-Calero E, Garda AL, Marin F, Puelles L (2006) Expression of Lrrn1 marks the prospective site of the zona limitans thalami in the early embryonic chicken diencephalon. Gene Expr Patterns 6:879-885.
- Hamano S, Ohira M, Isogai E, Nakada K, Nakagawara A (2004) Identification of novel human neuronal leucine-rich repeat (hNLRR) family genes and inverse association of expression of Nbla10449/hNLRR-1 and Nbla10677/hNLRR-3 with the prognosis of primary neuroblastomas. Int J Oncol 24:1457-1466.
- Krause C, Wolf C, Hemphala J, Samakovlis C, Schuh R (2006) Distinct functions of the leucine-rich repeat transmembrane proteins capricious and tartan in the Drosophila tracheal morphogenesis. Dev Biol 296:253-264.
- Mao Y, Kerr M, Freeman M (2008) Modulation of Drosophila retinal epithelial integrity by the adhesion proteins capricious and tartan. PLoS ONE 3:e1827.
- Milan M, Weihe U, Perez L, Cohen SM (2001) The LRR proteins capricious and Tartan mediate cell interactions during DV boundary formation in the Drosophila wing. Cell 106:785-794.
- Qu Y, Glasco DM, Zhou L, Sawant A, Ravni A, Fritzsch B, Damrau C, Murdoch JN, Evans S, Pfaff SL, Formstone C, Goffinet AM, Chandrasekhar A, Tissir F (2010) Atypical cadherins Celsr1-3 differentially regulate migration of facial branchiomotor neurons in mice. J Neurosci 30:9392-9401.
- Ravni A, Tissir F, Goffinet AM (2010) DeltaNp73 transcription factors modulate cell survival and tumor development. Cell Cycle 9.
- Ravni A, Qu Y, Goffinet AM, Tissir F (2009) Planar cell polarity cadherin Celsr1 regulates skin hair patterning in the mouse. J Invest Dermatol 129:2507-2509.
- Taguchi A, Wanaka A, Mori T, Matsumoto K, Imai Y, Tagaki T, Tohyama M (1996) Molecular cloning of novel leucine-rich repeat proteins and their expression in the developing mouse nervous system. Brain Res Mol Brain Res 35:31-40.
- Tissir F, Goffinet AM (2010) Planar cell polarity signaling in neural development. Curr Opin Neurobiol 20:572-577.
- Tissir F, Bar I, Goffinet AM, Lambert De Rouvroit C (2002a) Expression of the ankyrin repeat domain 6 gene (Ankrd6) during mouse brain development. Dev Dyn 224:465-469.
- Tissir F, De-Backer O, Goffinet AM, Lambert de Rouvroit C (2002b) Developmental expression profiles of Celsr (Flamingo) genes in the mouse. Mech Dev 112:157-160.
- Tissir F, Bar I, Jossin Y, De Backer O, Goffinet AM (2005) Protocadherin Celsr3 is crucial in axonal tract development. Nat Neurosci 8:451-457.
- Tissir F, Ravni A, Achouri Y, Riethmacher D, Meyer G, Goffinet AM (2009) DeltaNp73 regulates neuronal survival in vivo. Proc Natl Acad Sci U S A 106:16871-16876.
- Tissir F, Qu Y, Montcouquiol M, Zhou L, Komatsu K, Shi D, Fujimori T, Labeau J, Tyteca D, Courtoy P, Poumay Y, Uemura T, Goffinet AM (2010) Lack of cadherins Celsr2 and Celsr3 impairs ependymal ciliogenesis, leading to fatal hydrocephalus. Nat Neurosci 13:700-707.
- Zhou L, Goffinet AM, Tissir F (2008a) [Role of the cadherin Celsr3 in the connectivity of the cerebral cortex]. Med Sci (Paris) 24:1025-1027.
- Zhou L, Qu Y, Tissir F, Goffinet AM (2009) Role of the atypical cadherin Celsr3 during development of the internal capsule. Cereb Cortex 19 Suppl 1:i114-119.
- 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.
- Zhou L, Bar I, Achouri Y, Campbell K, De Backer O, Hebert JM, Jones K, Kessaris N, de Rouvroit CL, O’Leary D, Richardson WD, Goffinet AM, Tissir F (2008b) Early forebrain wiring: genetic dissection using conditional Celsr3 mutant mice. Science 320:946-949.

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Cellular crosstalks in amyotrophic lateral sclerosis : influence of neuroinflammation on astrocyte function and stem cell differentiation

Amyotrophic lateral sclerosis (ALS) is a devastating neurodegenerative disease. It essentially concerns the cerebral cortex, the brainstem and the spinal cord, where motor neurons progressively degenerate, causing muscle weakness in early stages and paralysis at later stages. Most frequently, ALS appears as sporadic cases, and biochemical studies have revealed several processes involved in the disease onset and progression. Thus, postmortem studies of ALS patients demonstrated high levels of oxidation and nitration of proteins, DNA, and lipids, indicating that free radical toxicity leads to nerve damages. Besides, glutamate excitotoxicity coinciding with insufficient glutamate clearance and local inflammation were also evidenced. In addition to these sporadic cases, epidemiology studies have highlighted a significant proportion of familial ALS cases in which mutations in the Cu/Zn-superoxide dismutase-1 gene (SOD1) have been identified. Expression of mutant SOD1 genes in transgenic rodents causes progressive paralysis and recapitulates the general features of ALS. Therefore, studies using these transgenic models provide insights into the mechanisms of motor neuron degeneration, which are useful in the design of putative treatment strategies. While the SOD1 mutations are associated with a gain of function, the loss of motor neurones appears also related to protein misfolding and aggregation, improper subcellular localization, and deficient catabolism. Also, as in the sporadic cases, production of free radicals, glutamate toxicity and mitochondrial dysfunction are also thought to play pathogenic roles in the transgenic rodent models. Finally, neuroinflammation involving accumulation of large numbers of activated microglia and astrocytes was observed in the brainstem and spinal cord of ALS cases and in mouse models of the disease.
Inflammatory responses within the central nervous system largely contribute to pathophysiology of several neurodegenerative diseases. Microglial cells play a crucial role in this process as these cells release a variety of inflammatory mediators including cytokines, prostanglandins, and reactive oxygen species which have diverse influences on the viability of neuronal cells. Focusing on amyotrophic lateral sclerosis, the aim of this project was to examine the influence of activated microglial cells and inflammatory mediators on protective/repair processes. Using cell models derived from healthy animals and from a transgenic rat strain developing the disease, the following aspects have been examined :

- Modulation of neuroinflammation on the activity of astrocytes and in particular their capacity to indirectly modulate glutamate transmission. In close relation with this line of research, we have also examined the pharmacological manipulation of the glutamate transmission
- Role of neuroinflammation in the differentiation and recruitment of stem cells

1. Pharmacological manipulation of astroglial glutamate transporters

Astrocytes are the most abundant cells found in the central nervous system. Beside providing a physical support to neurons, these cells play key roles in the control of ionic and metabolic homeostasis of the brain and the spinal cord in physiological conditions. They contribute to the regulation of neurotransmission through the release of several modulators but also through the active uptake of neuronally released glutamate. Excess of extracellular levels of this excitatory neurotransmitter causes profound damages to neuronal cells. The mechanisms underlying this glutamate-mediated excitotoxicity are complex, but primarily involve activation of proteases, generation of reactive oxygen species and mitochondrial

calcium overload, that act in synergy to damage cellular proteins, membranes, DNA and mitochondrial function, resulting in necrosis, apoptosis or both. While glutamate is essentially released by neurons, the sodium-dependent glutamate uptake by glial cells is the only natural mechanism of glutamate clearance from the extracellular space. The two major glial glutamate transporters are GLAST and GLT-1. Astrocytes further process glutamate to glutamine, which is transferred to neuronal cells to regenerate glutamate. Contradicting previous believes suggesting that glutamate transporters are always active, it is now known that uptake by astrocytes is dynamically regulated. Altered activity, cell trafficking and expression of these glial proteins appear to be closely associated with the development of several neurological disorders. A better understanding of the molecular and cellular mechanisms contributing to the regulation of glutamate handling by astrocytes (and putative pathological dysfunctions) should help to define new therapeutic approaches for treating excitotoxicity.

During the last 3 years, we have focused this part of our research on the regulation of glial glutamate transporters by neuropeptides from the VIP/PACAP family. Indeed, these peptides are widely expressed in the central and peripheral nervous systems. They act as neurotransmitters or neuromodulators and are endowed with neuroprotective and neurotrophic properties. Indeed, they promote early embryonic development and stimulate neuronal growth and survival. In relationship with the control of glutamate toxicity, the neuroprotective properties of these peptides were demonstrated in a model of excitotoxic lesions in newborn mice. Noteworthy, while some of the neuroprotective effects appear to result from a direct stimulation of receptors located on neuronal cells, protective activities have also been assigned to an influence on glial cells. The key finding of our first paper was the demonstration that the peptide PHI is able to induce a rapid and substantial increase in glutamate transporter activity in astrocytes. The peptide significantly increased the velocity of substrate uptake with a greater potency than VIP and investigations realized with available antagonists lead us to speculate that VPAC2 receptors could play a major role in the upregulation process. Finally, this upregulation was found to essentially reflect an increased cellular trafficking of the GLAST which is recruited at the cell surface of astrocytes, contributing to the increase in substrate uptake. These studies shed light on the possibility to consider the VIP/PACAP peptide family as either a key biological system which might be affected in nervous diseases or as a putative valuable target for pharmacological approaches of these disorders.

– Goursaud S., Maloteaux J.M. & Hermans E. (2008) Activation of VIP/PACAP type 2 receptor by the peptide histidine isoleucine in astrocytes influences GLAST-mediated glutamate uptake. J. Neurochem. 105, 1165-1175

In a related study, we have more specifically investigated the influence of these peptides on the regulation of the glutamate transporter GLT-1, both on astrocytes from wild-type animals and on astrocytes derived from the model of ALS. Indeed, preliminary observations indicated an altered expression of VPAC receptors found in astrocytes from these transgenic animals. This second paper provide evidence that the dysfunction of GLT-1 which is typically observed in animal models of ALS is also detected in primary cultured astrocytes from cortical grey matter tissue of these transgenic animals. Besides, the specific induction of the GLT-1b isoform in these cells constitutes original findings. Even though the molecular mechanisms supporting these regulatory crosstalks between VIP/PACAP transmission and glutamate handling in astrocytes remain unidentified, these data confirm the neuroprotective potential of these neuropeptides. The possibility to specifically regulate a single isoform of the high affinity transporter GLT-1 is an unprecedented observation which sheds light on new perspectives for the pharmacological manipulation of glutamate transmission in diseases such as ALS.

– Goursaud S., Maloteaux J.M., Hermans E. (2009) Distinct expression and regulation of the glutamate transporter isoforms GLT-1a and GLT-1b in cultured astrocytes from a rat model of amyotrophic lateral sclerosis (hSOD1G93A). Neurochem. Int. 55, 28-34

During this project, we have also developed an original research aiming at characterizing the properties of astrocytes from distinct regions of the nervous system. Indeed, as widely accepted for neuronal cells, it is likely that astrocytes are endowed with distinct functional properties, depending on their localization in the central nervous system. Focusing on the properties related to the regulation of glutamate transmission, we have compared astrocyte cultures derived from white matter (corpus callosum) and grey matter tissues (cortical structures). These populations of astrocytes showed clearly distinct phenotypes, adopting stellate or protoplasmic morphologies, respectively. In addition, white matter astrocytes showed high densities of the intermediate filament proteins GFAP, vimentin and nestin. At our surprise, the glutamate transporters GLAST and GLT-1, as well as glutamine synthetase, were found to be expressed at higher levels in white matter compared to grey matter astrocytes. Consistent with this aspartate uptake capacity was 3-4 fold higher in white matter cells, and the use of specific inhibitors revealed a substantial activity of GLT-1, contrasting with grey matter cells where this transporter appeared poorly functional. In addition, expression of type 5 metabotropic glutamate receptors was considerably higher in white matter astrocytes where the agonist DHPG triggered a large release of intracellular calcium. Differences in these astrocyte cultures were also observed when exposed to experimental conditions that trigger glial activation. This study highlights typical features of cultured astrocytes derived from white matter tissues, which appear constitutively adapted to handle excitotoxic insults. Moreover, the expression and activity of the astroglial components involved in the control of glutamatergic transmission are reinforced when these cells are maintained under conditions mimicking a gliotic environment.

– Goursaud S., Kozlova E., Maloteaux J.M., Hermans E. (2009) Cultured astrocytes derived from corpus callosum or cortical grey matter show distinct glutamate handling properties. J. Neurochem. 108, 1442-1452.

The robust expression of glutamate targets in the white matter tissue has encouraged us to further investigate the regulation of the glial glutamate transporters in the corpus callosum of ALS rats. Although the dysfunction affecting motor regions of the central nervous system are best characterized, altered activities in the white matter has also been evidenced. In very recent experiments, we have demonstrated the existence of complex regulatory processes affecting the expression and activity of GLT-1a and GLT-1b in this tissue during the progression of ALS and that the peptide from the VIP/PACAP family can profoundly affect these processes. We have accumulated evidence for a role of caspase enzymes in the breakdown of the glutamate this transporter in the model of ALS which can be inhibited by the neuropeptide. This study is now completed and submitted for publication.

– Goursaud S., Focant M.C., Berger J., Nizet Y., Maloteaux J.M., Hermans E. (2011) Up-regulation of glutamate transport by peptide histidine isoleucine in the corpus callosum of a rat model of amyotrophic lateral sclerosis (hSOD1G93A) involves inhibition of GLT-1a inactivation by caspase-3. Submitted

2. Influence of activated microglia and inflammation on the activity of astrocytes.

Representing approximately 10% of the cells of the brain parenchyma, the microglial cells (so-called macrophages of the CNS) constitute the first line of defence against pathogens and brain tissue injuries. Indeed, responding to diverse alterations in their local environment, microglia undergo noticeable morphological changes and modifications in their metabolic activities and gene expression. This phenotypic switch is accompanied by the release of a large variety of pro- and anti-inflammatory mediators, including cytokines, reactive oxygen species, nitric oxide (NO) prostanoids and complement factors. While anti-inflammatory mediators could contribute to neuroprotection, proinflammatory mediators appear to actively participate to the degeneration and the death of neurones, and their

involvement in diverse neurological disorders is well documented. With respect to the neuroprotective roles achieved by astrocytes, the control of glutamate transmission has received considerable interest and the physiological and pharmacological regulation of glial glutamate transporters has been deeply investigated. Proinflammatory mediators potentially released from activated microglia in pathological conditions have been shown to influence the efficiency of glial glutamate uptake. This reinforces our hypothesis for a mechanistic link between neuroinflammation and excitotoxicity, which frequently co-exist in neurological disorders.

As impaired glutamate uptake by astrocytes is a well characterized feature of ALS and other nervous disorders with an inflammatory component, we have investigated the involvement of neuroinflammation in this functional deficit. This part of the project was initiated in primary cultures of astrocytes and microglia cells derived from the rat cortex. By transferring conditioned media between cultures, we have examined the influence of soluble mediators released from activated microglia on the expression and activity of astrocyte glutamate transporters. This has involved functional studies of substrate uptake as well as immunodetections of the transporters and quantitative PCR analysis of transporter transcripts. Our experimental data on cells from wild type animals have revealed an unexpected increased capacity of glutamate uptake in astrocytes exposed to an inflammatory environment. These experiments were first realised with medium collected from lipopolysaccharide-activated microglia. In a second stage, we were able to demonstrate that the cytokine TNFalpha was causing the same regulation process. Finally, we have recently shown that TNFalpha distinctly affects the expression of the two isoforms of the high affinity glutamate transporter GLT-1 (isoforms a and b). This observation appears particularly relevant, considering the putative differential role played by these closely related transporter in ALS.

– Tilleux S., Goursaud S., Hermans E. (2009) Selective up-regulation of GLT-1 in cultured astrocytes exposed to soluble mediators released by activated microglia. Neurochem. Int. 55, 35-40
– Focant M.C., Goursaud S., Nizet Y., Hermans E. (2011) Differential regulation of C-terminal splice variants of the glutamate transporter GLT-1 by tumor necrosis factor-alpha in primary cultures of astrocytes. Submitted

3. Role of neuroinflammation in the differentiation and recruitment of stem cells

The modest capacity of endogenous repair processes in the central nervous system justifies the broad interest in the development of effective stem cell based therapies for neurodegenerative disorders and other acute or chronic lesions. Motivated by the ambitious expectation to achieve functional neuronal replacement, several studies have already evidenced a potential benefit of stem cell grafts in animal models of human disorders. Nevertheless, growing evidence suggests that the effects orchestrated by stem cells, in most experimental cases, are not necessarily associated with the generation of new neurons. This hypothesis correlates with the versatile properties of adult and embryonic stem cells. When introduced into the lesioned CNS, non-differentiated stem cells can have a positive influence through intrinsic neuroprotective capacities related to the production of neurotrophic factors, stimulation of endogenous neurogenesis and the modulation of neuroinflammation. Besides, stem cells are also endowed with a multipotent differentiation profile, suggesting that a positive outcome could result from the replacement of non-neuronal cell types, in particular astrocytes and oligodendrocytes.

The potential benefit of stem cell therapies specifically in the treatment of motor neuron diseases has been highlighted in several recent publications. Indeed, in ALS models, highly convincing are those studies showing that intraspinal injections of neural stem cells delayed the onset and prolonged animal survival through a neuroprotective potential. Exciting data suggest a non-autonomous mechanism for motor neuron death, implicating glial cells in the progression of the disease. Hence, targeting glial cells activation appears as a promising strategy to slow down ALS progression. Altogether, these studies

suggest that a positive outcome could be obtained by replacing or enriching abnormal glia with healthy glial cells. With respect to accumulating data showing that mesenchymal stem cells can transdifferentiate into astrocytes when grafted into the central nervous system, these cells represent an attractive tool for cell therapies aiming at ameliorating the neuronal environment. As an alternative to neural stem cells, adult mesenchymal stem cells have been shown to exhibit neuroprotective properties when introduced into the degenerating central nervous system through different putative mechanisms including secretion of growth factors and transdifferentiation.

In this part of our project, we have injected mesechymal stem cells into the cerebrospinal fluid of symptomatic hSOD1g93a rats, a transgenic animal model of familial ALS, expressing a mutated form of the human superoxide dismutase. Mesenchymal stem cells were found to infiltrate the nervous parenchyma and migrate substantially into the ventral grey matter where motor neurons degenerate. Even though overall astrogliosis was not modified, mesenchymal stem cells differentiated massively into astrocytes at the site of degeneration. The intrathecal delivery of mesenchymal stem cells and the subsequent generation of healthy astrocytes at symptomatic stage decreased motor neuron loss in the lumbar spinal cord, preserving motor functions and extending the survival of hSOD1g93a rats. This neuroprotection was correlated with decreased inflammation, as evidenced by a lower proliferation of microglial cells and a reduction of cox-2 and nox-2 expression. Together, these data highlight the protective capacity of adult mesenchymal stem cell-derived astrocytes when grafted into the central nervous system and illustrate an attractive strategy to target excessive inflammation in ALS.

An open question that remains to be answered is to clarify whether local inflammation within the nervous parenchyma is playing a role in the control of stem cell recruitment and differentiation. In a recent project which is still ongoing, we have delivered mesenchymal stem cells in the lumbar spinal cord fluid of adult rats undergoing partial sciatic nerve ligation. This surgery provokes a massive inflammatory response in the dorsal horn of the spinal cord, leading to the development of neuropathic pain. Up to now, we have not been able to evidence any change in the installation of this pain process in response to mesenchymal stem cells injection. Ongoing experiments aim at examining the local recruitment of the cells or the modulation of the endogenous inflammatory response.

– Boucherie C., Hermans E. (2009) Adult stem cell therapies for neurological disorders: benefits beyond neuronal replacement? J. Neurosci. Res. 15, 1509-21
– Boucherie C., Schäfer S., Lavand'homme P., Maloteaux J.M., Hermans E. (2009) Chimerisation of astroglial population in the lumbar spinal cord after mesenchymal stem cells transplantation prolongs survival in a rat model of amyotrophic lateral sclerosis. J. Neurosci. Res. 87, 1509-1521

Publications Group of Emmanuel HERMANS, UCL

Mentioning the support of the FMRE (project 2008-2010)

- BOUCHERIE C., HERMANS E. (2009) Adult stem cell therapies for neurological disorders: benefits beyond neuronal replacement? J. Neurosci. Res. 15, 1509-21
  IF 2,986
- TILLEUX S., GOURSAUD S., HERMANS E. (2009) Selective up-regulation of GLT-1 in cultured astrocytes exposed to soluble mediators released by activated microglia. Neurochem. Int. 55, 35-40
  IF 3,541
- GOURSAUD S., KOZLOVA E., MALOTEAUX J.M., HERMANS E. (2009) Cultured astrocytes derived from corpus callosum or cortical grey matter show distinct glutamate handling properties. J. Neurochem. 108, 1442-1452.
  IF 3,999
- BOUCHERIE C., SCHÄFER S., LAVAND'HOMME P., MALOTEAUX J.M., HERMANS E. (2009) Chimerisation of astroglial population in the lumbar spinal cord after mesenchymal stem cells transplantation prolongs survival in a rat model of amyotrophic lateral sclerosis. J. Neurosci. Res. 87, 1509-1521
  IF 2,986
- GOURSAUD S., MALOTEAUX J.M., HERMANS E. (2009) Distinct expression and regulation of the glutamate transporter isoforms GLT-1a and GLT-1b in cultured astrocytes from a rat model of amyotrophic lateral sclerosis (hSOD1G93A). Neurochem Int. 55, 28-34
  IF 3,541
- VANHOUTTE N., ABARCA-QUINONES J., JORDAN B.F., MALOTEAUX J.M. HERMANS E. (2009) Enhanced expression of the high affinity glutamate transporter GLT-1 in C6 glioma cells delays tumour progression in rat. Exp. Neurol. 218, 56-63
  IF 3,914
- BERGER J.V., DEUMENS R., GOURSAUD S., SCHÄFER S., LAVAND'HOMME P., JOOSTEN E.A., HERMANS E. (2011) Enhanced neuroinflammation and pain hypersensitivity after peripheral nerve injury in rats expressing mutated superoxide dismutase 1. Submitted
- GOURSAUD S., FOCANT M.C., BERGER J., NIZET Y., MALOTEAUX J.M., HERMANS E. (2011) Up-regulation of glutamate transport by peptide histidine isoleucine in the corpus callosum of a rat model of amyotrophic lateral sclerosis (hSOD1G93A) involves inhibition of GLT-1a inactivation by caspase-3. Submitted
- FOCANT M.C., GOURSAUD S., NIZET Y., HERMANS E. (2011) Differential regulation of C-terminal splice variants of the glutamate transporter GLT-1 by tumor necrosis factor-alpha in primary cultures of astrocytes. Submitted

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The representation of three-dimensional shape in posterior parietal and premotor cortex of the rhesus monkey

We investigated the coding of 2D- and 3D shape in parietal area AIP. We recorded single-unit activity in two passively fixating rhesus monkeys. The stimuli were images of real-world objects (both living and man-made objects) taken from two slightly different viewpoints. The two images of each object were presented alternately on a display to create the pattern of binocular disparity as present in the real object (=congruent disparity condition). To create the opposite pattern of disparities we exchanged the images between the eyes (= incongruent disparity condition). Control stimuli consisted of monocular presentations of the images, and binocular presentations of either the left-eye or the right-eye image. We searched for responsive AIP neurons during presentation of both congruent and incongruent stereo stimuli at the fixation point. If the neuron was responsive to at least one of the stimuli, we selected the object image evoking the strongest response (termed the preferred stimulus) and a different object image to which the neuron responded weakly (termed the non-preferred stimulus) for subsequent testing. To assess the effect of disparity, the preferred and non-preferred object images were presented in congruent stereo mode, incongruent stereo mode and no-stereo mode (both the left eye – and the right eye image), together with monocular presentations to the left and the right eye separately (Disparity test). Whenever a neuron showed selectivity to one of the stereo-conditions, we ran a Position-in-Depth Test, in which the congruent and incongruent stereo stimuli of the preferred object image were presented at five different positions in depth, ranging from -1° (near) to +1° (far) . All recorded neurons showing object-selective responses were subsequently studied in the Shape Test, consisting of the preferred and nonpreferred object images in congruent and incongruent stereo mode, the corresponding area-equalized 2D images (no-stereo mode) and the three contour versions (silhouette, outline and line drawing).

To assess the position invariance of neurons showing object-selective responses in the previous tests, we presented the preferred and nonpreferred object images in their optimal configuration (either congruent stereo, incongruent stereo or no-stereo mode) at 9 positions in the visual field spaced 5 deg apart around the fixation point (Position test). In this test all images were equal in surface area. Finally, a Size Test was also performed comparing responses to the area-equalized preferred and the non-preferred object images in their optimal configuration during foveal presentations with different sizes (12°, 9°, 6° and 3°). For most neurons the order of testing was: disparity test, position-in-depth test, shape test, position test and size test.

We recorded the activity of 140 AIP neurons responding selectively to at least one of the stimuli in the Search Test (103 neurons in monkey H, 37 neurons in monkey M). Approximately half of our population of AIP neurons was disparity-selective (68/140: 48.6%; 50.5% in monkey H, 43.2% in monkey M). For all stereo-selective cells, disparity selectivity could not be simply explained by the response to the monocular images, and moderate but significant preference for congruent disparities was observed ($_{Ncongruent}$=43; $_{Nincongruent}$=25). Ninety-one neurons tested with two-dimensional images (91/97: 93.8%) showed significant selectivity to at least one of the stimulus types presented in the Shape Test (2D images, silhouette, outline and line drawing). Nine neurons (9/91: 9.9%) were only selective for the two-dimensional images containing surface information while the majority of shape-tuned cells (90.1%) were selective for one or more of the two-dimensional contours.

A total of 13 out of 49 neurons tested in the Position test were classified as strongly position-invariant in our study (13/49: 26.5%; MNRW ≤ 0.50), while 12 cells presented weak position invariance (12/49: 24.5%; MNRW < 0.75). In contrast, 24 cells showed position dependency (24/49: 49%) presenting highly irregular RFs with additional local maxima outside the global maximum.

The possible interaction between size and shape selectivity was measured in 33 AIP neurons. In the Size Test, the same two images selected for the Shape-Tuning and the Shape-Position Test were now presented foveally in 4 different sizes: 12deg, 9deg, 6deg and 3deg. Considering the AIP population at the Size Test, 57.6% neurons (19/33) showed significant shape selectivity for more than one size, and was therefore considered as size-invariant. Eight of these neurons preserved their selectivity across all sizes (8/19: 42.1%), being classified as strongly size-invariant. Thus AIP neurons exhibit 2D-shape selectivity that can be tolerant to changes in frontoparallel position and retinal size.

In a second study, we trained three rhesus monkeys in a delayed visually-guided grasping task. In this task, an object is illuminated on a carroussel in front of the monkey, and after a delay of 1000 ms a sound indicates that the animal has to grasp the object, lift and hold the object for 500 ms until the reward is administered. As a control, we also employed a grasping task in the dark (memory-guided grasping). The second task in this study was a passive fixation task on a display while disparity-defined curved surfaces were presented. We wanted to determine to what extent 3D-shape selective neurons in areas AIP and F5a are also involved in visually-guided grasping. We recorded single- and multiunit activity in AIP and F5a during presentation of the 3D surfaces. When we encountered 3D-shape selective neural activity, we switched to the grasping task and recorded single- and multiunit activity. In almost every experiment (14/15), the neural activity at the 3D-shape selective site was also responsive during grasping. Remarkably, we observed all three types of previously described AIP and F5a neurons: visual-dominant, visuo-motor and motor-dominant. The latter type of neuron was active during both visually-guided and memory-guided grasping in complete darkness. We also observed visual-dominant neurons in F5a, a neuronal subtype that has not been described before. Thus 3D-shape selective neurons in AIP and F5a are also involved in visually-guided and memory-guided grasping of objects.

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Characterization of spontaneous brain activity nin unconscious participants by multimodal functional neuroimaging (EEG, fMRI, PET)

1. Introduction

Our project aimed at characterizing the spontaneous brain activity and its impact on perception and cognition in humans, especially when the level or content of consciousness is altered. Our work focused on both physiological conditions (sleep) and pathological situations (unconscious patients).

2. Characterization of spontaneous brain activity during normal human sleep

In humans, sleep consists of two drastically different states : non rapid eye movement (NREM) sleep and rapid eye movement (REM) sleep. The cellular processes that generate the various NREM sleep oscillations (spindles, K-complexes, delta and slow rhythms) are described in great detail in animals. In humans, positron emission tomography (PET) allows the description of the gross functional neuroanatomy of NREM sleep. However, the poor temporal resolution of this technique does not allow the accurate characterization of the cerebral responses to the transient sleep events described above. For instance, slow wave activity is classically related to a decrease in cerebral energy metabolism and blood flow when measured by PET, whereas we were able to identify increases in activity associated with slow waves during NREM sleep.

2.1. Regional brain activity related to slow waves during non rapid eye movement sleep

Dang-Vu TT, Schabus M, Desseilles M, Albouy G, Boly M, Darsaud A, Gais S, Rauchs G, Sterpenich V, Vandewalle G, Carrier J, Moonen G, Balteau E, Degueldre C, Luxen A, Phillips C, Maquet P (2008) Spontaneous neural activity during human slow wave sleep. Proc Natl Acad Sci U S A 105:15160-15165.

Slow wave sleep (SWS) is associated with spontaneous brain oscillations that are thought to participate in sleep homeostasis and to support the processing of information related to the experiences of the previous awake period. At the cellular level, during SWS, a slow oscillation (<1 Hz) synchronizes firing patterns in large neuronal populations and is reflected on electroencephalography (EEG) recordings as large-amplitude, low-frequency waves. By using simultaneous EEG and event-related functional magnetic resonance imaging (fMRI), we characterized the transient changes in brain activity consistently associated with slow waves (>140 microV) and delta waves (75-140 microV) during SWS in 14 non-sleep-deprived normal human volunteers. Significant increases in activity were associated with these waves in several cortical areas, including the inferior frontal, medial prefrontal, precuneus, and posterior cingulate areas. Compared with baseline activity, slow waves are associated with significant activity in the parahippocampal gyrus, cerebellum, and brainstem, whereas delta waves are related to frontal responses. No decrease in activity was observed. This study demonstrates that SWS is not a state of brain quiescence, but rather is an active state during which brain activity is consistently synchronized to the slow oscillation in specific cerebral regions. The partial overlap between the response pattern related to SWS waves and the waking default mode network is consistent with the fascinating hypothesis that brain responses synchronized by the slow oscillation restore microwake-like activity patterns that facilitate neuronal interactions.

2.2. Interplay between spontaneous and induced brain activity during normal human sleep

We recently conducted an experiment assessing sound processing during NREM sleep to explore the apparent discrepancy between human and animal data concerning this issue. Recent evidence points out that cortical processing of external information still persists during sleep in humans. Cellular recordings in animals however suggest that the brain reactivity to external stimulation is inhibited during specific phasic activities of non-rapid-eye-movement (NREM) sleep, especially during sleep spindles. Using simultaneous electroencephalography (EEG) / functional magnetic resonance imaging (fMRI) in 13 non-sleep deprived normal human volunteers, the present study aims at assessing how spindles modulate the processing of auditory stimuli at the systems level in humans. Brain responses to pure tones were categorized in 3 types according to their occurrence during waking (TW), NREM sleep but outside spindles (TN), or spindles (TS). Expectedly, TW and TN activated the thalamus and the primary auditory cortex. Among TN, the primary auditory cortex was even more activated when the tone was followed by an evoked K-complex. By contrast, no significant brain activation was associated with TS. These results confirm that external information can be conveyed up to the cortical level during NREM sleep, a process which is associated with the production of evoked K-complexes. Furthermore, our findings demonstrate that spindles block the processing of sensory information during NREM sleep in humans, possibly contributing to the preservation of sleep continuity. **These results are summarized in a publication which is submitted for publication.**

2.3. Methodological developments required by EEG/fMRI studies

High quality EEG recordings during fMRI acquisitions required the development of novel methods for cleaning raw data from scanner and pulse-related artifacts.

Leclercq Y, Balteau E, Dang-Vu T, Schabus M, Luxen A, Maquet P, Phillips C (2009) Rejection of pulse related artefact (PRA) from continuous electroencephalographic (EEG) time series recorded during functional magnetic resonance imaging (fMRI) using constraint independent component analysis (cICA). Neuroimage 44:679-691.

Rejection of the pulse related artefact (PRA) from electroencephalographic (EEG) time series recorded simultaneously with fMRI data is difficult, particularly during NREM sleep because of the similarities between sleep slow waves and PRA, in both temporal and frequency domains and the need to work with non-averaged data. Here we introduce an algorithm based on constrained independent component analysis (cICA) for PRA removal. This method has several advantages: (1) automatic detection of the components corresponding to the PRA; (2) stability of the solution and (3) computational treatability. Using multichannel EEG recordings obtained in a 3 T MR scanner, with and without concomitant fMRI acquisition, we provide evidence for the sensitivity and specificity of the method in rejecting PRA in various sleep and waking conditions.

3. Regulation of sleep and wakefulness

Waking performance results at any point in time from the interaction between a circadian signal and the sleep need accrued during the preceding waking period. The neural correlates of this interaction a hardly known, especially in humans. During the last years, we initiated a new research project which precisely aimed at characterizing the neural correlates of the regulation of sleep and wakefulness

3.1. Influence of the chronotype on daytime performance and its neural correlates

Schmidt C, Collette F, Leclercq Y, Sterpenich V, Vandewalle G, Berthomier P, Berthomier C, Phillips C, Tinguely G, Darsaud A, Gais S, Schabus M, Desseilles M, Dang-Vu TT, Salmon E, Balteau E, Degueldre C, Luxen A, Maquet P, Cajochen C, Peigneux P (2009) Homeostatic sleep pressure and responses to sustained attention in the suprachiasmatic area. Science 324:516-519.

See also :

Schmidt C, Peigneux P, Maquet P, Phillips C (2010) Response to comment on "Homeostatic Sleep Pressure and Responses to Sustained Attention in the Suprachiasmatic Area". Science 316:309c-.

Throughout the day, cognitive performance is under the combined influence of circadian processes and homeostatic sleep pressure. Some people perform best in the morning whereas others are more alert in the evening. These chronotypes provide a unique way to study the effects of sleep/wake regulation on the cerebral mechanisms supporting cognition. Using functional magnetic resonance imaging in extreme chronotypes we found that maintaining attention in the evening was associated with higher activity in evening than morning chronotypes in a region of the locus coeruleus and in a suprachiasmatic area (SCA) including the circadian master clock. Activity in the SCA decreased with increasing homeostatic sleep pressure. This result shows the direct influence of the homeostatic and circadian interaction on the neural activity underpinning human behavior.

3.2. Neural bases of genetically-determined vulnerability to sleep loss

Vandewalle G, Archer SN, Wuillaume C, Balteau E, Degueldre C, Luxen A, Maquet P, Dijk DJ (2009) Functional magnetic resonance imaging-assessed brain responses during an executive task depend on interaction of sleep homeostasis, circadian phase, and PER3 genotype. J Neurosci 29:7948-7956.

Cognition is regulated across the 24-h sleep-wake cycle by circadian rhythmicity and sleep homeostasis through unknown brain mechanisms. We investigated these mechanisms in an fMRI study of executive-function during a normal sleep-wake cycle and during sleep-loss. The study population was stratified according to homozygosity for a variable-number (4 or 5) tandem-repeat polymorphism in the coding region of the clock gene PERIOD3 (PER3). This polymorphism confers vulnerability to sleep-loss and circadian misalignment through its effects on sleep homeostasis. In the less-vulnerable genotype, no changes were observed in brain responses during the normal-sleep wake cycle. During sleep-loss, these individuals recruited supplemental anterior frontal, temporal and subcortical regions and thalamo-prefrontal connectivity was enhanced, while executive function was maintained. By contrast, in the vulnerable genotype, activation in a posterior prefrontal area was already reduced when comparing the evening to the morning during a normal sleep-wake cycle. Furthermore, in the morning after a night of sleep-loss, widespread reductions in activation in prefrontal, temporal, parietal and occipital areas were observed in this genotype. These differences occurred in the absence of genotype-dependent differences in circadian phase. The data show that dynamic changes in brain responses to an executive-task evolve across the sleep-wake and circadian cycles in a regionally-specific manner that is determined by a polymorphism which affects sleep homeostasis. The findings support a model of individual differences in executive control, in which the allocation of prefrontal resources through thalamic activation is constrained by sleep pressure and circadian phase.

3.3. Non classical photoreception

The activity of the master clock, located in the suprachiasmatic nucleus (SCN), spontaneously fluctuates with a period of about 24 hours and is synchronized by light to the alternation of days and night. This synchronization depends upon a new photoreception system which has recently been described and is sensitive to ambient light levels. It involves photosensitive retinal ganglion cells which directly project on the SCN. In addition, non visual effects of light are not restricted to adjusting the circadian phase. They can elicit rapid alerting effects and can therefore swiftly modify regional brain responsiveness. Using fMRI, we were the first to describe the non visual effects of lights onto regional brain responses.

Vandewalle G, Maquet P, Dijk DJ (2009) Light as a modulator of cognitive brain function. Trends Cogn Sci 13:429-438.

Humans are a diurnal species usually exposed to light while engaged in cognitive tasks. Light not only guides performance on these tasks through vision but also exerts non-visual effects that are mediated in part by recently discovered retinal ganglion cells maximally sensitive to blue light. We review recent neuroimaging studies which demonstrate that the wavelength, duration and intensity of light exposure modulate brain responses to (non-visual) cognitive tasks. These responses to light are initially observed in alertness-related subcortical structures (hypothalamus, brainstem, thalamus) and limbic areas (amygdala and hippocampus), followed by modulations of activity in cortical areas, which can ultimately affect behaviour. Light emerges as an important modulator of brain function and cognition.

Vandewalle G, Schwartz S, Grandjean D, Wuillaume C, Balteau E, Degueldre C, Schabus M, Phillips C, Luxen A, Dijk DJ, Maquet P (2010) Spectral quality of light modulates emotional brain responses in humans. Proc Natl Acad Sci U S A 107:19549-19554.

Light therapy is an effective treatment for mood disorders. Here we asked whether exposure to light influences emotional brain function. During functional magnetic resonance imaging, healthy volunteers listened to emotional and neutral voices while being exposed to alternating 40s-periods of blue or green ambient light. Blue (relative to green) light increased responses to emotional stimuli in the voice area of the temporal cortex and in the hippocampus. During emotional processing, the functional connectivity between the voice area, the amygdala and the hypothalamus was selectively enhanced in the context of blue illumination. These results demonstrate the acute influence of light on emotional brain processing and identify a network merging affective and irradiance/light information. The superiority of blue over green light suggests the involvement of melanopsin-dependent photoreception in emotion regulation.

4. Influence of sleep on memory

Recent memories are actively reprocessed during sleep and this offline processing is involved in memory consolidation. During the last years, we have explored a number of different memory systems. Our last works deal with motor sequence learning, spatial memory, emotional memory, as well as more unusual aspects of memory, such as false memories and insight.

4.1. Correlates of sleep-related offline motor sequence processing

Albouy G, Sterpenich V, Balteau E, Vandewalle G, Desseilles M, Dang-Vu T, Darsaud A, Ruby P, Luppi PH, Degueldre C, Peigneux P, Luxen A, Maquet P (2008) Both the hippocampus and striatum are involved in consolidation of motor sequence memory. Neuron 58:261-272.

Functional magnetic resonance imaging (fMRI) was used to investigate the cerebral correlates of motor sequence memory consolidation. Participants were scanned while training on an implicit oculomotor sequence learning task and during a single testing session taking place 30 min, 5 hr, or 24 hr later. During training, responses observed in hippocampus and striatum were linearly related to the gain in performance observed overnight, but not over the day. Responses in both structures were significantly larger at 24 hr than at 30 min or 5 hr. Additionally, the competitive interaction observed between these structures during training became cooperative overnight. These results stress the importance of both hippocampus and striatum in procedural memory consolidation. Responses in these areas during training seem to condition the overnight memory processing that is associated with a change in their functional interactions. These results show that both structures interact during motor sequence consolidation to optimize subsequent behavior.

Intrigued by these results, we checked in a follow up study whether this hippocampal activity elicited during initial training could predict the gain in performance overnight in another motor sequence learning task, the finger tapping task. We also tested the hypothesis that the initial hippocampal activity is selectively related to sleep-dependent motor sequence consolidation. Functional magnetic resonance imaging was used to specify the implication of the hippocampus in sleep-dependent consolidation of motor memories. Participants were scanned during practice of a finger tapping task or a visuo-motor adaptation task. They were retested three days later, with either sleep or total sleep deprivation during the first post-training night. During initial motor sequence learning, late hippocampal activity predicts the gain in performance observed at retest in sleepers but not in sleep-deprived subjects. In sleepers, responses increase in the hippocampus and medial prefrontal cortex whereas in sleep-deprived subjects, responses increase in the putamen and cingulate cortex. In contrast, for the adaptation task, performance was only maintained after sleep whereas sleep deprivation resulted in performance deterioration. No hippocampal responses were detected during training. These results stress the importance of hippocampal activity during initial practice for subsequent sleep-dependent consolidation of motor skills that imply the acquisition of a motor sequence.

Hotermans C, Peigneux P, de Noordhout AM, Moonen G, Maquet P (2008) Repetitive transcranial magnetic stimulation over the primary motor cortex disrupts early boost but not delayed gains in performance in motor sequence learning. Eur J Neurosci

In humans the consolidation of recently learned motor skills is a multi-step process. We previously showed that performance on the finger-tapping task (FTT; i.e. a sequential motor skill) temporarily improves early on, 5-30 min after practice has ended, but not 4 h later. In the absence of any further practice to the task, this early boost in performance was predictive of the performance levels eventually achieved 48 h later, suggesting its functional relevance for long-term memory consolidation [Hotermans, Peigneux, Maertens de Noordhout, Moonen, and Maquet (2006) Early boost and slow consolidation in motor skill learning. Learn. Mem., 13, 580-583]. Here, we focused on the role of the primary motor cortex (M1) in consolidation using repetitive transcranial magnetic stimulation (rTMS) applied immediately before testing at 30 min, 4 or 24 h after practice of the FTT. Immediately after learning, rTMS over M1 depressed the early boost in performance, but did not affect the delayed improvement observed 48 h later. Four and 24 h after practice, rTMS did not disrupt performance anymore. These results suggest that M1 supports performance during the early post-training phase of motor skill consolidation, but is no longer mandatory in the subsequent, delayed stages of consolidation.

4.2. Sleep and topographical memory

Rauchs G, Orban P, Schmidt C, Albouy G, Balteau E, Degueldre C, Schnackers C, Sterpenich V, Tinguely G, Luxen A, Maquet P, Peigneux P (2008) Sleep modulates the neural substrates of both spatial and contextual memory consolidation. PLoS ONE 3:e2949.

It is known that sleep reshapes the neural representations that subtend the memories acquired while navigating in a virtual environment. However, navigation is not process-pure, as manifold learning components contribute to performance, notably the spatial and contextual memory constituents. In this context, it remains unclear whether post-training sleep globally promotes consolidation of all of the memory components embedded in virtual navigation, or rather favors the development of specific representations. Here, we investigated the effect of post-training sleep on the neural substrates of the consolidation of spatial and contextual memories acquired while navigating in a complex 3D, naturalistic virtual town. Using fMRI, we mapped regional cerebral activity during various tasks designed to tap either the spatial or the contextual memory component, or both, 72 h after encoding with or without sleep deprivation during the first post-training night. Behavioral performance was not dependent upon post-training sleep deprivation, neither in a natural setting that engages both spatial and contextual memory processes nor when looking more specifically at each of these memory representations. At the neuronal level however, analyses that focused on contextual memory revealed distinct correlations between performance and neuronal activity in frontal areas associated with recollection processes after post-training sleep, and in the parahippocampal gyrus associated with familiarity processes in sleep-deprived participants. Likewise, efficient spatial memory was associated with posterior cortical activity after sleep whereas it correlated with parahippocampal/medial temporal activity after sleep deprivation. Finally, variations in place-finding efficiency in a natural setting encompassing spatial and contextual elements were associated with caudate activity after post-training sleep, suggesting the automation of navigation. These data indicate that post-training sleep modulates the neural substrates of the consolidation of both the spatial and contextual memories acquired during virtual navigation.

4.3. Effects of sleep and lack of sleep on remote memories

Sterpenich V, Albouy G, Darsaud A, Schmidt C, Vandewalle G, Dang Vu TT, Desseilles M, Phillips C, Degueldre C, Balteau E, Collette F, Luxen A, Maquet P (2009) Sleep promotes the neural reorganization of remote emotional memory. J Neurosci 29:5143-5152.

Sleep promotes memory consolidation, a process by which fresh and labile memories are reorganized into stable memories. Emotional memories are usually better remembered than neutral ones, even at long retention delays. In this study, we assessed the influence of sleep during the night following encoding onto the neural correlates of recollection of emotional memories six months later. After incidental encoding of emotional and neutral pictures, half of the subjects were allowed to sleep, whereas the others were totally sleep deprived, on the first post-encoding night. During subsequent retest fMRI sessions taking place three days and six months later, subjects made recognition memory judgments about the previously studied and new pictures. Between these retest sessions, all participants slept as usual at home. At six-month retest, recollection was associated with significantly larger responses in subjects allowed to sleep than in sleep deprived subjects, in the ventral medial prefrontal cortex (vMPFC) and the precuneus, two areas involved in memory retrieval, as well as in the amygdala and the occipital cortex, two regions the response of which was modulated by emotion at encoding. Moreover, the functional connectivity was enhanced between the vMPFC and the precuneus, as well as between the amygdala, the vMPFC and the occipital cortex in the sleep group relative to the sleep-deprived group. These results suggest that sleep during the first post-encoding night profoundly influences the long-term systems-

level consolidation of emotional memory and modifies the functional segregation and integration associated with recollection in the long term.

4.4. Does sleep promotes false memories ?

Darsaud A, Dehon H, Lahl O, Sterpenich V, Boly M, Dang-Vu T, Desseilles M, Gais S, Matarazzo L, Peters F, Schabus M, Schmidt C, Tinguely G, Vandewalle G, Luxen A, Maquet P, Collette F (2010) Does Sleep Promote False Memories? J Cogn Neurosci.

Memory is constructive in nature so that it may sometimes lead to the retrieval of distorted or illusory information. Sleep facilitates accurate declarative memory consolidation but might also promote such memory distortions. We examined the influence of sleep and lack of sleep on the cerebral correlates of accurate and false recollections using functional magnetic resonance imaging (fMRI). After encoding lists of semantically related word associates, half of the participants were allowed to sleep, whereas the others were totally sleep deprived on the first post-encoding night. During a subsequent retest fMRI session taking place three days later, participants made recognition memory judgments about the previously studied associates, critical theme words (which had not been previously presented during encoding) and new words unrelated to the studied items. Sleep, relative to sleep deprivation, enhanced accurate and false recollections. No significant difference was observed in brain responses to false or illusory recollection between sleep and sleep deprivation conditions. However, after sleep but not after sleep deprivation (exclusive masking), accurate and illusory recollections were both associated with responses in the hippocampus and retrosplenial cortex. The data suggest that sleep does not selectively enhance illusory memories but rather tends to promote systems-level consolidation in hippocampo-neocortical circuits of memories subsequently associated with both accurate and illusory recollections. We further observed that during encoding, hippocampal responses were selectively larger for items subsequently accurately retrieved, than for material leading to illusory memories. The data indicate that the early organization of memory during encoding is a major factor influencing subsequent production of accurate or false memories.

4.5. Neural Precursors of Delayed Insight

Darsaud A, Wagner U, Balteau E, Desseilles M, Sterpenich V, Vandewalle G, Albouy G, Dang-Vu T, Collette F, Boly M, Schabus M, Degueldre C, Luxen A, Maquet P Neural Precursors of Delayed Insight. J Cogn Neurosci. (2010)

The solution of a problem left unresolved in the evening can sometimes pop into mind as a sudden insight after a night of sleep in the following morning. Although favorable effects of sleep on insightful behavior have been experimentally confirmed, the neural mechanisms determining this delayed insight remain unknown. Here, using functional magnetic resonance imaging (fMRI), we characterize the neural precursors of delayed insight in the number reduction task (NRT), in which a hidden task structure can be learned implicitly, but can also be recognized explicitly in an insightful process, allowing immediate qualitative improvement in task performance. Normal volunteers practiced the NRT during two fMRI sessions (training and retest), taking place 12 hours apart after a night of sleep. After this delay, half of the subjects gained insight into the hidden task structure ("solvers," S), whereas the other half did not ("nonsolvers," NS). Already at training, solvers and nonsolvers differed in their cerebral responses associated with implicit learning. In future solvers, responses were observed in the superior frontal sulcus, posterior parietal cortex, and the insula, three areas mediating controlled processes and supporting early learning and novice performance. In contrast, implicit learning was related to significant responses in the hippocampus in nonsolvers. Moreover, the hippocampus was functionally coupled with the basal ganglia in nonsolvers and with the superior frontal sulcus in solvers, thus potentially biasing participants' strategy towards implicit or controlled processes of memory encoding, respectively. Furthermore, in solvers but not

in nonsolvers, response patterns were further transformed overnight, with enhanced responses in ventral medial prefrontal cortex, an area previously implicated in the consolidation of declarative memory. During retest in solvers, before they gain insight into the hidden rule, significant responses were observed in the same medial prefrontal area. After insight, a distributed set of parietal and frontal areas is recruited among which information concerning the hidden rule can be shared in a so-called global workspace.

4.6. Sleep and visual perceptual learning

Matarazzo L, Franko E, Maquet P, Vogels R (2008) Offline processing of memories induced by perceptual visual learning during subsequent wakefulness and sleep: A behavioral study. J Vis 8:7 1-9.

To characterize perceptual memory consolidation during sleep, we used a coarse orientation discrimination task in which participants had to discriminate the orientation of orthogonal gratings occluded by increasing levels of noise. In a first study (N = 11), we showed that the learning effect in this task is retinotopic (position-specific) and orientation specific. In a second experiment, we assessed the effect of nocturnal sleep, as opposed to the effect of time, on perceptual learning. A first group of participants was trained in the morning, tested in the evening and retested the next morning (morning-evening-morning, MEM, N = 11); a second group was trained in the evening, tested the next morning, and retested in the evening (evening-morning-evening; EME; N = 12). Between training and testing, EME subjects improved significantly more (after a night of sleep) than MEM subjects (after 12 waking hours). Similarly, between test and retest, performance of MEM subjects (after a full night of sleep) improved significantly more than in EME subjects (after 12 further waking hours). These results suggest a beneficial effect of sleep on coarse orientation discrimination. Further studies are needed to characterize the neural correlates of this perceptual learning and the offline consolidation of perceptual memory.

5. Characterization of induced brain activity in unconscious patients

In parallel to our research work on sleep, our team also investigated spontaneous and induced brain activities in unconscious or minimally conscious patients

Boly M, Faymonville ME, Schnakers C, Peigneux P, Lambermont B, Phillips C, Lancellotti P, Luxen A, Lamy M, Moonen G, Maquet P, Laureys S (2008) Perception of pain in the minimally conscious state with PET activation: an observational study. Lancet Neurol 7:1013-1020.

Patients in a minimally conscious state (MCS) show restricted self or environment awareness but are unable to communicate consistently and reliably. Therefore, better understanding of cerebral noxious processing in these patients is of clinical, therapeutic, and ethical relevance. We studied brain activation induced by bilateral electrical stimulation of the median nerve in five patients in MCS (aged 18-74 years) compared with 15 controls (19-64 years) and 15 patients (19-75 years) in a persistent vegetative state (PVS) with (15)O-radiolabelled water PET. By way of psychophysiological interaction analysis, we also investigated the functional connectivity of the primary somatosensory cortex (S1) in patients and controls. Patients in MCS were scanned 57 (SD 33) days after admission, and patients in PVS 36 (9) days after admission. Stimulation intensities were 8.6 (SD 6.7) mA in patients in MCS, 7.4 (5.9) mA in controls, and 14.2 (8.7) mA in patients in PVS. Significant results were thresholded at p values of less than 0.05 and corrected for multiple comparisons. In patients in MCS and in controls, noxious stimulation activated the thalamus, S1, and the secondary somatosensory

or insular, frontoparietal, and anterior cingulate cortices (known as the pain matrix). No area was less activated in the patients in MCS than in the controls. All areas of the cortical pain matrix showed greater activation in patients in MCS than in those in PVS. Finally, in contrast with patients in PVS, those in MCS had preserved functional connectivity between S1 and a widespread cortical network that includes the frontoparietal associative cortices. Cerebral correlates of pain processing are found in a similar network in controls and patients in MCS but are much more widespread than in patients in PVS. These findings might be objective evidence of a potential pain perception capacity in patients in MCS, which supports the idea that these patients need analgesic treatment.

Vanhaudenhuyse A, Noirhomme Q, Tshibanda LJ, Bruno MA, Boveroux P, Schnakers C, Soddu A, Perlbarg V, Ledoux D, Brichant JF, Moonen G, Maquet P, Greicius MD, Laureys S, Boly M Default network connectivity reflects the level of consciousness in non-communicative brain-damaged patients. Brain 133:161-171.
The 'default network' is defined as a set of areas, encompassing posterior-cingulate/precuneus, anterior cingulate/mesiofrontal cortex and temporo-parietal junctions, that show more activity at rest than during attention-demanding tasks. Recent studies have shown that it is possible to reliably identify this network in the absence of any task, by resting state functional magnetic resonance imaging connectivity analyses in healthy volunteers. However, the functional significance of these spontaneous brain activity fluctuations remains unclear. The aim of this study was to test if the integrity of this resting-state connectivity pattern in the default network would differ in different pathological alterations of consciousness. Fourteen non-communicative brain-damaged patients and 14 healthy controls participated in the study. Connectivity was investigated using probabilistic independent component analysis, and an automated template-matching component selection approach. Connectivity in all default network areas was found to be negatively correlated with the degree of clinical consciousness impairment, ranging from healthy controls and locked-in syndrome to minimally conscious, vegetative then coma patients. Furthermore, precuneus connectivity was found to be significantly stronger in minimally conscious patients as compared with unconscious patients. Locked-in syndrome patient's default network connectivity was not significantly different from controls. Our results show that default network connectivity is decreased in severely brain-damaged patients, in proportion to their degree of consciousness impairment. Future prospective studies in a larger patient population are needed in order to evaluate the prognostic value of the presented methodology.

6. Spontaneous brain activity during hypnosis and general anesthesia

Our team also investigated other states of altered consciousness, such as general anesthesia. Although the work is still ongoing, the first data were published during the last 3 years.

Bonhomme V, Maquet P, Phillips C, Plenevaux A, Hans P, Luxen A, Lamy M, Laureys S (2008) The effect of clonidine infusion on distribution of regional cerebral blood flow in volunteers. Anesth Analg 106:899-909.
BACKGROUND: Through their action on the locus coeruleus, alpha2-adrenoceptor agonists induce rapidly reversible sedation while partially preserving cognitive brain functions. Our goal in this observational study was to map brain regions whose activity is modified by clonidine infusion so as to better understand its loci of action, especially in relation to sedation. METHODS: Six ASA I-II right-handed volunteers were recruited. Electroencephalogram (EEG) was monitored continuously.

After a baseline H2(15)O activation scan, clonidine infusion was started at a rate ranging from 6 to 10 microg x kg(-1) x h(-1). A sequence of 11 similar scans was then performed at 8 min intervals. Plasma clonidine concentration was measured. Using statistical parametric mapping, we sought linear correlations between normalized regional cerebral blood flow (rCBF), an indicator of regional brain activity, and plasma clonidine concentration or spindle EEG activity. RESULTS: Clonidine induced clinical sedation and EEG patterns (spindles) comparable to early stage nonrapid eye movement sleep. A significant negative linear correlation between clonidine concentration and rCBF or spindle activity was observed in the thalamus, prefrontal, orbital and parietal association cortex, posterior cingulate cortex, and precuneus. CONCLUSIONS: The EEG patterns and decreases in rCBF of specific brain regions observed during clonidine-induced sedation are similar to those of early stage nonrapid eye movement sleep. Patterns of deactivated brain regions are also comparable to those observed during general anesthesia or vegetative state, reinforcing the hypothesis that alterations in the activity of a common network occur during these modified conscious states.

7. Other research works supported by the Queen Elisabeth Medical Foundation

Attention deficits in major depression

Desseilles M, Balteau E, Sterpenich V, Dang-Vu TT, Darsaud A, Vandewalle G, Albouy G, Salmon E, Peters F, Schmidt C, Schabus M, Gais S, Degueldre C, Phillips C, Luxen A, Ansseau M, Maquet P, Schwartz S (2009) Abnormal neural filtering of irrelevant visual information in depression. J Neurosci 29:1395-1403.

The pathophysiology of major depressive disorder (MDD) includes both affective and cognitive dysfunctions. We aimed to clarify how regions regulating affective processing interact with those involved in attention, and how such interaction impacts on perceptual processing within sensory cortices. Based on previous work showing that top-down influences from attention can determine the processing of external inputs within early sensory cortices, we tested with functional MRI (fMRI) whether MDD alters attentional ('top-down') effects on the neural filtering of irrelevant, non-emotional visual stimuli. The present fMRI study was conducted in 14 non-medicated patients with a first episode of unipolar MDD and 14 matched controls. During scanning, subjects performed two tasks imposing two different levels of attentional load at fixation (easy or difficult), while irrelevant colored stimuli were presented in the periphery. Analyses of fMRI data revealed that MDD patients show (i) an abnormal filtering of irrelevant information in visual cortex, (ii) an altered functional connectivity between fronto-parietal networks and visual cortices, and (iii) a hyperactivity in subgenual cingulate/ medial orbitofrontal cortex that was modulated by attentional load. These results demonstrate that biological abnormalities contribute to the cognitive deficits seen in major depression, and clarify how neural networks implicated in mood regulation influence executive control and perceptual processes. These findings do not only improve our understanding of the pathophysiological mechanisms underlying cognitive dysfunctions in MDD, but also shed new light on the interaction between cognition and mood regulation.

Desseilles M, Schwartz S, Dang-Vu TT, Sterpenich V, Ansseau M, Maquet P, Phillips C Depression alters "top-down" visual attention: a dynamic causal modeling comparison between depressed and healthy subjects. Neuroimage 54:1662-1668.

Using functional magnetic resonance imaging (fMRI), we recently demonstrated that nonmedicated patients with a first episode of unipolar major depression (MDD) compared to matched controls

exhibited an abnormal neural filtering of irrelevant visual information (Desseilles et al., 2009). During scanning, subjects performed a visual attention task imposing two different levels of attentional load at fixation (low or high), while task-irrelevant colored stimuli were presented in the periphery. In the present study, we focused on the visuo-attentional system and used "Dynamic Causal Modeling" (DCM) on the same dataset to assess how attention influences a network of three dynamically-interconnected brain regions (visual areas V1 and V4, and intraparietal sulcus (P), differentially in MDD patients and healthy controls. Bayesian model selection (BMS) and model space partitioning (MSP) were used to determine the best model in each population. The best model for the controls revealed that the increase of parietal activity by high attention load was selectively associated with a negative modulation of P on V4, consistent with high attention reducing the processing of irrelevant colored peripheral stimuli. The best model accounting for the data from the MDD patients showed that both low and high attention levels exerted modulatory effects on P. The present results document abnormal effective connectivity across visuo-attentional networks in MDD, which likely contributes to deficient attentional filtering of information.

Related references

- ALBOUY, G., STERPENICH, V., BALTEAU, E., VANDEWALLE, G., DESSEILLES, M., DANG-VU, T., DARSAUD, A., RUBY, P., LUPPI, P. H., DEGUELDRE, C., PEIGNEUX, P., LUXEN, A. & MAQUET, P. (2008) Both the hippocampus and striatum are involved in consolidation of motor sequence memory. Neuron, 58, 261-72.
- ALBOUY, G. & MAQUET, P. (2008) [Hippocampus, striatum and sequences]. Med Sci (Paris), 24, 921-2.
- BOLY, M., PHILLIPS, C., BALTEAU, E., SCHNAKERS, C., DEGUELDRE, C., MOONEN, G., LUXEN, A., PEIGNEUX, P., FAYMONVILLE, M. E., MAQUET, P. & LAUREYS, S. (2008) Consciousness and cerebral baseline activity fluctuations. Hum Brain Mapp, 29, 868-874.
- BOLY, M., PHILLIPS, C., TSHIBANDA, L., VANHAUDENHUYSE, A., SCHABUS, M., DANG-VU, T. T., MOONEN, G., HUSTINX, R., MAQUET, P. & LAUREYS, S. (2008) Intrinsic brain activity in altered states of consciousness: how conscious is the default mode of brain function? Ann N Y Acad Sci, 1129, 119-29.
- BOLY, M., FAYMONVILLE, M. E., SCHNAKERS, C., PEIGNEUX, P., LAMBERMONT, B., PHILLIPS, C., LANCELLOTTI, P., LUXEN, A., LAMY, M., MOONEN, G., MAQUET, P. & LAUREYS, S. (2008) Perception of pain in the minimally conscious state with PET activation: an observational study. Lancet Neurol, 7, 1013-20.
- BONHOMME, V., MAQUET, P., PHILLIPS, C., PLENEVAUX, A., HANS, P., LUXEN, A., LAMY, M. & LAUREYS, S. (2008) The effect of clonidine infusion on distribution of regional cerebral blood flow in volunteers. Anesth Analg, 106, 899-909, table of contents.
- DANG-VU, T. T., SCHABUS, M., DESSEILLES, M., ALBOUY, G., BOLY, M., DARSAUD, A., GAIS, S., RAUCHS, G., STERPENICH, V., VANDEWALLE, G., CARRIER, J., MOONEN, G., BALTEAU, E., DEGUELDRE, C., LUXEN, A., PHILLIPS, C. & MAQUET, P. (2008) Spontaneous neural activity during human slow wave sleep. Proc Natl Acad Sci U S A, 105, 15160-5.
- DESSEILLES, M., DANG-VU, T., SCHABUS, M., STERPENICH, V., MAQUET, P. & SCHWARTZ, S. (2008) Neuroimaging insights into the pathophysiology of sleep disorders. Sleep, 31, 777-94.
- HOTERMANS, C., PEIGNEUX, P., DE NOORDHOUT, A. M., MOONEN, G. & MAQUET, P. (2008) Repetitive transcranial magnetic stimulation over the primary motor cortex disrupts early boost but not delayed gains in performance in motor sequence learning. Eur J Neurosci.
- MATARAZZO, L., FRANKO, E., MAQUET, P. & VOGELS, R. (2008) Offline processing of memories induced by perceptual visual learning during subsequent wakefulness and sleep: A behavioral study. J Vis, 8, 7 1-9.
- RAUCHS, G., ORBAN, P., BALTEAU, E., SCHMIDT, C., DEGUELDRE, C., LUXEN, A., MAQUET, P. & PEIGNEUX, P. (2008) Partially segregated neural networks for spatial and contextual memory in virtual navigation. Hippocampus, 18, 503-518.
- RAUCHS, G., ORBAN, P., SCHMIDT, C., ALBOUY, G., BALTEAU, E., DEGUELDRE, C., SCHNACKERS, C., STERPENICH, V., TINGUELY, G., LUXEN, A., MAQUET, P. & PEIGNEUX, P. (2008) Sleep modulates the neural substrates of both spatial and contextual memory consolidation. PLoS ONE, 3, e2949.
- VANHAUDENHUYSE, A., BOVEROUX, P., BOLY, M., SCHNAKERS, C., BRUNO, M. A., KIRSCH, M., DEMERTZI, A., LAMY, M., MAQUET, P., LAUREYS, S. & FAYMONVILLE, M. E. (2008) [Hypnosis and pain perception]. Rev Med Liege, 63, 424-8.
- BOLY, M., TSHIBANDA, L., VANHAUDENHUYSE, A., NOIRHOMME, Q., SCHNAKERS, C., LEDOUX, D., BOVEROUX, P., GARWEG, C., LAMBERMONT, B., PHILLIPS, C., LUXEN, A., MOONEN, G., BASSETTI, C., MAQUET, P. & LAUREYS, S. (2009) Functional connectivity in the default network during resting state is preserved in a vegetative but not in a brain dead patient. Hum Brain Mapp, 30, 2393-400.
- DANG-VU, T. T., DESSEILLES, M., SCHWARTZ, S. & MAQUET, P. (2009) Neuroimaging of narcolepsy. CNS Neurol Disord Drug Targets, 8, 254-63.
- DESSEILLES, M., BALTEAU, E., STERPENICH, V., DANG-VU, T. T., DARSAUD, A., VANDEWALLE, G., ALBOUY, G., SALMON, E., PETERS, F., SCHMIDT, C., SCHABUS, M., GAIS, S., DEGUELDRE, C., PHILLIPS, C., LUXEN, A., ANSSEAU, M., MAQUET, P. & SCHWARTZ, S. (2009) Abnormal neural filtering of irrelevant visual information in depression. J Neurosci, 29, 1395-403.
- LECLERCQ, Y., BALTEAU, E., DANG-VU, T., SCHABUS, M., LUXEN, A., MAQUET, P. & PHILLIPS, C. (2009) Rejection of pulse related artefact (PRA) from continuous electroencephalographic (EEG) time series recorded during functional magnetic resonance imaging (fMRI) using constraint independent component analysis (cICA). Neuroimage, 44, 679-91.
- MASCETTI, L., FORET, A., BONJEAN, M., MATARAZZO, L., DANG-VU, T. & MAQUET, P. (2009) Some facts about sleep relevant for Landau-Kleffner syndrome. Epilepsia, 50 Suppl 7, 43-6.
- SCHMIDT, C., COLLETTE, F., LECLERCQ, Y., STERPENICH, V., VANDEWALLE, G., BERTHOMIER, P., BERTHOMIER, C., PHILLIPS, C., TINGUELY, G., DARSAUD, A., GAIS, S., SCHABUS, M., DESSEILLES, M., DANG-VU, T. T., SALMON, E., BALTEAU, E., DEGUELDRE, C., LUXEN, A., MAQUET, P., CAJOCHEN, C. & PEIGNEUX, P. (2009) Homeostatic sleep pressure and responses to sustained attention in the suprachiasmatic area. Science, 324, 516-9.
- STERPENICH, V., ALBOUY, G., DARSAUD, A., SCHMIDT, C., VANDEWALLE, G., DANG VU, T. T., DESSEILLES, M., PHILLIPS, C., DEGUELDRE, C., BALTEAU, E., COLLETTE, F., LUXEN, A. & MAQUET, P. (2009) Sleep promotes the neural reorganization of remote emotional memory. J Neurosci, 29, 5143-52.

– VANDEWALLE, G., ARCHER, S. N., WUILLAUME, C., BALTEAU, E., DEGUELDRE, C., LUXEN, A., MAQUET, P. & DIJK, D. J. (2009) Functional magnetic resonance imaging-assessed brain responses during an executive task depend on interaction of sleep homeostasis, circadian phase, and PER3 genotype. J Neurosci, 29, 7948-56.
– VANDEWALLE, G., MAQUET, P. & DIJK, D. J. (2009) Light as a modulator of cognitive brain function. Trends Cogn Sci, 13, 429-38.
– DARSAUD, A., DEHON, H., LAHL, O., STERPENICH, V., BOLY, M., DANG-VU, T., DESSEILLES, M., GAIS, S., MATARAZZO, L., PETERS, F., SCHABUS, M., SCHMIDT, C., TINGUELY, G., VANDEWALLE, G., LUXEN, A., MAQUET, P. & COLLETTE, F. (2010) Does Sleep Promote False Memories? J Cogn Neurosci.
– SCHMIDT, C., PEIGNEUX, P., MAQUET, P. & PHILLIPS, C. (2010) Response to comment on «Homeostatic Sleep Pressure and Responses to Sustained Attention in the Suprachiasmatic Area». Science, 316, 309c-.
– VANDEWALLE, G., SCHWARTZ, S., GRANDJEAN, D., WUILLAUME, C., BALTEAU, E., DEGUELDRE, C., SCHABUS, M., PHILLIPS, C., LUXEN, A., DIJK, D. J. & MAQUET, P. (2010) Spectral quality of light modulates emotional brain responses in humans. Proc Natl Acad Sci U S A, 107, 19549-54.
– BOVEROUX, P., VANHAUDENHUYSE, A., BRUNO, M. A., NOIRHOMME, Q., LAUWICK, S., LUXEN, A., DEGUELDRE, C., PLENEVAUX, A., SCHNAKERS, C., PHILLIPS, C., BRICHANT, J. F., BONHOMME, V., MAQUET, P., GREICIUS, M. D., LAUREYS, S. & BOLY, (2010) M. Breakdown of within- and between-network resting state functional magnetic resonance imaging connectivity during propofol-induced loss of consciousness. Anesthesiology, 113, 1038-53.
– COLOGAN, V., SCHABUS, M., LEDOUX, D., MOONEN, G., MAQUET, P. & LAUREYS, S. (2010) Sleep in disorders of consciousness. Sleep Med Rev, 14, 97-105.
– PHILLIPS, C. L., BRUNO, M. A., MAQUET, P., BOLY, M., NOIRHOMME, Q., SCHNAKERS, C., VANHAUDENHUYSE, A., BONJEAN, M., HUSTINX, R., MOONEN, G., LUXEN, A. & LAUREYS, S. (2010) «Relevance vector machine» consciousness classifier applied to cerebral metabolism of vegetative and locked-in patients. Neuroimage.
– VANHAUDENHUYSE, A., NOIRHOMME, Q., TSHIBANDA, L. J., BRUNO, M. A., BOVEROUX, P., SCHNAKERS, C., SODDU, A., PERLBARG, V., LEDOUX, D., BRICHANT, J. F., MOONEN, G., MAQUET, P., GREICIUS, M. D., LAUREYS, S. & BOLY, M. (2010) Default network connectivity reflects the level of consciousness in non-communicative brain-damaged patients. Brain, 133, 161-71.

Final report of the research group of

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Exploration of the memory enhancing effects of angiotensin IV and unravelling its mechanism of action

I. Introduction

I.1. Ang IV and its binding site, the IRAP enzyme

In this project, we investigated the hexapeptide angiotensin IV (Ang IV), primarily because of its reported facilitatory role in memory acquisition and retrieval (see I.2). This peptide is a fragment of the cardiovascular hormone angiotensin II (Ang II) known from its effects on the regulation of sodium excretion, body fluid volumes and arterial blood pressure. While Ang IV binds with low affinity to the classical Ang II receptors (AT_1 and AT_2), there is now clear evidence that most of its effects are mediated via inhibition of an enzyme, insulin-regulated aminopeptidase (IRAP) or cystinyl aminopeptidase (EC 3.4.11.3), also known as placental leucine aminopeptidase (P-LAP) and oxytocinase (Otase) (Albiston et al., 2001; Albiston et al., 2009*).* IRAP is a type II integral membrane protein homologous to aminopeptidase N (APN), and other Zn^{2+}-dependent enzymes of the gluzincin aminopeptidase family (Rogi et al., 1996, for review see Vauquelin et al., 2002). LVV-hemorphin-7 (LVV-H7), an endogenous central nervous system peptide obtained by hydrolysis of the beta chain of hemoglobin (Møeller et al., 1997), was also found to be a potent inhibitor of IRAP.

I.2. Role of Ang IV in memory and learning paradigms

Ang IV and related ligands have been described to display beneficiary effects in animal models for cognitive impairment (for review see De Bundel et al., 2008). These exciting findings initiated the search for their mechanism of action. The key finding of Ang IV to enhance memory acquisition and recall was reported in passive and conditioned avoidance response studies (Braszko et al., 1998, Wright et al., 1993, 1996, Tchekalarova et al., 2001). Intracerebroventricular (i.c.v.) administration of Nle^1-Ang IV facilitated the ability to solve a spatial learning task in the circular water maze (Wright et al., 1999). Moreover, Ang IV and LVV-H7 facilitated potassium-evoked acetylcholine release from rat hippocampal slices (Lee et al., 2001), suggesting an Ang IV-acetylcholine interaction. Electrophysiological and biochemical studies revealed that the cognitive effects of Ang IV analogues were mediated via the hippocampus. Ang IV and its analogues significantly enhanced hippocampal long-term potentiation (LTP) in the dentate gyrus and the CA1 field, both in vitro (Kramar et al., 2001) and in vivo (Wayner et al., 2001). Moreover, autoradiographic studies revealed that Ang IV binding sites are prominent in brain structures important to cognitive processing, including hippocampus (Miller-Wing et al., 1993).

II. Initial working hypotheses and aims of the project

(i) First we aimed to clarify the function of IRAP in memory processes by comparing the effects of Ang IV in **IRAP knockout (KO) mice** with those in wild-type (WT) mice in several memory tasks (in collaboration with S. Chai and A. Albiston, Australia). Similar experiments with Ang IV administration were performed in **transgenic mice** that manifest characteristic behavioural and neuropathological features of Alzheimer's disease.
(ii) The second aim was to further explore by which **mechanisms of action** the Ang IV-IRAP interaction is able to trigger physiologically relevant intra- as well as extracellular processes and how its facilitates memory functioning. This approach was initially focused on intact-cell experiments and relevant

outcomes could then serve as a rationale (or tools) for dedicated *in vivo* tests.
(iii) Finally, **more stable Ang IV ligands** were designed and evaluated. We first investigated their stability and affinity for IRAP *in vitro*. The most promising compounds could then be administered *in vivo* to WT and Alzheimer mice and tested for their memory-promoting effects.

III. Results with regard to the initial working hypotheses

III 1.1. IRAP KO mice unexpectedly show an age-related deficit in spatial memory

Since several years we have a fruitful collaboration with Anthony Albiston and Siew Chai from the University of Melbourne (Australia). Our main joint hypothesis is that the memory enhancing effects of Ang IV and LVV-H7 are due to pharmacological inhibition of IRAP. We possess a strain of mice with a targeted deletion of the IRAP gene in collaboration with these Australian colleagues. One of our team members, Dimitri De Bundel, also performed one year of experimental work in the Australian lab in the frame of this collaboration. During the course of this project, Chai and co-workers made a lot of progress in conducting a comprehensive analysis of the behavioural phenotype of the IRAP KO mice and in testing the memory and learning capacities of the IRAP KO mice in various tasks for memory and learning. Therefore this work package described in the initial project proposal was not repeated within our laboratory. Moreover, our Australian colleagues did an unexpected finding. Inconsistent with our common hypothesis, they found that permanent deletion of IRAP in mice resulted in an age-related deficit in spatial memory (Albiston et al., 2009). A significant genotype difference was detected in performance of 6 month old IRAP KO mice in the Y maze tasks but was without effect in two other memory paradigms, i.e. novel object recognition and T maze spontaneous alternation (Albiston et al., 2009). IRAP KO mice thus experience a more rapid onset in spatial memory deficits in comparison with their WT litter mates. It would be interesting to investigate whether similar effects would be found in an inducible KO/knockdown mouse. Unfortunately such a mouse strain has not yet been developed.

III 1.2. Pilot study trying to unravel possible effects of Ang IV in the APP23 mouse model for Alzheimer's disease

Next we examined possible effects of Ang IV in a murine model of Alzheimer's disease in collaboration with Prof. De Deyn and Dr. Van Dam of the University of Antwerp. Their APP23 model (Van Dam and De Deyn, 2006) provides a unique opportunity to asses IRAP as a target for the development of drugs for the treatment of Alzheimer's disease. Twenty six male heterozygous APP23 mice with an average age of 3 months old were used. They were all stereotaxically implanted with an injection guide cannula aimed at the lateral ventricle since this route of administration has best been described for the memory enhancing effects of Ang IV. The mice were allowed to recover for a minimum of 7 days and were daily handled for 1 minute during this time. Then they were subjected to the standard Morris water maze protocol by researchers blinded to the genetic and treatment status of the animals. The mice received a daily injection of aCSF, 1 nmol Ang IV or 1 nmol LVV-H7 10 minutes before the start of each trial series. Acquisition training consisted of eight sessions of four daily trials (15-min intertrial interval) starting from four different positions of the circular pool in a semi-random order. Unfortunately many technical problems hampered us to draw conclusions from this study. The mice with a guide cannula in the lateral ventricle had severe difficulties with swimming and the dental cement caps to secure the injection cannulas got loose in many mice. These are also the main reasons why this pilot experiment failed. Indeed, up till now memory enhancing effects of several drugs, e.g. memantine, within the APP23 mouse model have always been shown after systemic administration (Van Dam et al., 2005).

III.2.1. Glucose hypothesis as mechanism of memory promoting action of Ang IV, in vitro and in vivo approaches

The IRAP ligands Ang IV and LVV-H7 enhance performance in a range of memory paradigms in normal rats and ameliorate memory deficits in rat models for amnesia. The mechanism by which these peptides facilitate memory remains however to be elucidated. In recent in vitro experiments, it was demonstrated that Ang IV and LVV-H7 potentiated activity-evoked glucose uptake into hippocampal neurons (Fernando et al., 2008). This raises the possibility that IRAP ligands may facilitate memory in hippocampus-dependent tasks through enhancement of hippocampal glucose uptake. This is a sound hypothesis since IRAP and the GLUT4 glucose transporter co-exist in neurons (Fernando et al., 2007). Moreover, both proteins are co-transported between intracellular stores and the cell surface. In fact, IRAP is the only protein identified that has the same insulin-regulated trafficking characteristics as GLUT4 (Keller et al., 1995; Ross et al., 1996; Sumitani et al., 1997). In adipocytes IRAP is known to be localised almost exclusively with GLUT4 in specialised vesicles (Keller et al., 1995). This makes Ang IV a unique ligand that may interfere with IRAP recycling and by this way enhance or prolong the exposure of GLUT4 at the cell surface. The thereby increased glucose uptake could then elicit an increased learning capacity. Indeed, trials with both rodents and humans have demonstrated that glucose enhances cognitive performance. Interestingly, this is particularly the case in elderly subjects and in patients with Alzheimer's disease (review McNay and Gold, 2002).

Previous studies of the insulin-mediated translocation of IRAP to the cell surface have been hampered by the laborious detection of IRAP at the cell surface (Garza and Birnbaum, 2000; Johnson et al., 1998; Nakamura et al., 2000). We aimed to develop a more direct and faster method to detect IRAP. To this end, we used model systems with well-characterized IRAP: CHO-K1 cells expressing endogenous IRAP and recombinant HEK293 cells expressing human IRAP. A more widespread application of the method was demonstrated by the use of 3T3-L1 adipocytes. After stimulation of the cells with insulin, internalization of IRAP was inhibited by the addition of phenyl arsine oxide (PAO). Then, cell-surface IRAP was detected by the high-affinity binding of radiolabelled angiotensin (Ang) IV (either 125I or 3H). We monitored the time- and concentration dependence of insulin-mediated translocation of IRAP in both cell lines and 3T3-L1 adipocytes. A plateau was reached between 6 and 8 min, and 10^{-7} M insulin led to the highest amount of IRAP at the cell surface. Based on the capacity of the IRAP apoenzyme to display high affinity for radiolabelled Ang IV and on the ability of PAO to inhibit IRAP internalization, we developed a more direct and faster method to measure insulin-mediated translocation of IRAP to the cell surface (Demaeght et al., 2008).

We next demonstrated for the first time *in vivo* that Ang IV and LVV-H7 also enhanced spatial working memory in the plus maze spontaneous alternation task but found no *in vivo* evidence for enhanced hippocampal glucose uptake or altered cerebral blood flow (De Bundel et al., 2009). Indeed,
acute i.c.v. administration of 1 nmol Ang IV or 0.1 nmol LVV-H7 in 3 months-old Sprague-Dawley rats enhanced spatial working memory in the plus maze spontaneous alternation task. Extracellular hippocampal glucose levels were monitored before, during and after behavioral testing using *in vivo* microdialysis. Extracellular hippocampal glucose levels decreased significantly to about 70% of baseline when the animals explored the plus maze, but remained constant when the animals were placed into a novel control chamber. Ang IV and LVV-H7 did not significantly alter hippocampal glucose levels compared to control animals in the plus maze or control chamber. Both peptides had no effect on hippocampal blood flow as determined by laser Doppler flowmetry, excluding that either peptide increased the hippocampal supply of glucose.

III.2.2. Involvement of the angiotensin AT_1 receptor in the memory enhancing effects of Ang IV

We thus further investigated whether other mechanisms may be involved in the central memory promoting effects of Ang IV and LVV-H7, and determined the effects of i.c.v. administration of Ang IV or LVV-H7 on hippocampal neurotransmitter levels using microdialysis in rats. Spatial working memory enhancing effects of IRAP ligands are typically observed within 30 min following their i.c.v. administration (De Bundel et al., 2009). Ang IV (1-10 nmol) and LVV-H7 (0.1-1 nmol) did not alter hippocampal dopamine, serotonin, GABA or acetylcholine levels within this time frame, suggesting that modulation of these neurotransmitters was not involved in the spatial working memory effects of Ang IV and LVV-H7. A clear and sustained decrease in hippocampal acetylcholine levels was however observed 60-100 min following i.c.v. injection of Ang IV. While increased hippocampal acetylcholine are known to be required for encoding of spatial information, decreased hippocampal acetylcholine has been proposed to be required for consolidation and retrieval of spatial information (Hasselmo, 2006).
Interestingly, our experiments also revealed that Ang IV modulated hippocampal acetylcholine levels, whereas LVV-H7 did not. Given that both Ang IV and LVV-H7 are potent competitive inhibitors of IRAP (Lew et al., 2003; Demaegdt et al., 2004), this discrepancy suggests that Ang IV may exert its effect on hippocampal acetylcholine through a binding site different from IRAP. The AT1 receptor was a strong candidate since we previously reported that this receptor mediates pressor effects following i.c.v. administration of Ang IV (Yang et al., 2008). We next demonstrated that LVV-H7 does not bind to the AT1 receptor in contrast to Ang IV which is a low affinity agonist of the AT1 receptor. The observation that the potent and selective AT1 receptor antagonist candesartan reversed the effect of Ang IV on hippocampal acetylcholine prompted us to investigate the involvement of the AT1 receptor in the effect of Ang IV on spatial working memory in the plus maze spontaneous alternation task. And indeed, pretreatment of the rats with candesartan also abolished the spatial working memory enhancing effect of Ang IV. However, the AT1 receptor was clearly not involved in the spatial memory facilitating effect of LVV-H7.

Following i.c.v. administration, ^{123}I-Ang IV did not diffuse to the hippocampus, suggesting an extrahippocampal site of Ang IV-mediated action. The obtained orbital pinhole SPECT image suggested that ^{123}I-Ang IV and/or its radioactive degradation products are retained around the site of administration and did not show a homogenous distribution throughout the brain.

In conclusion, we demonstrated that the AT_1 receptor is involved in the effects of Ang IV on hippocampal acetylcholine levels and spatial working memory (De Bundel et al., 2010). This paves the way for the AT_1 receptor to be involved in other cognitive effects of Ang IV as well. This does however not exclude that Ang IV mediates other central effects independently of AT_1 receptor activation and potentially through IRAP binding. In this context it has indeed been demonstrated that Ang IV protects rats against limbic seizures (Stragier et al., 2006) and experimental ischaemic stroke (Faure et al., 2006) independently of AT_1 receptor activation. Furthermore, the present findings do not exclude a potential role of IRAP as a target for memory enhancing drugs. Indeed, LVV-H7 has no affinity for the AT_1 receptor but nevertheless enhanced spatial working memory (De Bundel et al., 2009), spatial reference memory (Albiston et al., 2004) and passive avoidance memory (Albiston et al., 2004). Moreover, a newly synthesized inhibitor of IRAP that showed no affinity for the AT_1 receptor, HFI419, also enhanced object recognition and spatial working memory (Albiston et al., 2008).

III.3.1 Characterisation of the ligand binding and enzymatic properties of novel Ang IV ligands in neuronal cell lines and other cell lines

An important handicap for the studies dealing with the physiological role of Ang IV is its rapid degradation by different proteases. Hence, there is a need for metabolically stabilized Ang IV analogues. Especially

for the studies that are focused on IRAP, these analogues should also preferably display pronounced selectivity for this enzyme, not only versus AP-N but also versus the AT_1-type receptors for Ang II. We therefore started collaboration a few years ago with two chemistry departments (Prof. D. Tourwe – DSCH, VUB and Prof. M. Hallberg - Uppsala, Sweden) and developed stable, high affinity, IRAP-selective ligands (Andersson et al., 2008, 2010, Axén et al., 2006, 2007; Lukaszuk et al., 2008, 2009. Screening revealed that one of such synthetic compounds (further denoted as 'AL-11') met the desired specifications. AL-11 has recently been tritiated (coll. with Prof. Toth - Szeged, Hungary) and its binding properties have been explored.

The interaction of two new radioligands ([^{3}H]Ang IV and [^{3}H]AL-11) with IRAP was studied in different cell lines, including naïve CHO cells and a hippocampal neuronal cell line (P40H1). We first examined whether tritiated Ang IV displayed the same IRAP binding characteristics as the earlier commonly used iodinated form of Ang IV. The reason for this alternative labelling is that iodine is a large and hydrophobic atom, capable of changing the pharmacological and physicochemical properties of the natural ligand. The pharmacological profile of the [^{3}H]Ang IV-labelled sites was established by competition binding assays with unlabelled IRAP ligands (Demaegdt et al., 2008, 2009). This profile was similar to that obtained with [^{125}I]Ang IV competition binding assays in different cell lines (Demaegdt et al., 2004a; Demaegdt et al., 2006, Lew et al., 2003, Lee et al., 2003). Of note is that IRAP could only be detected by both labelled forms of Ang IV when divalent cation chelators EDTA and 1,10-PHE (E/P) were included in the medium (Demaegdt et al., 2004b, 2006). Very recently, we established that, irrespective of the marked susceptibility of Ang IV to proteolytic degradation (Lukaszuk et al., 2008), this ligand only binds with high affinity to the catalytic Zn^{2+}- depleted IRAP apo-enzyme (Demaegdt et al., 2010a). A novel Ang IV analogue, AL-11, resisted to proteolytic degradation and was found to bind with the same high affinity to the catalytically active and apo-forms of IRAP. It also displayed pronounced selectivity towards IRAP versus the IRAP-alike aminopeptidase N enzyme as well as the AT_1 receptor (Lukaszuk et al., 2008). AL-11 was custom-tritiated and this new radioligand was indeed able to label both forms of IRAP (i.e. the apo-form in presence of E/P and the active form in the absence of the chelators) in cell membranes as well as in intact cells (Demaegdt et al., 2009, Demaegdt et al., 2010b). In agreement with the findings for unlabelled AL-11 (Lukaszuk et al., 2008), [^{3}H]AL-11 only labelled IRAP in P40H1 cell membranes whereas enzyme inhibition assays (using the synthetic colorigenic substrate l-Leu-pNA) pointed to the concomitant presence of IRAP and APN in those membrane preparations. Hence, [^{3}H]AL-11 represents a prototype of a class of metabolically stable radioligands that display high potency and selectivity for native IRAP. Binding studies with this (and a potential next generation of even more potent radioligands like [^{3}H]AL-40, Lukaszuk et al., 2009) makes it now possible to examine IRAP by under physiologically relevant conditions.

IRAP has hitherto nearly invariably been investigated on membrane preparations of naïve- and recombinant IRAP- expressing cells. As our main goal is to better understand the physiological role of IRAP with special reference to its alleged involvement in the cognitive effects of Ang IV and potential role in immunoregulation, we wanted to develop techniques to investigate the pharmacological and dynamic properties of this enzyme in intact cell systems. We compared the binding properties of [^{3}H] Ang IV and [^{3}H]AL-11 to intact CHO-K1 cells in the absence of E/P (Demaegdt et al., 2010b). Whereas [^{3}H]Ang IV bound with low affinity to IRAP and another yet undisclosed cellular recognition site, [^{3}H] AL-11 displayed high affinity and specificity for IRAP and, most interestingly, a very low degree of non-specific binding. Subsequent research was focused on the kinetic properties of [^{3}H]AL-11 binding, the ensuing subcellular localization of this radioligand and the repercussion of pre-treating the cells with distinct IRAP ligands thereon. A most striking observation was that pre-treating the cells with AL-11 and

AL-40 decreased the ensuing surface binding and resulting intracellular accumulation of [^{3}H]AL-11 while the natural peptide ligands, Ang IV and LVV-H7, were unable to produce this effect. After elimination of simpler explanations, it is proposed that metabolically stable ligands undergo semi-continuous cycling between the cell surface and endosomal compartments. The *in vivo* efficacy of stable and unstable IRAP ligands could therefore differ.

IV. Ang IV, involvement in memory mechanisms, synaptic plasticity, epilepsy and affective disorders

Since especially the experiments described under III.1. could not be properly finished, we extended the aims of the current project also other fields of neuroscience in which we are very active, i.e. ***epilepsy and affective disorders.***

The link between ***memory mechanisms and epilepsy mechanisms*** is not at all farfetched. Clinically, cognitive decline is a well-known co-morbidity of epilepsy (Hermann et al., 2008) and epileptic seizures occur in patients with dementia at a higher prevalence than among healthy elderly individuals (Mendez and Lim, 2003). Also at the level of synaptic transmission and plasticity there are strong similarities between memory and epilepsy mechanisms. Indeed, long-term potentiation (LTP) and long-term depression (LTD) are defined as a persisting enhancement or suppression of synaptic efficacy and are posited as the underlying cellular mechanism for memory formation and extinction respectively (Royer and Pare, 2003). These forms of synaptic plasticity are strikingly similar to the synaptic rearrangements observed in the kindling model for epileptogenesis and are recognized factors in the evolution of epilepsy. Last but not least, there is a large body of evidence that the peptide of our interest, Ang IV, has cognitive enhancing properties, affects clearly synaptic efficacy in the hippocampus and has a modulatory action on seizures and epileptogenesis. We have reviewed in detail the effects of Ang IV on synaptic transmission and plasticity, learning, memory, and epileptic seizure activity (De Bundel et al., 2008).

Ang IV was previously found to attenuate pentylenetetrazole-induced ***seizures*** (Tchekalarova et al., 2001). Furthermore, Ang IV showed an anti-epileptogenic effect as it not only suppressed the maintenance of the generalization phenomenon during the kindling procedure but also blocked the development of an epileptic-like state in mice (Tchekalarova et al., 2005a,b). We have previously shown that i.c.v. administered Ang IV is anticonvulsant in the acute pilocarpine model for focal epilepsy in rats (Stragier et al., 2006). This was accompanied by a concomitant increase of the hippocampal extracellular ***dopamine and serotonin*** concentration. Possibly, this plays a role in the anticonvulsant effect of Ang IV. Indeed, several well-known anti-epileptic drugs can elicit a monoaminergic stimulation. Moreover, it was shown in our laboratory that intrahippocampally administered dopamine and serotonin protected rats against pilocarpine induced convulsions via respectively D_2 and 5-HT_{1A} receptor activation (Clinckers et al., 2004).

The anticonvulsant effect of Ang IV against limbic seizures could be reversed by somatostatin sst_2 receptor antagonism (Stragier et al., 2006). This suggests that Ang IV may exert an anticonvulsive effect via inhibition of IRAP, resulting in an increase of the anticonvulsive neuropeptide somatostatin-14. Besides somatostatin, other IRAP neuropeptide substrates might also be involved in protection from seizures. IRAP is indeed an aminopeptidase known to metabolize several substrate neuropeptides *in vitro*, including somatostatin, oxytocin, vasopressin, lys-bradykinin, met-enkephalin, dynorphin A 1-8,

neurokinin A, neuromedin B and cholecystokinin 8 ((Rogi et al., 1996; Lew et al., 2003). It was therefore suggested that Ang IV may mediate some or all of its central effects through inhibition of IRAP and accumulation of its neuropeptide substrates, i.e. the ***so-called neuropeptide hypothesis***.

Some of these various neuropeptide substrates, ***somatostatin and oxytocin,*** deserved further attention within this project.

The ***neuropeptide somatostatin*** is indeed a powerful modulator of hippocampal excitability. Somatostatin-expressing neurons constitute a major subtype of hippocampal GABA-ergic interneurons. Loss of these neurons in the hilus of the dentate gyrus is a hallmark of the hippocampus in patients with temporal lobe epilepsy and in corresponding animal models. A possible role for somatostatin in controlling hippocampal excitability is further supported by the observation that somatostatin reduces epileptiform activity in the CA1 and CA3 regions of rat hippocampal slices. Moreover, somatostatin is anticonvulsive in several rodent models for limbic seizures, and as described above, we were able to demonstrate a clear interaction between the anticonvulsive effects of Ang IV and the somatostatin system.

Interactions between Ang IV and the ***neuropeptide oxytocin*** have also been proposed to be responsible for some of the behavioural observations produced by administration of Ang IV and its synthetic analogues in animal models (Albiston et al. 2003). Both Ang IV and oxytocin enhanced learning and memory in rodents (Gard, 2007). In the light of the proposed effects of Ang IV on oxytocinase/ Otase (see introduction), the effects of Ang IV on cognition could result from elevated levels of oxytocin due to IRAP/Otase inhibition. Moreover, Beyer and colleagues just recently demonstrated that Ang IV elevated oxytocin levels in the rat amygdala and produced anxiolytic-like activity through subsequent oxytocin receptor activation (Beyer et al., 2010). Finally, oxytocin has also been demonstrated to possess antidepressant-like activities in rodents (Gard, 2007).

V. Extended working hypotheses and aims of the project with regard to the involvement of IRAP in epileptic seizures and antidepressant-like activities

(i) First we aimed to clarify the function of IRAP in seizure mechanisms by comparing the threshold for epileptic seizures in **IRAP knockout (KO) mice** and their wild-type (WT) litter mates in several chemoconvulsant seizure models. Similary we used **IRAP KO mice** and their WT litter mates to investigate the role of IRAP in possible anti-depressant-like effects.
(ii) The second aim was to further explore by which **mechanisms of action** the Ang IV-IRAP interaction is able to induce anticonvulsant effects, and whether IRAP could be involved in some of the known *in vivo* oxytocin-mediated effects.
(iii) Finally, **novel developed and stable Ang IV ligands** could be evaluated in various seizure models.

VI. Results with regard to the extended working hypotheses

VI.1.1. IRAP is unequivocally involved in seizure generation

To unequivocally unravel the involvement of IRAP in seizure generation, IRAP KO mice and their WT littermates were subjected to an intravenous tail infusion of pentylenetetrazole, which is an established acute model of generalised seizures. Compared to male WT mice, male KO mice showed significantly increased pentylenetetrazole thresholds for myoclonic twitch, clonus without loss of reflexes and clonus with loss of reflexes (Loyens et al, submitted). We also tested the IRAP KO mice in a model for limbic seizures, i.e. the pilocarpine tail infusion model. IRAP KO mice had a significantly higher pilocarpine threshold compared to WT animals for the first onset of tremor, clonic convulsions, tonic convulsions and death. These data again unequivocally show that mice lacking functional IRAP are partially protected against pilocarpine-induced seizures and toxicity. In conclusion, IRAP is thus clearly involved in seizure generation, since male IRAP KO mice are less sensitive to the development of generalized seizures following pentylenetetrazole administration or limbic seizures following pilocarpine administration.

VI.1.2. Results obtained on the mechanism of anticonvulsive action by an Ang IV-IRAP interaction

Since we clarified the involvement of IRAP in seizure mechanisms, we subsequently investigated the local effects of two peptide inhibitors of IRAP, Ang IV and LVV-H7, on seizures evoked by intrahippocampal pilocarpine administration in rats. Intrahippocampal administration of Ang IV or LVV-H7 protected rats against pilocarpine induced seizures. We clearly excluded the involvement of other potential binding sites, essentially the angiotensin AT_1 receptor for Ang IV and the opioid μ/κ receptor for LVV-H7. We demonstrated that the anticonvulsive effects of both locally applied Ang IV and LVV-H7 are reversed by the sst_2 receptor antagonist cyanamid 154806. Intrahippocampal administration of somatostatin was anticonvulsive in the same model. We then hypothesized that the anticonvulsive effects of Ang IV and LVV-H7 would result from inhibition of somatostatin degradation via IRAP. We therefore initiated collaboration with Prof. Kiki Thermos of the University of Heraklion (Greece). One investigator of our team performed the necessary microdialysis experiments in the laboratory of Heraklion and the samples were analysed for somatostatin content by a validated radioimmunoassay. Nevertheless, Ang IV and LVV-H7 did not increase the extracellular somatostatin levels as such and even suppressed high potassium-evoked somatostatin release in the hippocampus of freely moving rats. Moreover, we observed no differences in the degradation of somatostatin in a cerebral membrane preparation from IRAP KO compared to WT mice. The degradation profile of labelled somatostatin in these homogenates was determined at different time intervals (0, 30, 60, 120 and 180 minutes) by an in-house validated radioimmunoassay. Taken together, all these experiments strongly suggest that the effects of Ang IV and LVV-H7 do not result from accumulation of somatostatin due to inhibition of the catalytic domain of IRAP and thus at the moment we reject the so-called neuropeptide hypothesis as a mechanism of anticonvulsive action of the IRAP ligands.

We therefore propose that inhibition of IRAP may directly affect sst_2 receptor signalling and/or trafficking. We are still investigating this new hypothesis in more detail with *ex vivo* experiments. We posses a CHO cell line with stable expression of the sst2 receptor. We already demonstrated that this cell line has a clear endogenous expression of IRAP and thus this cell line is an ideal tool to unravel further the mechanism of action. We also excluded direct binding of Ang IV and LVV-H7 to sst_2 receptors within a well validated radioligand binding assay.

We can also exclude the involvement of oxytocin in the mechanism of anticonvulsant action of Ang IV within the pentylenetetrazole model. To date, the role of the IRAP substrate oxytocin in seizure mechanisms has not been well described in literature. We demonstrated recently that oxytocin has proconvulsant effects within the pentylenetetrazole model for generalised seizures. Indeed, oxytocin at a dose of 0.25 mg/kg, which we demonstrated in another set of experiments to possess antidepressant-like effects within the forced swim test and which is thus centrally active at this dose, significantly lowered the seizure threshold for ear twitch, myoclonic twitch and forelimb clonus in mice.
Finally, we can report that, in the frame of the experiments we performed in collaboration with Prof. Kiki Thermos of the University of Heraklion, we demonstrated that another somatostatin receptor subtype, the sst_1 receptor, is not involved in the anticonvulsive action of somatostatin and thus most probably also not in the mechanism of action of IRAP ligands. Interestingly, these experiments pointed for the first time *in vivo* that the hippocampal sst_1 receptors act as inhibitory autoreceptors. Indeed, intrahippocampal administration of the sst_1 receptor antagonist SRA880 led to a robust, but transient increase in hippocampal somatostatin levels without affecting the GABA levels. It is well known that somatostatin is a co-transmitter of the hippocampal GABAergic interneurones. Our data demonstrate that the observed effects on hippocampal somatostatin levels did not result from increased phasic firing of somatostatin-containing interneurons, but rather involved a specific loss of negative feedback control on tonic somatostatin release (De Bundel et al., 2010).

VI.2.1. Evaluation of the role of IRAP in animal models for depression

We have also explored whether IRAP is implicated in an animal model for depression-like activiy. For this purpose adult male and female control and IRAP KO mice were subjected to the tail suspension test. Two independent researchers of which one was blinded for the treatment manually recorded the immobility time (in s) of each mouse during the 5-min testing period. To validate this system, mice received an i.p. injection of the antidepressant imipramine 30 minutes before the test. As expected, imipramine significantly decreased the mean immobility time as compared to the control group. However, there were no differences in immobility time between WT and IRAP knockout mice of both sexes. These experiments did not allow us to conclude that IRAP might be involved in antidepressant-like effects. Nevertheless, these data are in agreement with the findings that - unlike oxytocin - Ang IV alone had also no effect on learned helplessness in the forced swim test, another test often used to predict potential antidepressant efficacy in humans (Gard, 2007).

VI.2.2. IRAP is involved in the antidepressant-like effects of oxytocin

As stated already above, oxytocin acts as a hormone and a neuromodulator with antidepressant-like activities. Whereas the complete oxytocin structure is required for full endocrine activity, oxytocin metabolites are known to exert strong central activities. Oxytocin has been shown to be rapidly cleaved by IRAP *in vitro*. We recently investigated *in vivo* whether IRAP deletion can modulate the antidepressant-like effects of oxytocin and whether ageing interferes with antidepressant -like effects of oxytocin. For this purpose, male and female C57Bl/6 mice (3 months and 14 months) were used in the forced swim test to optimize the dose of oxytocin with an antidepressant -like effect. For the first swim, no drugs or saline were administered. After a period of four days the same mice underwent a second forced swim, one hour after subcutaneous injection of either saline (10 ml/kg) or oxytocin (0.05 mg/kg, 0.15 mg/kg, 0.25 mg/kg, 0.35 mg/kg or 0.5 mg/kg). Immobility was timed during the second forced swim. The tricyclic antidepressant imipramine was used as a positive control. Young (3 to 6 months old) and middle-aged (12 to 18 months) male and female IRAP WT and KO mice were subjected to the forced swim test, as described above. All mice were first placed in an open field to study locomotor behaviour. Young male C57Bl/6 mice showed a decreased immobility time following the administration of 0.15 mg/

kg and 0.25 mg/kg oxytocin. The effect of 0.25 mg/kg oxytocin was reproduced in young male IRAP WT mice, but not in IRAP KO mice. Oxytocin had no effect in either young female C57Bl/6 mice or in young female IRAP WT and KO mice. However, we found an antidepressant-like effect of 0.15 mg/kg oxytocin in middle-aged female C57Bl/6 mice and IRAP WT mice, compared to saline treated controls. In addition, middle-aged female KO mice treated with saline or oxytocin did not show significant differences in immobility. Oxytocin did not influence the locomotor behaviour of the mice within the open filed, indicating that the observed antidepressant-like effects are not due to locomotor problems. These data attribute an important role to IRAP as a mediator of antidepressant-like effects of oxytocin, since the immobility time in IRAP KO mice did not change following oxytocin treatment. We suggest that IRAP could be responsible for the *in vivo* degradation of oxytocin into its metabolites. We hypothesize that oxytocin metabolites rather than the intact neuropeptide are responsible for antidepressant-like activity. In addition, the absence of oxytocin effects in younger female mice indicates that the antidepressant-like effects of oxytocin are age-dependent in female mice. These differences could be explained by a difference in circulating estrogen and oxytocin levels. However, to confirm these hypotheses, further work is required.

VII. 2008-2010 Publication List

2008

- Albiston, A.L., Morton, C.J., Ng, H.L., Pham, V., Yeatman, H.R., Ye, S., Fernando, R.N., De Bundel, D., Ascher, D.B., Mendelsohn, F.A., Parker, M.W., Chai, S.Y. Identification and characterization of a new cognitive enhancer based on inhibition of insulin-regulated aminopeptidase. FASEB J. 2008, 22(12): 4209-4217. (SCI impact factor=6.80)
- Andersson, H., Demaegdt, H., Vauquelin, G., Lindeberg, G., Hallberg, A., Karlén, A., Hallberg, M. Ligan ds to the (IRAP)/AT4 receptor encompassing a 4-hydroxydiphenylmethane scaffold replacing Tyr2. Bioorg Med Chem. 2008, 16, 6924-6935. (SCI impact factor: 2.62)
- De Bundel, D., Smolders, I., Vanderheyden, P., Michotte, Y. Ang II and Ang IV: unraveling the mechanism of action on synaptic plasticity, memory, and epilepsy. CNS Neurosci Ther. 2008,14(4):315-339. (SCI impactfactor = 3.80)
- Demaegdt, H., Smitz, L., De Backer, J.P., Le, M.T., Bauwens, M., Szemenyei, E., Tóth, G., Michotte, Y., Vanderheyden, P.M.L., Vauquelin, G. Translocation of the Insulin Regulated Aminopeptidase to the cell surface: detection by radioligand binding. Brit J Pharmacol, 2008, 154, 872-881. (SCI impactfactor: 3.82)
- Demaegdt, H. Chapter IV: How is the Insulin Regulated Aminopeptidase involved in the physiological and in vitro effects of Angiotensin IV? Review. 'Angiotensin Research Progress', Eds: Hina Miura and Yuuto Sasaki, Nova Publishers group, pp 93-119.
- Lukaszuk, A., Demaegdt, H., Szemenyei, E., Tóth, G., Tymecka, T., Misicka, A., Karoyan, P., Vanderheyden, P.M.L., Vauquelin, G., Tourwé, D. beta-homo-amino acid scan of Angiotensin IV. J Med Chem, 2008, 51, 2291-2296. (SCI impact factor: 5.11)
- Stragier, B., De Bundel, D., Sarre, S., Smolders, I., Vauquelin, G., Dupont, A., Michotte, Y., Vanderheyden, P. Involvement of insulin-regulated aminopeptidase in the effects of the renin-angiotensin fragment angiotensin IV: a review. Heart Fail Rev. 2008, 13(3):321-337. (SCI impactfactor = 2.12)

2009

- De Bundel D, Smolders I, Yang R, Albiston AL, Michotte Y, Chai SY. Angiotensin IV and LVV-haemorphin 7 enhance spatial working memory in rats: effects on hippocampal glucose levels and blood flow. Neurobiol Learn Mem. 2009; 92(1):19-26. (SCI impact factor = 3.80)
- Demaegdt H, Lukaszuk A, De Buyser E, De Backer JP, Szemenyei E, Tóth G, Chakravarthy S, Panicker M, Michotte Y, Tourwé D, Vauquelin G. Selective labeling of IRAP by the tritiated AT(4) receptor ligand [3H]Angiotensin IV and its stable analog [3H]AL-11. Mol Cell Endocrinol. 2009 , 311(1-2):77-86. (SCI impact factor = 3.50)
- Lukaszuk A, Demaegdt H, Feytens D, Vanderheyden P, Vauquelin G, Tourwé D. The replacement of His(4) in angiotensin IV by conformationally constrained residues provides highly potent and selective analogues. J Med Chem. 2009, 52(18):5612-8. (SCI impact factor = 4.80)
- Vanderheyden PM, From angiotensin IV binding site to AT4 receptor., Mol Cell Endocrinol. 2009, 302(2):159-66. (SCI impact factor = 3.50)

2010

- Andersson H, Demaegdt H, Vauquelin G, Lindeberg G, Karlén A, Hallberg M, Erdélyi M, Hallberg A. Disulfide cyclized tripeptide analogues of angiotensin IV as potent and selective inhibitors of insulin-regulated aminopeptidase (IRAP). J Med Chem. 2010, 53(22):8059-71 (SCI impact factor = 4.80)
- De Bundel D, Demaegdt H, Lahoutte T, Caveliers V, Kersemans K, Ceulemans AG, Vauquelin G, Clinckers R, Vanderheyden P, Michotte Y, Smolders I. Involvement of the AT1 receptor subtype in the effects of angiotensin IV and LVV-haemorphin 7 on hippocampal neurotransmitter levels and spatial working memory. J Neurochem. 2010, 112(5):1223-34. (SCI impactFactor = 3.99)
- De Bundel D, Aourz N, Kiagiadaki F, Clinckers R, Hoyer D, Kastellakis A, Michotte Y, Thermos K, Smolders I. Hippocampal sst1 receptors are autoreceptors and do not affect seizures in rats. Neuroreport, 2010; 10;21(4):254-8.(SCI impact factor = 1.90).

Submitted

- Demaegdt H., De Backer J.-P., Lukaszuk A., Tóth G, Szemenyei E., Tourwé D. and Vauquelin G. Angiotensin IV only displays low affinity for native insulin-regulated amonipeptidase (IRAP) . Fund. Clin. Pharmacol. (submitted)
- Demaegdt H., Gard P., De Backer J.-P., Lukaszuk A., Szemenyei E., Tóth G, Tourwé D. and Vauquelin G. Binding of “AT4 receptor” ligands to the insulin regulated aminopeptidase (IRAP) in intact Chinese hamster ovary cells. Mol. Cell. Endocrinol. (submitted)
- Loyens E, Schallier A, Chai SY, De Bundel D, Michotte Y, Vanderheyden P, Smolders I. Deletion of insulin-regulated aminopeptidase in mice decreases susceptibility to pentylenetetrazol-induced generalized seizures. Seizure (submitted)

VIII. References

- Albiston A.L., McDowall S.G., Matsacos D., Sim P., Clune E., Mustafa T., Lee J., Mendelsohn F.A.O., Simpson R.G., Connolly L., Chai S.Y. Evidence that the Angiotensin IV (AT4) receptor is the enzyme Insulin-regulated aminopeptidase. J. Biol. Chem. 276: 48623-48626, 2001.
- Albiston AL, Mustafa T, McDowall SG, Mendelsohn FA, Lee J, Chai SY. AT4 receptor is insulin-regulated membrane aminopeptidase: potential mechanisms of memory enhancement. Trends Endocrinol Metab. 14(2):72-7. Review, 2003.
- Albiston AL, Pederson ES, Burns P, Purcell B, Wright JW, Harding JW, Mendelsohn FA, Weisinger RS, Chai SY. Attenuation of scopolamine-induced learning deficits by LVV-hemorphin-7 in rats in the passive avoidance and water maze paradigms. Behav Brain Res. Sep 23;154(1):239-43, 2004.
- Albiston AL, Morton CJ, Ng HL, Pham V, Yeatman HR, Ye S, Fernando RN, De Bundel D, Ascher DB, Mendelsohn FA, Parker MW, Chai SY. Identification and characterization of a new cognitive enhancer based on inhibition of insulin-regulated aminopeptidase. FASEB J. Dec;22(12):4209-17. Epub 2008 Aug 20, 2008.
- Albiston A.L., Fernando R.N., Yeatman H.R., Burns P., Ng L., Daswani D., Diwakarla S., Pham V., Chai S.Y. Gene knockout of insulin-regulated aminopeptidase: Loss of the specific binding site for angiotensin IV and age-related deficit in spatial memory. Neurobiol. Learning Memory, doi:.2009.07.011, 2009.
- Andersson H., Demaegdt H., Erdélyi M., Vauquelin G., Lindeberg G., Hallberg M., Karlén A. and Hallberg A. Disulfide cyclized tripeptide analogues of angiotensin IV as potent and selective inhibitors of insulin-regulated aminopeptidase (IRAP). J. Med. Chem. (in press), 2010.
- Andersson H., Demaegdt H., Vauquelin G., Lindeberg G., Hallberg A., Karlén A. and Hallberg M. Ligands to the (IRAP)/AT4 receptor encompassing a 4-hydroxydiphenyl-methane scaffold replacing Tyr2. Bioorg. Med. Chem. 16, 6924-6935, 2008.
- Axén A., Lindeberg G., Demaegdt H., Vauquelin G., Karlén A. and Hallberg M. Cyclic insulin-regulated aminopeptidase (IRAP)/AT4 receptor ligands. J. Pept. Sci. 12, 705-713, 2006.
- Axén A, Andersson H, Lindeberg G, Rönnholm H, Kortesmaa J, Demaegdt H, Vauquelin G, Karlén A, Hallberg M. Small potent ligands to the insulin-regulated aminopeptidase (IRAP)/AT(4) receptor. J Pept Sci. Jul;13(7):434-44, 2007.
- Beyer CE, Dwyer JM, Platt BJ, Neal S, Luo B, Ling HP, Lin Q, Mark RJ, Rosenzweig-Lipson S, Schechter LE. Angiotensin IV elevates oxytocin levels in the rat amygdala and produces anxiolytic-like activity through subsequent oxytocin receptor activation. Psychopharmacology (Berl). 209(4):303-11, 2010.
- Braszko J.J. , Kupryszewski G., Witczuk B., Wisniewski K. Angiotensin II (3-8)-hexapeptide affects motor activity, performance of passive avoidance and a conditioned avoidance response in rats. Neuroscience 27: 777-783, 1988.
- Chai S.Y., Bastias M.A., Clune E.F., Matsacos D.J., Mustafa T., Lee J.H., McDowall S.G., Mendelsohn F.A., Albiston A.L., Paxinos G. Distribution of angiotensin IV binding sites (AT4 receptor) in the human forebrain, midbrain and pons as visualised by in vitro receptor autoradiography. J. Chem. Neuroanat. 20(3-4): 339-348, 2000.
- Clinckers R, Smolders I., Meurs A., Ebinger G., Michotte Y., Anticonvulsant action of hippocampal dopamine and serotonin

is independently mediated by D2 and 5-HT1A receptors. J. Neurochem. 89:834-843, 2004.
– De Bundel D., Smolders I., Vanderheyden P.M.L. and Michotte Y. Ang II and Ang IV: unraveling the mechanism of action on synaptic plasticity, memory and epilepsy. CNS Neurosci. Ther. 14(4), 315-339, 2008.
– De Bundel D, Smolders I, Yang R, Albiston AL, Michotte Y, Chai SY. Angiotensin IV and LVV-haemorphin 7 enhance spatial working memory in rats: effects on hippocampal glucose levels and blood flow. Neurobiol Learn Mem. Jul;92(1):19-26. Epub 2009 Feb 20, 2009.
– De Bundel D, Demaegdt H, Lahoutte T, Caveliers V, Kersemans K, Ceulemans AG, Vauquelin G, Clinckers R, Vanderheyden P, Michotte Y, Smolders I. Involvement of the AT1 receptor subtype in the effects of angiotensin IV and LVV-haemorphin 7 on hippocampal neurotransmitter levels and spatial working memory. J Neurochem. Mar;112(5):1223-34, 2010.
– Demaegdt H., Laeremans H., De Backer J.P., Mosselmans S, Le MT, Kersemans V, Michotte Y., Vauquelin G. and Vanderheyden P.M.L. Synergistic modulation of cystinyl aminopeptidase by divalent cation chelators. Biochem. Pharmacol. 68:893-900, 2004.
– Demaegdt, H., Smitz, L., De Backer, J.P., Le, M.T., Bauwens, M., Szemenyei, E., Tóth, G., Michotte, Y., Vanderheyden, P., Vauquelin, G. Translocation of the insulin-regulated aminopeptidase to the cell surface: detection by radioligand binding. Br. J. Pharmacol. 154, 872-881, 2008.
– Demaegdt, H, Lukaszuk, A, De Buyser, E, De Backer, JP, Szemenyei, E, Tóth, G, Chakravarthy, S, Panicker, P, Michotte, Y, Tourwé, D, Vauquelin, G. Selective labeling of IRAP by the tritiated AT_4 receptor ligands [^{3}H]Angiotensin IV and its stable analog [^{3}H]AL-11. Mol. Cell. Endocrinol, 311, 77-86, 2009.
– Demaegdt H., De Backer J.-P., Lukaszuk A., Tóth G, Szemenyei E., Tourwé D. and Vauquelin G. Angiotensin IV only displays low affinity for native insulin-regulated amonipeptidase (IRAP) . Fund. Clin. Pharmacol. (submitted), 2010a.
– Demaegdt H., Gard P., De Backer J.-P., Lukaszuk A., Szemenyei E., Tóth G, Tourwé D. and Vauquelin G. Binding of "AT4 receptor" ligands to the insulin regulated aminopeptidase (IRAP) in intact Chinese hamster ovary cells. Mol. Cell. Endocrinol. (submitted), 2010b.
– Faure S, Chapot R, Tallet D, Javellaud J, Achard JM, Oudart N. Cerebroprotective effect of angiotensin IV in experimental ischemic stroke in the rat mediated by AT(4) receptors. J Physiol Pharmacol. Sep;57(3):329-42, 2006.
– Fernando R.N., Luff S.E., Albiston A.L., Chai S.Y., Sub-cellular localization of insulin-regulated membrane aminopeptidase, IRAP to vesicles in neurons. J. Neurochem. 102: 967-976, 2007.
– Fernando R.N., Albiston A.L., Chai S.Y., The insulin-regulated aminopeptidase IRAP is colocalised with GLUT4 in the mouse hippocampus, potential role in modulation of glucose uptake in neurones? Eur. J. Neurosci. 28(3):588-598, 2008.
– Gard PR, Daw P, Mashhour ZS, Tran P. Interactions of angiotensin IV and oxytocin on behaviour in mice. J Renin Angiotensin Aldosterone Syst. 2007 Sep;8(3):133-8.
– Garza, L.A., Birnbaum, M.J., 2000. Insulin-responsive aminopeptidase trafficking in 3T3-L1 adipocytes. J Biol. Chem. 275, 2560-2567.
– Grillo CA, Piroli GG, Hendry RM, Reagan LP. Insulin-stimulated translocation of GLUT4 to the plasma membrane in rat hippocampus is PI3-kinase dependent. Brain Res. 1296:35-45, 2009.
– Hasselmo M. E. The role of acetylcholine in learning and memory. Current opinion in neurobiology 16, 710-715, 2006.
– Hermann B, Seidenberg M, Jones J. The neurobehavioural comorbidities of epilepsy: can a natural history be developed? Lancet Neurol. Feb;7(2):151-60. Review, 2008.
– Johnson, A.O., Subtil, A., Petrush, R., Kobylarz, K., Keller, S.R., McGraw, T.E., 1998. Identification of an insulin-responsive, slow endocytic recycling mechanism in Chinese hamster ovary cells. J. Biol. Chem. 273, 17968-17977.
– Keller S. R., Scott H.M., Mastick C.C., Aebersold R., Lienhard G.E. Cloning and characterization of a novel insulin-regulated membrane aminopeptidase from Glut4 vesicles. J. Biol. Chem. 270, 23612-23618, 1995.
– Kramár E.A., Armstrong D.L., Ikeda S., Wayner M.J., Harding J.W., Wright J.W. The effects of angiotensin IV analogs on long-term potentiation within the CA1 region of the hippocampus in vitro. Brain Res. 897: 114-121, 2001.
– Lee J., Chai S., Mendelsohn F.A., Morris M.J., Allen A.M. Potentiation of cholinergic transmission in the rat hippocampus by angiotensin IV and LVV-hemorphin-7. Neuropharmacology 40: 618-623, 2001.
– Lew R.A., Mustafa T., Ye S., McDowall S.G., Chai S.Y. and Albiston A.L. Angiotensin AT4 ligands are potent, competitive inhibitors of insulin regulated aminopeptidase (IRAP). J Neurochem. 86: 344-350, 2003.
– Lukaszuk, A., Demaegdt, H., Szemenyei, E., Tóth, G., Tymecka, T., Misicka, A., Karoyan, P., Vanderheyden, P.M.L., Vauquelin, G., Tourwé, D. beta-homo-amino acid scan of Angiotensin IV. J. Med. Chem., 51, 2291-2296, 2008.
– Lukaszuk, A, Demaegdt, H, Feytens, D, Vanderheyden, P, Vauquelin, G, Tourwé, D. The replacement of His(4) in angiotensin IV by conformationally constrained residues provides highly potent and selective analogues. J Med Chem, 52, 5612-8, 2009.
– McNay EC, Gold PE. Food for thought: fluctuations in brain extracellular glucose provide insight into the mechanisms of memory modulation. Behav Cogn Neurosci Rev. 1(4):264-80. Review, 2002.
– Mendez M, Lim G. Seizures in elderly patients with dementia: epidemiology and management. Drugs Aging. 20(11):791-803. Review, 2003.
– Miller-Wing A.V., Hanesworth J.M., Sardinia M.F., Hall K.L., Wright J.W., Speth R.C., Grove K.L., Harding J.W. Central

angiotensin IV binding sites: distribution and specificity in guinea pig brain. J. Pharmacol. Exp. Ther. 266: 1718-1726, 1993.
- Møeller I., Lew R.A., Mendelsohn F.A., Smith A.I., Brennan M.E., Tetaz T.J., Chai S.Y. The globin fragment LVV-hemorphin-7 is an endogenous ligand for the AT4 receptor in the brain. J. Neurochem. 68: 2530-2537, 1997.
- Nakamura, H., Itakuara, A., Okamura, M., Ito, M., Iwase, A., Nakanishi, Y., Okada, M., Nagasaka, T., Mizutani, S. 2000. Oxytocin stimulates the translocation of oxytocinase of human vascular endothelial cells via activation of oxytocin receptors. Endocrinology 141, 4481-4485.
- Rogi T., Tsujimoto M., Nakazato H., Mizutani S., Tomoda Y. Human placental leucine aminopeptidase/oxytocinase. A new member of type II membrane spanning zinc metallopeptidase family. J. Biol. Chem. 271: 56–61, 1996.
- Ross S.A., Scott H.M., Morris N.J., Leung W.Y., Mao F., Lienhard G.E., Keller S.R., Characterization of the insulin-regulated membrane aminopeptidase in 3T3–L1 adipocytes. J. Biol. Chem. 271, 3328-3332, 1996.
- Royer S, Paré D. Conservation of total synaptic weight through balanced synaptic depression and potentiation. Nature. Apr 3;422(6931):518-22, 2003.
- Stragier B., Clinckers R., Meurs A., De Bundel D., Sarre S., Ebinger G., Michotte Y., Smolders I. Involvement of the somatostatin 2 receptor in the anticonvulsant effect of angiotensin IV against pilocarpine-induced limbic seizures in rats. J. Neurochem. 98(4): 1100-1113, 2006.
- Sumitani S., Ramlal T., Somwar R., Keller S.R., Klip A.. Insulin regulation and selective segregation with glucose transporter-4 of the membrane aminopeptidase vp165 in rat skeletal muscle cells. Endocrinology. 138, 1029-1034, 1997.
- Tchekalarova J., Kambourova T., Georgiev V. Interaction between angiotensin IV and adenosine A(l) receptor related drugs in passive avoidance conditioning in rats. Behav. Brain Res. 123: 113-116, 2001.
- Tchekalarova J., Georgiev V., Angiotensin peptides modulatory system: how is it implicated in the control of seizure susceptibility? Life Sci. 76:955-970, 2005a.
- Tchekalarova J., Sotiriou E., Georgiev V., Kostopoulos G., Angelatou F., Up-regulation of adenosine A1 receptor binding in pentylenetetrazol kindling in mice: effects of angiotensin IV. Brain Res. 1032:94-103, 2005b.
- Van Dam D, Abramowski D, Staufenbiel M, De Deyn PP. Symptomatic effect of donepezil, rivastigmine, galantamine and memantine on cognitive deficits in the APP23 model. Psychopharmacology (Berl). 180(1):177-90, 2005.
- Van Dam D., De Deyn P.P., Drug discovery in dementia: the role of rodent models. Nat Rev Drug Discov. 11, 956-970, 2006.
- Vauquelin G., Michotte Y., Smolders I., Sarre S., Ebinger G., Dupont A., Vanderheyden P. Cellular targets for angiotensin II fragments: pharmacological and molecular evidence. J Renin Angiotensin Aldosterone Syst. 3(4):195-204, 2002.
- Wayner M.J.A.D., Phelix C.F., Wright J.W., Harding J.W. Angiotensin IV enhances LTP in rat dentate gyrus in vivo. Peptides 22: 1403-1414, 2001.
- Wright J.W., Miller-Wing A.V., Shaffer M.J., Higginson C., Wright D.E., Hanesworth J.M., Harding J.W. Angiotensin II(3-8) (ANG IV) hippocampal binding: potential role in the facilitation of memory. Brain Res. Bull. 32(5): 497-502, 1993.
- Wright J.W., Clemens J.A., Panetta J.A., Smalstig E.B., Stubley-Weatherly L.A., Kramár E.A., Pederson E.S., Mungall B.H., Harding J.W. Effects of LY231617 and angiotensin IV on ischemia-induced deficits in circular water maze and passive avoidance performance in rats. Brain Res. 717: 1-11, 1996.
- Wright J.W., Stubley L., Pedersen E.S., Kramar E.A., Hanesworth J.M., Harding J.W. Contributions to the brain angiotensin IV-AT4 receptor subtype system to spatial learning. J. Neurosci. 19: 3952-3961, 1999.
- Yang R, Smolders I, De Bundel D, Fouyn R, Halberg M, Demaegdt H, Vanderheyden P, Dupont AG.
- Brain and peripheral angiotensin II type 1 receptors mediate renal vasoconstrictor and blood pressure responses to angiotensin IV in the rat. J Hypertens. May;26(5):998-1007, 2008.

Final report of the research group of

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Characterization of new cellular and molecular mechanisms underlying the migration of interneurons in the telencephalon

Backround

The complex organization of the cerebral cortex reflects the elaborated patterns of cell migration that contributed to its development. This structure contains neurons that are distributed within layers and are regionally organized into specialized areas that underlie sophisticated motor, cognitive and perceptual abilities. Cortical lamination follows an «inside-out» sequence of neuronal placement and maturation that arises from the successive birth and orderly migration of pyramidal projection neurons born in the dorsal telencephalon and GABAergic interneurons generated in the ganglionic eminences (GE)(Marin and Rubenstein, 2003). Experimental observations show that most projection neurons migrate radially within the cortical wall, whereas interneurons migrate from the GE through multiple tangential pathways to reach the developing cortex. Recent studies suggest that defects in neuronal migration may lead to several impairments, which, in human, are characterised, by learning disabilities, mental retardation or epilepsy (Levitt et al., 2004; Pancoast et al., 2005). Moreover, converging experimental and clinical evidence suggests that altered interneuron development may underlie part of the pathophysiological processes that ultimately lead to bipolar disorder, schizophrenia and autism (Benes and Berretta, 2001; Levitt et al., 2004). Defining how cortical neurons migrate and integrate into specific circuits is, therefore, essential for understanding the biological basis of these disorders.
The following report summarizes the work performed during the past three years thanks to the generous funding from the FMRE/GSKE and provides the perspectives of our future research.

Unravelling the functions of Cip/Kip proteins during the migration and differentiation of cortical interneurons

The generation of cortical interneurons is a complex process that relies on the decision of ventral progenitors to leave the cell cycle, migrate tangentially to appropriate locations and differentiate into mature neurons that are stably positioned and are actively extending axonal and dendrite branches. Importantly, these concurrent steps imply dynamic cell shape remodelling which largely depends on the regulation of cytoskeleton components. Our current experiments are aimed at identifying key molecules that control cytoskeleton remodelling during the migration and branching of cortical interneurons. Indeed, migration of interneurons results from successive cycles of morphological changes that couple the saltatory progression of their cell body with the dynamic remodelling of their leading process. The nucleus alternates resting phases that correlate with the elongation of the leading process and moving phases associated with growth cone-like structure (named growth cone further in the text) splitting and branching of that process.
First identified as cell cycle inhibitors mediating the growth inhibitory cues of upstream signalling pathways, the cyclin-CDK inhibitors of the Cip/Kip family have emerged as multifonctional proteins with roles extending beyond the cell cycle regulation. A prime example is $p27^{Kip1}$ as reported by our previous work. It promotes both neuronal differentiation and migration of cortical projection neurons through distinct and separable cell-cycle independant mechanisms (Nguyen et al., 2006). Our recent analyses performed on embryonic brains revealed the expression of $p27^{Kip1}$ in progenitors located in the subventricular zone of the medial (MGE) and caudal ganglionic eminence (CGE) as well as in postmitotic

cortical interneurons. Interestingly, $p27^{Kip1}$ was expressed in both the nucleus and the cytoplasm of these neurons, suggesting that it could play cell cycle-unrelated functions. In order to test this hypothesis we analysed the generation and migration of interneurons in the cortex of p27 knockout mice (Fero et al., 1996; Nakayama et al., 1996). Surprisingly, the lack of p27 expression did not impair the proliferation nor the cell cycle exit of GE interneurons progenitors, suggesting that $p57^{Kip2}$ (according to its expression pattern) might compensate for the lack of $p27^{Kip1}$. However, we found a reduced number of Lhx6-positive interneurons in the lateral cortex of p27 knockout E14 embryos that suggests a defect in tangential migration. To test this hypothesis we conditionally removed p27 in Dlx5,6-positive cortical interneurons (generated by Dlx5,6 Cre-IRES-GFP (Stenman et al., 2003) X $p27^{lox/lox}$ (Chien et al., 2006) breedings) and confirmed defects in tangential migration. We performed time lapse experiments on cultured slices from E12 embryos from similar breedings and observed a significant reduction of the speed of tangential migration when p27 was conditionally removed (33.0 +/- 1.9 μm/hour, n=46 cells versus 45.9 + /- 1.6 μm/hour, n=59 for control). These data prompt us to analyse the detailed morphology of GABAergic interneurons during their migration out of the MGE. For this purpose we cultured MGE explants from transgenic mice on wild type cortical feeders and carefully recorded the front of migration using time-lapse analyses. Cells that lack p27 were undergoing unusualy quick but less efficient nucleokinesis, as the total distance travelled by the nucleus was reduced. In addition, the absence of p27 resulted in an uncoordinated production of branches on the leading process. These cellular defects likely account for the reduced migration speed of the GABAergic interneurons that are invading the cerebral cortex of Dlx5,6 Cre-IRES-GFP ; $p27^{lox/lox}$ embryos in vivo. In addition, we demonstrated that p27 regulates the tangential migration of interneurons through cell cycle-independent activities as its acute knockdown by electroporation-based transfection of shRNAs in postmitotic interneurons resulted in defective tangential migration and, cortical interneurons generated in $p27^{ck-}$ embryos (knock-in mouse where the coding sequence of $p27^{Kip1}$ has been swapped with a mutant version of $p27^{Kip1}$ ($p27^{ck-}$) that cannot promote cell cycle exit ; (Besson et al., 2004; Nguyen et al., 2006) did not show obvious migration defects. Real time imaging studies revealed that kinetics of both nucleokinesis and leading process branching were disrupted in the absence of p27. Thus, we have analyzed the molecular pathways triggered by $p27^{Kip1}$ that controls the nucleokinesis and the dyamic branching of cortical interneurons during tangential migration. Tangential migration occurs as a series of saltatory movements and dynamic branching of the leading process during which the neuronal cytoskeleton undergoes complex structural changes. At the molecular level, we found that this protein controled actomyosin contractions that drive nucleus forward translocation and growth cone splitting. Furthermore, we showed that $p27^{Kip1}$ is a microtubule-associated protein that stimulates the polymerisation and increases the stability of microtubules, thereby promoting neurite elongation during migration. Thus, $p27^{Kip1}$ cell-autonomously controls interneuron migration by coupling specific regulations of the actin and microtubule cytoskeletons.
This work will be submitted for publication during 2011.

Defining Elongator functions during the migration and differentiation of cortical interneurons

The dynamic remodelling of the cytoskeleton provides the driving force required for cell migration. Several molecules that interact with either actin filaments or microtubules have been identified as part of the molecular machinery that underlie the radial migration of projection neurons. We have recently identified the Elongator complex as a new element of this machinery.
Elongator is composed by 6 subunits (Elp1-Elp6) and assembled by its scaffold protein Elp1 (Close et al., 2006; Petrakis et al., 2004). Elp3 is the catalytic subunit which acetylates histone H3 (Hawkes

et al., 2002; Kim et al., 2002; Winkler et al., 2002). Elongator associates with several nascent RNAs in yeast (Kristjuhan and Svejstrup, 2004) and is preferentially recruited to the transcribed regions of human genes (Close et al., 2006; Kouskouti and Talianidis, 2005), which supports a role in transcript elongation. Other reports also provided evidences for a role in exocytosis and tRNA modification in the cytoplasm (Esberg et al., 2006; Huang et al., 2005; Rahl et al., 2005). Elongator deficiency in humans causes familial dysautonomia (FD), an autosomal recessive disease characterized by defects in the development and maintenance of neurons of the autonomic and sensory systems (Axelrod, 2004; Slaugenhaupt and Gusella, 2002). While neuropathological reports have mostly described lesions in the peripheral nervous system (PNS) (Pearson and Pytel, 1978; Pearson et al., 1978), functional neuroimaging analyses supported central defects with unusual activities in specific cortical areas (Axelrod et al., 2000). In addition, we showed that the depletion of Elongator cell-autonomously delayed radial migration and impaired branching of cortical projection neurons by reducing the acetylation of α tubulin (Creppe et al., 2009).

Our current results show that Elongator subunits are also expressed in the GE, where cortical GABAergic interneurons are generated. Thus, we will assess if Elongator takes part to the regulation of the migration and branching of cortical interneurons. For this purpose, we have generated an $Elp3^{lox/lox}$ transgenic mouse line and received another line $Elp1^{lox/lox}$ (KOMP repository) that will be used for conditional removal of Elp3 and Elp1 in Dlx5,6:Cre-IRES-GFP interneurons. This strategy will allow us to analyse the generation, migration and branching of Elongator deficient cortical interneurons.

We recently identified α tubulin as the first cytoplamic target of Elp3. Its proper acetylation by Elp3 is required for the regulation of both, branching and migration of cortical projection neurons (Creppe et al., 2009). While a variety of cytoplasmic and mitochondrial proteins are known to be acetylated, the role of such modification and the identity of the acetylase often remains unknown (Kim et al., 2006). We are currently characterizing other relevant cytoplasmic substrates of Elp3 that can be acetylated and as such underlie neurogenesis in the brain. To unravel this issue, we are following two experimental approaches. First, in order to characterise the Elp3-dependent acetylome (all proteins that undergo acetylation in the cytoplasm) of the developing cortex, we are using a conditional gene knockout approach ($Elp3^{lox/lox}$; FoxG1:Cre) to prevent the expression of Elp3 in cortical projection neurons. Dorsal or ventral progenitors and newborn neurons will be isolated prior or after *crelox*-mediated invalidation and then processed by MS-based quantitative proteomics (using iTRAQ reagents, Applied Biosystems) to characterize the acetylome and uncover specific non-core histone targets of Elp3. We will follow a complementary approach to validate the acetylation of proteins that have recently been described in large acetylome screens (Choudhary et al., 2009; Kim et al., 2006) and that promote brain neurogenesis. Among them, Filamin A has recently been shown to bind Elp1, an interaction required to target filamin A to membrane ruffles and thus promotes neuron migration (Johansen et al., 2008). As such, Filamin A is a strong acetylation target of Elp3. To confirm this hypothesis, we will look for filamin A acetylation in cortical neurons isolated from $Elp3^{lox/lox}$; FoxG1:Cre mouse embryos (E14) and analyse the morphology and migration of cortical projection neurons expressing key acetylated K-to-R filamin A proteins. We will also assess if filamin A is a direct target of Elp3 by performing *in vitro* acetylation assays (Creppe et al., 2009). The remaining candidate proteins identified by MS-based quantitative proteomics will be validated by *in vitro* acetylation assays (Creppe et al., 2009) and the biological significance of their modification will be experimentally addressed *in vivo*. Their expression pattern will be analysed by immunohistochemistry when commercial antibodies are available or by *in situ* hybridization. The candidates showing the most promising expression pattern regarding the regulation of key developmental steps in corticogenesis (proliferation, migration and differentiation), will be analysed in more details. Thus, the endogenous candidate proteins will be silenced using shRNA encoding plasmids and replaced by shRNA refractory mutant protein harbouring key acetylated K-to-R mutations. The resulting phenotype will be analysed

by immunohistochemistry. Our preliminary results obtained with conditional Elp3 knockout embryos revealed a dramatic reduction in the cortex thickness that came with a significant decrease of α tubulin acetylation, as previously reported after RNAi-induced acute depletion of Elp3 in cortical neurons. We are currently analysing all aspect of corticogenesis, including cell proliferation, cell cycle exit, neuronal differentiation and migration in the cortical plate. We are also setting up the iTRAQ methods to analyse the Elp3-dependent acetylome. In addition, we are currently analysing the Elp3-dependent acetylome in N2A cells with SILAC, another MS-based quantitative proteomic technique. By combining both in vitro (SILAC) and in vivo (iTRAQ) approaches, we hope to find higly conserved targets that are acetylated by Elp3 and that may contribute to neurogenesis.

Bibliography

- Axelrod, F.B. (2004). Familial dysautonomia. Muscle Nerve *29*, 352-363.
- Axelrod, F.B., Zupanc, M., Hilz, M.J., and Kramer, E.L. (2000). Ictal SPECT during autonomic crisis in familial dysautonomia. Neurology *55*, 122-125.
- Benes, F.M., and Berretta, S. (2001). GABAergic interneurons: implications for understanding schizophrenia and bipolar disorder. Neuropsychopharmacology *25*, 1-27.
- Besson, A., Gurian-West, M., Schmidt, A., Hall, A., and Roberts, J.M. (2004). p27Kip1 modulates cell migration through the regulation of RhoA activation. Genes Dev *18*, 862-876.
- Chien, W.M., Rabin, S., Macias, E., Miliani de Marval, P.L., Garrison, K., Orthel, J., Rodriguez-Puebla, M., and Fero, M.L. (2006). Genetic mosaics reveal both cell-autonomous and cell-nonautonomous function of murine p27Kip1. Proc Natl Acad Sci U S A *103*, 4122-4127.
- Choudhary, C., Kumar, C., Gnad, F., Nielsen, M.L., Rehman, M., Walther, T.C., Olsen, J.V., and Mann, M. (2009). Lysine acetylation targets protein complexes and co-regulates major cellular functions. Science *325*, 834-840.
- Close, P., Hawkes, N., Cornez, I., Creppe, C., Lambert, C.A., Rogister, B., Siebenlist, U., Merville, M.P., Slaugenhaupt, S.A., Bours, V.*, et al.* (2006). Transcription impairment and cell migration defects in elongator-depleted cells: implication for familial dysautonomia. Mol Cell *22*, 521-531.
- Creppe, C., Malinouskaya, L., Volvert, M.L., Gillard, M., Close, P., Malaise, O., Laguesse, S., Cornez, I., Rahmouni, S., Ormenese, S.*, et al.* (2009). Elongator controls the migration and differentiation of cortical neurons through acetylation of alpha-tubulin. Cell *136*, 551-564.
- Esberg, A., Huang, B., Johansson, M.J., and Bystrom, A.S. (2006). Elevated levels of two tRNA species bypass the requirement for elongator complex in transcription and exocytosis. Mol Cell *24*, 139-148.
- Fero, M.L., Rivkin, M., Tasch, M., Porter, P., Carow, C.E., Firpo, E., Polyak, K., Tsai, L.H., Broudy, V., Perlmutter, R.M.*, et al.* (1996). A syndrome of multiorgan hyperplasia with features of gigantism, tumorigenesis, and female sterility in p27(Kip1)-deficient mice. Cell *85*, 733-744.
- Hawkes, N.A., Otero, G., Winkler, G.S., Marshall, N., Dahmus, M.E., Krappmann, D., Scheidereit, C., Thomas, C.L., Schiavo, G., Erdjument-Bromage, H.*, et al.* (2002). Purification and characterization of the human elongator complex. J Biol Chem *277*, 3047-3052.
- Heng, J.I., Moonen, G., and Nguyen, L. (2007). Neurotransmitters regulate cell migration in the telencephalon. Eur J Neurosci *26*, 537-546.
- Huang, B., Johansson, M.J., and Bystrom, A.S. (2005). An early step in wobble uridine tRNA modification requires the Elongator complex. Rna *11*, 424-436.
- Johansen, L.D., Naumanen, T., Knudsen, A., Westerlund, N., Gromova, I., Junttila, M., Nielsen, C., Bottzauw, T., Tolkovsky, A., Westermarck, J.*, et al.* (2008). IKAP localizes to membrane ruffles with filamin A and regulates actin cytoskeleton organization and cell migration. J Cell Sci *121*, 854-864.
- Kim, J.H., Lane, W.S., and Reinberg, D. (2002). Human Elongator facilitates RNA polymerase II transcription through chromatin. Proc Natl Acad Sci U S A *99*, 1241-1246.
- Kim, S.C., Sprung, R., Chen, Y., Xu, Y., Ball, H., Pei, J., Cheng, T., Kho, Y., Xiao, H., Xiao, L.*, et al.* (2006). Substrate and functional diversity of lysine acetylation revealed by a proteomics survey. Mol Cell *23*, 607-618.
- Kouskouti, A., and Talianidis, I. (2005). Histone modifications defining active genes persist after transcriptional and mitotic inactivation. Embo J *24*, 347-357.
- Kristjuhan, A., and Svejstrup, J.Q. (2004). Evidence for distinct mechanisms facilitating transcript elongation through chromatin in vivo. Embo J *23*, 4243-4252.
- Levitt, P., Eagleson, K.L., and Powell, E.M. (2004). Regulation of neocortical interneuron development and the implications

for neurodevelopmental disorders. Trends Neurosci *27*, 400-406.
– Marin, O., and Rubenstein, J.L. (2003). Cell migration in the forebrain. Annu Rev Neurosci *26*, 441-483.
– Nakayama, K., Ishida, N., Shirane, M., Inomata, A., Inoue, T., Shishido, N., Horii, I., and Loh, D.Y. (1996). Mice lacking p27(Kip1) display increased body size, multiple organ hyperplasia, retinal dysplasia, and pituitary tumors. Cell *85*, 707-720.
– Nguyen, L., Besson, A., Heng, J.I., Schuurmans, C., Teboul, L., Parras, C., Philpott, A., Roberts, J.M., and Guillemot, F. (2006). p27kip1 independently promotes neuronal differentiation and migration in the cerebral cortex. Genes Dev *20*, 1511-1524.
– Pancoast, M., Dobyns, W., and Golden, J.A. (2005). Interneuron deficits in patients with the Miller-Dieker syndrome. Acta Neuropathol (Berl) *109*, 400-404.
– Pearson, J., and Pytel, B.A. (1978). Quantitative studies of sympathetic ganglia and spinal cord intermedio-lateral gray columns in familial dysautonomia. J Neurol Sci *39*, 47-59.
– Pearson, J., Pytel, B.A., Grover-Johnson, N., Axelrod, F., and Dancis, J. (1978). Quantitative studies of dorsal root ganglia and neuropathologic observations on spinal cords in familial dysautonomia. J Neurol Sci *35*, 77-92.
– Petrakis, T.G., Wittschieben, B.O., and Svejstrup, J.Q. (2004). Molecular architecture, structure-function relationship, and importance of the Elp3 subunit for the RNA binding of holo-elongator. J Biol Chem *279*, 32087-32092.
– Rahl, P.B., Chen, C.Z., and Collins, R.N. (2005). Elp1p, the yeast homolog of the FD disease syndrome protein, negatively regulates exocytosis independently of transcriptional elongation. Mol Cell *17*, 841-853.
– Slaugenhaupt, S.A., and Gusella, J.F. (2002). Familial dysautonomia. Curr Opin Genet Dev *12*, 307-311.
– Stenman, J., Toresson, H., and Campbell, K. (2003). Identification of two distinct progenitor populations in the lateral ganglionic eminence: implications for striatal and olfactory bulb neurogenesis. J Neurosci *23*, 167-174.
– Winkler, G.S., Kristjuhan, A., Erdjument-Bromage, H., Tempst, P., and Svejstrup, J.Q. (2002). Elongator is a histone H3 and H4 acetyltransferase important for normal histone acetylation levels in vivo. Proc Natl Acad Sci U S A *99*, 3517-3522.

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2010

– Heng, J.I.-T., Chariot, A., and **Nguyen, L.[CA]:** Molecular layers underlying cytoskeleton remodeling during cortical development. ***Trends Neurosci*** (2010), 33(1): 38-47 (I.F. 2009= 12.794)
– Close P., Creppe, C., Gillard, M., Ladang, A., Chapelle, J.-P., **Nguyen, L.**, and Chariot, A. The emerging role of protein acetylation outside of the nucleus. ***Cell Mol Life Sci*** (2010), 67(8): 1255-64 (I.F. 2009= 6.090)
– Creppe, C., Malinouskaya, L., Volvert, M.-L., Close, P., Laguesse, S., Gillard, M., Chariot, A., and **Nguyen, L.[CA]** Elongator orchestrate la neurogenèse du cortex cérébral ***Médecine sciences*** (2010), 26(2): 135-7
– **Nguyen, L.[CA]**, Humbert, S., Saudou, F[CA]., and Chariot, A.[CA]: Elongator – an emerging role in different neurological diseases. ***Trends Mol Med*** (2010), 16(1): 1-6 (I.F. 2009= 11.049)

2009

– Creppe, C.*, Malinouskaya, L.*, Volvert, M.-L., Gillard, M., Close, P., Malaise, O., Laguesse, S., Cornez, I., Rahmouni, S., Ormenese, S., Belachew, S., Malgrange, B., Chapelle, J.-P., Siebenlist, U., **Moonen, G.**, Chariot, A.[CA,*] and **Nguyen, L.[CA,*]** : Elongator Controls the Migration and Differentiation of Cortical Neurons through Acetylation of Alpha Tubulin. ***Cell*** (2009), 132: 551-564 (I.F. 2008=31.253)
– Borgs, L., Beukelears, P., Vandenbosch, R., **Nguyen, L.**, **Moonen, G.**, Maquet, P., Albrecht, U., Belachew, S., and Malgrange, B[CA].: Period 2 regulates neural/stem progenitor cell proliferation in the adult hippocampus. ***BMC Neurosci*** (2009), 10(1): 30 (I.F. 2008=3.144)
– Vandenbosch, R., Borgs, L., Beukelaers, P., Belachew, S., **Moonen, G.**, **Nguyen, L.**, and Malgrange, B.: Adult Neurogenesis and the diseased brain. ***Curr Med Chem*** (2009), 16(6): 652-66 (I.F. 2008= 4.823)
– Sacheli, R., **Nguyen, L.**, Borgs, L., Vandenbosch, R., Bodson, M., Lefebvre, P.P., and Malgrange, B.: Expression patterns of miR-96, miR-182 and miR-183 in the developing inner ear. ***Gene Expr Patterns*** (2009), 9(5): 364-70 (I.F. 2008=2.112)

Other publications of the laboratory (2008-2010)

2010

- Poirrier, A.-L., Van Den Ackerveken, Kim, T.S., Vandenbosch, R., **Nguyen, L.**, Lefebvre, P.P., and Malgrange, B. [CA] : Ototoxic drugs: Difference in sensitivity between mice and guinea pigs ***Toxicol Lett*** (2010), in press (I.F. 2009= 3.479)
- Godin, J., Colombo, K., Charrin, B., Keryer, G., Volvert, M.-L., Guillemot, F., Bellaiche, Y., Saudou, F., **Nguyen, L.**, and Humbert, S. [CA] : Huntingtin is required for proper Spindle Orientation during mitosis in Drosophila and Mammals. ***Neuron*** (2010), 67: 1-15 (I.F. 2009= 13.260)
- Breuskin, I., Bodson, M., Thelen, N., Borgs, L., **Nguyen, L.**, Stolt, C., Wegner, M., Levebvre**,** P.P., and Malgrange, B. [CA] : Glial but not neuronal development in the cochleo-vestibular ganglion requires Sox10. ***J Neurochem*** (2010), in press (I.F. 2009= 3.999)
- Malgrange, B. [CA], Borgs, L., Grobarczkj, B., Purnelle, A., Ernst, P., **Moonen, G.**, and **Nguyen, L.** [CA]: Using pluripotent stem cells to untangle neurodegenerative disease mechanisms. ***Cell Mol Life Sci*** (2010), in press (I.F. 2009= 6.090)

2009

- De Nijs, L., Léon, C., **Nguyen, L.**, LoTurco, J.J., Delguado-Escueta, A.V., Grisar, T [CA]., and Lakaye, B. : EFHC1, a protein mutated in juvenil myoclonic epilepsy, is a microtubule-associated-protein involved in cell division and early neuronal migration. ***Nat Neurosci*** (2009), 12(10): 1266-74 (I.F. 2008=14.164)
- Breuskin, I., Bodson, M., Thielen, N., Thiry, M., Borgs, L., **Nguyen, L.**, Lefebvre, P.P., and Malgrange, B. [CA] : Sox10 promotes the survival of cochlear progenitors during the establishment of the organ of Corti. ***Dev Biol*** (2009), 335(2):327-39 (I.F. 2008=4.416)

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Characterization of G protein-coupled receptors involved in drug addiction and motor diseases

1. The glucocorticoid-induced receptor (GIR) receptors

Glucocorticoid-induced receptor (GIR or GPR83) is a receptor with predominant expression in brain and thymus. High levels of GIR expression have been described in limbic forebrain and hypothalamic regions of the brain of mouse, rat and human, suggesting a role for GIR in memory, cognition, stress, reward or the control of emotion. We have generated a knock-out model for GIR.

GIR is expressed in cholinergic interneurons of the striatum. In our targeting vector, part of the GIR coding region was replaced by a tau-lacZ fusion gene (placed under control of the natural GIR promoter) and selection cassettes. The tau-lacZ fusion protein was visualised by X-Gal staining of brain slice in heterozygous animals. We observed a strong cellular staining in olfactory bulb, olfactory tubercle, nucleus of the olfactory tract, piriform cortex and scattered cells in dorsal striatum. Few cells were also stained in the thalamus, hypothalamus and CA3 region of hippocampus. In all cases, the neuronal processes were also stained. In the olfactory bulb, the localisation and shape of processes indicated the peri-glomerular identity for the GIR expressing-neurons. In the dorsal striatum, the size of the cell bodies and the arborisation suggested that GIR was expressed in large aspiny cholinergic interneurons. Co-immunostaining revealed 89% co-localization of GIR with choline acetyl-transferase (CHAT), a marker of cholinergic neurons. However cholinergic interneurons are also located in the ventral striatum where very few GIR-positive neurons were found, indicating the existence of a subclass of cholinergic interneurons in the dorsal striatum expressing GIR. Few other GIR-positive neurons did not colocalise with CHAT and had a distinctive elongated cell shape, with a single process.

GIR invalidation decreases motor learning ability. Cholinergic interneurons of the striatum have been shown to be involved in motor learning. We assessed the locomotor ability of GIR knock-out mice in four tests probing striatal and/or cerebellar function. In the rota-rod test, designed to assess motor coordination, knock-out mice displayed a reduced aptitude for coordinated movements and did not manage to learn the test even after 5 days of conditioning. Similarly, knock-out mice were inefficient compared to wild type in the strength-grip test, a test that involves muscular strength and coordination. The runway test and the foot print test are designed to explore cerebellar deficiencies. Wild type and knock-out mice displayed similar abilities in both tests. In addition to motor control, striatum also participates to motivational control and rewarding effects of drug of abuse. Dopaminergic activity in nucleus accumbens is primarily implicated in the rewarding properties of drugs such as cocaine. We assessed the rewarding effects of cocaine in the conditioned place preference test but did not observed differences between wild type and knock-out mice.

GIR invalidation affects the activity of the striatal network. Dopaminergic and cholinergic afferences modulate the GABAergic medium spiny neurons (MSNs). MSNs are commonly divided in two major subsets on the basis of their axonal projections and on the expression of neuropeptides and dopamine receptor subtypes. Striatonigral MSNs co-express D_1 dopaminergic receptors, substance P and dynorphin, while striatopallidal MSNs co-express D_2 dopaminergic receptors, A_{2a} adrenergic receptors and enkephalin. Changes in striatal network activity are typically correlated with the expression level of these neuropeptides. Quantitative in-situ hybridization showed that enkephalin transcripts are slightly decreased while substance P and dynorphin trannscripts were significantly increased in the caudate

putamen of knock-out mice. In situ binding suggested that D1 and D2 receptors were not significantly modified in the striatum or cortex of GIR knock-out mice.

The number and distribution of cholinergic neurons in the striatum were not modified in knockout mice. We monitored the acetylcholine secretion *in vivo* in the dorsal striatum with a microdialysis probe. We observed a decrease in basal acetylcholine secretion in knock-out mice as compared to wild type mice. Scopolamine, an M2 receptor antagonist that suppresses the autocrine negative feedback of acetylcholine, increased acetylcholine secretion in both genotypes, but knock-out mice returned faster to a lower basal level. Induction of early genes such as c-fos or Zif-268 has been proposed to reflect neuronal activity. Zif-268 transcript levels were increased in the caudate putamen of knock-out mice, more particularly in the most superficial parts of the structure, where most GIR-positive cholinergic interneurons are located.

GIR invalidation affects the stress axis. Glucocorticoids and forskolin were initially described to induce GIR expression in a thymoma cell line. In the brain however, GIR expression is decreased by glucocorticoids in hypothalamus, suggesting a possible role in the regulation of stress. Plasma corticosteroid concentrations were significantly lower in knock-out mice in basal conditions, and the difference between genotypes became larger in stressful situations (LPS administration, restraint, open field test).

Motor control in stressful conditions. We monitored the spontaneous locomotor activity in actimetry boxes. On day 1, wild type and knock-out mice displayed similar locomotor activities. After habituation and learning (day 2 and 3), knock-out mice displayed a lower activity for both the horizontal and vertical components. In the open field test, which is more stressful, GIR knock-out mice displayed a significantly higher locomotor activity during the three days of the test and spent more time in the central area. These results suggest an anxiolytic-like phenotype. Mice were tested in behavioral tests probing more specifically anxiety, namely the light and dark box and the elevated plus maze. Although the knockout animals displayed higher locomotor activity in these arenas, the parameters reflecting anxiety were not significantly different between genotypes. We assessed the effect of the D1 agonist SKF38393 and the M2 antagonist scopolamine on the locomotory activity of wild type and knock-out mice in the open-field test. Both drugs resulted in a similar biphasic effect on GIR knockout mice. At low doses, they did not affect wild type mice but decreased the locomotor activity of knockout mice to the same level as wild type animals. At higher doses, they increased in parallel the activity in both genotypes. Finally, we also tested knock-out mice in tests commonly used for probing antidepressant compounds. In the tail suspension test and the forced swimming test, knock-out mice displayed a depressive-like behaviour: they spent significantly more time immobile than wild type mice. In both tests, the injection of fluoxetine, an anti-depressant compound, reduced the time spent immobile for wild type and knock-out mice.

Pharmacological activation of GIR by poly-unsaturated fatty acids and NPY peptides. Peptides of the NPY family have been described to bind and activate GIR expressed in COS-7 cells (NPY(3-36) > PYY (3-36) > NPY > Leu,Pro-NPY), suggesting that GIR is a novel NPY receptor with a Y2-like pharmacology. Independently, several poly-unsaturated fatty acids (PUFA) were reported in a patent to act on GIR, following their isolation from pig brain. Both hypotheses were tested, following the expression of human GIR (hGIR) in CHO-K1 cells also expressing Gα16 and apoaequorin. hGIR-overexpressing cells were specifically activated by docosahexanoic acid (DHA), 9-cis retinoic acid (9-cRA), and arachidonic acid but not NPY or PYY (3-36). A cell line expressing the Y2 receptor was activated by NPY and PYY (3-36) but not by DHA or 9-cRA, while control cell lines expressing other receptor did not respond

to these ligands. We next assessed the effect of combinations of PUFAs and Y2 agonists on hGIR-overexpressing cell. DHA in combination with PYY (3-36) or NPY induced a stronger activation. In the presence of DHA, the EC_{50} for PYY-3-36 decreased to the nanomolar range and the E_{max} was increased.

We also monitored the internalisation of GIR following activation in COS-7 cells transiently expressing hGIR or a fusion of hGIR tagged with GFP at its C-terminus. We observed the GPCR Internalisation was detected either by fluorescent confocal microscopy or FACS. DHA at 10 µM promoted internalisation of hGIR (62% at 45 min), which clustered in a peri-nuclear endosomal compartment. A control receptor similarly tagged with GFP did not internalise in the same conditions. NPY or PYY (3-36) alone did not internalise hGIR but combinations of DHA and PYY (3-36) promoted faster internalisation and at lower concentrations than DHA alone. These results suggest that Y2 agonists can activate hGIR, but only in the presence of DHA.

We tested by in situ binding assay whether GIR could bind NPY peptides in mouse brain. Using ^{125}I-PYY (3-36) as a tracer and Leu,Pro-NPY as a Y1 competitor, we could determine Y2-like binding sites in specific brain regions. We observed a significant reduction of ^{125}I-PYY (3-36) binding sites in the hippocampus and amygdala of GIR knock-out mice as compared to wild type animals. Binding in striatum was very low and we were uable to observe differences in this region. Similarly, no difference was seen in septum, where GIR is not expressed.

We also tested whether part of the physiological effects of PYY could be mediated by GIR. Following i.p. injection, PYY (3-36) decreased locomotory activity of wild type mice, but not of GIR knock-out mice, suggesting that GIR is required for the locomotory effects of PYY(3-36) in vivo. Finally we quantified NPY expression in striatum and hypothalamus of wild type and knock-out mice. We observed a significant increase in NPY expression in the striatum (but not hypothalamus) of knock-out as compared to wild type mice.

Altogether, we have shown that GIR is expressed by cholinergic interneurons of dorsal striatum, and that inactivation of this receptor is associated with cholinergic hypoactivity in the striatum (Laurent et al. In preparation). This leads to an alteration of coordinated movement and increased locomotory activity in stressful conditions. We also showed that GIR is activated in a cooperative manner by NPY peptides and polyunsaturated fatty acids, and that the receptor is involved in some of the activities of NPY peptides in vivo (Laurent et al. submitted).

2. The GPR3 receptor in emotional-like responses

GPR3 is an orphan G-protein-coupled receptor (GPCR) which, upon transfection in various mammalian cell lines, causes strong constitutive activation of adenylyl cyclase, in the absence of any added agonist. In mouse oocytes, GPR3 contributes to maintenance of cAMP concentrations at a level required to ensure meiotic arrest in prophase I until the LH surge. Whether cAMP accumulation is the result of a true constitutive activity of the receptor or the consequence of the chronic stimulation by a ubiquitous unknown ligand, is still debated. Sphingosine 1-phoshate was proposed as an agonist of the rat GPR3 homologue but this has not been confirmed yet. GPR3 transcripts are also widely expressed in the mouse brain, in areas related to different physiological functions. More specifically, GPR3 receptor is expressed in the main brain structures involved in stress-related behaviors such as habenula but also hippocampus, amygdala, limbic system and cortex. Interestingly, the highest levels of expression were

found in the habenula. The habenular complex is an important relay station between the limbic forebrain and the midbrain. It has been clearly shown to participate in the regulation of ascending monoamine and acetylcholine transmission towards hippocampus and frontal cortex. Serotonin and noradrenaline systems are known to play a role in the regulation of several central activities including mood and anxiety. Dysregulation of these systems appears to have a role in the pathophysiology of depression and anxiety disorders. Given the ability of GPR3 to activate the cAMP regulatory cascade in a tonic way in areas involved in stress-related behaviors, we hypothesized that GPR3 could play a role in the control of the corresponding behavioral responses.

We investigated therefore the consequences of genetic deletion of GPR3 in several behavioral paradigms and on neurotransmission. Compared to wild-type, hippocampal neurons from Gpr3$^{-/-}$ mice displayed lower basal intracellular cAMP levels, consistent with the strong constitutive activity of GPR3 in transiently transfected cells. Gpr3$^{-/-}$ mice developed and behaved normally with neither major changes in locomotion under basal conditions nor impairment in motor coordination. No deficits in avoidance learning were evidenced in Gpr3$^{-/-}$ mice, which exhibited similar performance than wild-type mice in the active avoidance paradigm. This suggests that the lack of GPR3 does not affect the learning of fear which primarily develops through a conditioned process. However, Gpr3$^{-/-}$ mice exhibited a higher level of anxiety-related responses after exposure to unfamiliar stressful environment in the open-field and the elevated plusmaze paradigms, including a behavioral inhibition observed as a reduced activity in the open-field test. The anxiety-like phenotype observed in the elevated plus-maze was sensitive to the effect of benzodiazepines since the administration of diazepam reversed the anxiogenic response. In addition, an increase in the number of attacks and a decrease in the latency period for the first attack were observed in the resident-intruder test, revealing a higher level of aggressiveness in mutant mice. Thus, the behavioral phenotype argues for a possible link between emotional reactivity and aggressive behavior in mutant mice. It also suggests that Gpr3$^{-/-}$ mice could be more susceptible to develop a ''depression-related'' behavior when exposed to a stressful situation from which they cannot escape. Indeed Gpr3$^{-/-}$ mice exhibited a behavioral despair as evidenced by an increased duration of immobility in the tail suspension and the forced swim tests, which are widely used to assess the efficacy of antidepressant drugs and genetic manipulation relevant to depression. We did not notice any change in serum corticosterone levels in Gpr3$^{-/-}$ mice under basal conditions or in response to the stress induced by the tail suspension test. Therefore, the mechanism underlying the behavioral characteristics of Gpr3$^{-/-}$ mice does not seem to be related to an alteration of the HPA axis activity.

Monoaminergic neurotransmission is thought to modulate mood states and the stress response. Important alterations of the brain monoaminergic systems have been involved in mood disorders, and animal studies proposed an inverse relationship between the activity of the brain 5-HT system and aggressive behavior. Therefore, we analyzed the tissue levels of 5-HT, norepinephrine (NE) and dopamine (DA) in several brain structures of Gpr3$^{-/-}$ mice. We found that Gpr3$^{-/-}$ mice exhibit abnormally low levels of monoamines in various brain areas under basal conditions. In particular, the dramatic decrease in 5-HT content observed in hippocampus, hypothalamus and frontal cortex could well account for the behavioral despair and aggressiveness displayed by the Gpr3$^{-/-}$ mice and indicate a role for GPR3 in modulating the serotonergic system. The observation of a significant decrease in NE in cortex and hypothalamus could also account for the behavioral phenotype of knockout mice. Because the metabolites of 5-HT and DA were also decreased in Gpr3$^{-/-}$ mice, it seems likely that the primary target of the regulation by GPR3 is the synthesis or reuptake of these neurotransmitters. Conversely, in case of NE, the primary metabolite NM was found to be increased indicating that its metabolism is affected by Gpr3 deletion.

The expression of GPR3 showed the highest level in the habenula. The habenular complex is an evolutionarily conserved diencephalic structure linking the forebrain with midbrain and hindbrain structures. It has been shown to participate in the regulation of monoamine transmission. Through a habenulo-raphe pathway it modulates serotonergic activity in many structures including the hippocampus and influences the noradrenergic activity through a connexion with the locus coeruleus. Our results suggest that such a control is modulated at least in part through GPR3.

Interestingly, mice deficient in phosphodiesterase-4D (PDE-4D) and animals treated with the PDE-4 inhibitor rolipram display an attenuated despair behavior when exposed to the same behavioral models used in the present study. Since GPR3 and PDE-4D are both highly expressed in medial habenula and have antagonistic action on the intracellular cAMP level, these data strengthen the notion that basal levels of cAMP in habenular neurons are an important parameter for the emotional-like behaviors under the control of the limbic system.

This study demonstrates therefore that GPR3 plays an important role in modulating several responses in animal models consistently employed to evaluate emotional disorders including anxiety, depression-like disorders, and aggressiveness, probably by tuning the monoaminergic neurotransmission in various brain regions. In consequence, GPR3 dysfunction could be involved in the etiology of disorders associated with emotional disturbances, thereby representing a novel actor of the cAMP-dependent signaling pathway linked to behavioral responses (Valverde et al. 2009).

3. In vivo function of the adenosine A_{2A} receptor

The A_{2A} adenosine receptor is involved in the regulation of addiction induced by different drugs of abuse. The specific role of A_{2A} receptors in the behavioural and neurochemical responses to morphine associated with its motivational properties were tested. The acute administration of morphine induced a similar enhancement of locomotor activity and antinociceptive responses in both A_{2A} knockout and control mice. However, the rewarding effects induced by morphine were completely blocked in A_{2A} KO mice. Also, naloxone did not induce place aversion in animals lacking A_{2A}. The results demonstrate that the rewarding and aversive effects associated with morphine abstinence were abolished in A_{2A} KO mice, supporting a differential role of the A_{2A} adenosine receptor in the somatic and motivational effects of morphine addiction. This provides evidence for the role of A_{2A} receptors as general modulators of the motivational properties of drugs of abuse (Castañé et al. 2008).

We have shown previously that mice lacking the adenosine A_{2A} receptor generated on a CD1 background self-administer more ethanol and exhibit hyposensitivity to acute ethanol. We have now shown that these A_{2A} KO mice display a reduced ethanol-induced conditioned place preference (CPP) and an increased sensitivity to the anxiolytic and locomotor stimulant effects of ethanol, but they did not show alteration in ethanol-induced conditioned taste aversion and locomotor sensitization. However, in A_{2A} KO mice on a C57BL/6J background, no difference was observed in terms of ethanol consumption and preference, ethanol-induced CPP and locomotor-stimulant effects. Nevertheless the A_{2A} agonist CGS 21680 reduced ethanol consumption and preference in C57BL/6J mice. Despite genetic background differences, the results show that inactivation of the A2a receptor leads to high ethanol consumption, increased sensitivity to the locomotor-stimulant/anxiolytic effects of ethanol and a decrease in ethanol-induced CPP (Houchi et al. 2008).

Adenosine has been proposed as an endogenous anticonvulsant and long-term caffeine intake has been reported to decrease the susceptibility to convulsants in mice. We investigated the occurrence of seizures following long-term oral administration of caffeine in adenosine A_{2A} receptor knockout and control mice. Clonic seizures induced by acute pentylenetetrazol (PTZ) were significantly attenuated in KO mice and also reduced by a 14-day caffeine treatment in WT mice. We showed also a protecting effect of a 21-day caffeine treatment in WT mice against kindled seizures induced by PTZ in an increasing dose schedule. The protective effects against PTZ-induced seizures occurring when the adenosine A_{2A} receptor is absent or chronically blocked by caffeine is likely due to a decreased neuronal excitability in these conditions (El Yacoubi et al. 2008).

We also investigated the consequences of deleting the adenosine A_{2A} receptor in different experimental models of epilepsy. A_{2A}R KO mice were not protected against seizures originating from brainstem structures, namely electroshock-induced seizures. The intensities of seizures induced by pentylenetetrazol or pilocarpine, as well as the percentages of convulsing mice, were significantly reduced in A_{2A} receptor knockout (A_{2A}R KO) animals. A_{2A}R KO mice exhibited reduced pentylenetetrazol-induced kindled seizures, demonstrating an important role of the A_{2A} receptor in the acquisition of kindling. These data suggest that adenosine stimulating A_{2A} receptors modulates excitatory neurotransmission and exacerbates limbic seizures. It is therefore suggested that adenosine A_{2A} receptor antagonists might offer protection from some epileptic syndromes (El Yacoubi et al. 2009).

Peripheral nerve injury produces a persistent neuropathic pain state characterized by spontaneous pain, allodynia and hyperalgesia. The possible involvement of adenosine receptors in the development of neuropathic pain and the expression of microglia and astrocytes in the spinal cord after sciatic nerve injury was evaluated. Partial ligation of the sciatic nerve was performed in A_{2A} knockout mice and wild-type littermates. The development of mechanical and thermal allodynia, as well as thermal hyperalgesia was evaluated by using the von Frey filament model, the cold-plate test and the plantar test, respectively. In wild-type animals, sciatic nerve injury led to a neuropathic pain syndrome that was revealed in these three nociceptive behavioural tests. However, a significant decrease of the mechanical allodynia and a suppression of thermal hyperalgesia and allodynia were observed in A_{2A}R deficient mice. Taken together, these results demonstrate the involvement of A_{2A}Rs in the control of neuropathic pain (Bura et al. 2008).

Mice lacking the adenosine A_{2A} receptor are less sensitive to nociceptive stimuli, and A_{2A} receptor antagonists have antinociceptive effects. We have previously shown a marked reduction in the behavioural responses to formalin injection in A_{2A} receptor knockout mice. This may be due to the presence of pronociceptive A_{2A} receptors on sensory nerves, and if so spinal cords from A_{2A} receptor knockout mice may have altered neurochemical responses to a nociceptive stimulus. We tested this hypothesis by studying two parameters known to change with spinal cord activity, NMDA glutamate receptor binding and [^{14}C]-2-deoxyglucose uptake, following intraplantar formalin injection in wild-type and A_{2A} receptor knockout mice. In naïve untreated A_{2A} knockout mice [^{14}C]-2-deoxyglucose uptake in all regions of the spinal cord was significantly lower compared to the wild-type, similar to the reduced NMDA receptor binding that we have previously observed. Following formalin treatment, there was an decrease in [^{3}H]-MK801 binding to NMDA receptors and an increase in [^{14}C]-2-deoxyglucose uptake in the spinal cords of wild-type mice, and these changes were significantly reduced in the A_{2A} knockout mice. In addition to altered behavioural responses, there are therefore corresponding reductions in spinal cord neurochemical changes induced by formalin in mice lacking adenosine A_{2A} receptors. These observations support the hypothesis that activation of A_{2A} receptors enhances nociceptive input into the spinal cord and suggests a possible role for A_{2A} antagonists as analgesics (Hussey et al. 2010).

Adenosine A_{2A}, cannabinoid CB_1 and metabotropic glutamate 5 (mGlu5) receptors are all highly expressed in the striatum. We investigated whether, and by which mechanisms, these receptors interact in the regulation of striatal synaptic transmission. By extracellular field potentials recordings in corticostriatal slices, we demonstrated that the ability of the selective CB_1 agonist WIN55,212-2 to depress synaptic transmission was prevented by the pharmacological blockade or the genetic inactivation of A_{2A}Rs. Such a permissive effect of A_{2A}Rs towards CB1Rs does not seem to occur pre-synaptically as the ability of WIN55,212-2 to increase the R2/R1 ratio under a protocol of paired-pulse stimulation was not modified by ZM241385. Furthermore, the effects of WIN55,212-2 were reduced in slices from mice lacking post-synaptic striatal A_{2A}Rs. The selective mGlu5R agonist CHPG potentiated the synaptic effects of WIN55,212-2, and such a potentiation was abolished by A_{2A}R blockade. Unlike the synaptic effects, the ability of WIN55,212-2 to prevent NMDA-induced toxicity was not influenced by ZM241385. These results show that the state of activation of A_{2A}Rs regulates the synaptic effects of CB1Rs and that A_{2A}Rs may control CB_1 effects also indirectly, namely through mGlu5Rs (Tebano et al. 2009).

Dopamine D_2 and adenosine A_{2A} receptors are highly enriched in striatal neurons and exhibit strong interactions. By performing perforated-patch-clamp recordings on brain slices, it was shown that membrane potential transitions and firing patterns in striatal neurons are tightly controlled by D_2 and A_{2A} receptors through specific protein-protein interactions including A2A-D2 receptors heteromerization (Azdad et al. 2009).

Huntington's disease (HD) is a progressive neurodegenerative genetic disorder that leads to motor, cognitive, and psychiatric disturbances. The primary neuropathological hallmark is atrophy of the striatum. HD preferentially affects efferent striato-pallidal neurons that express enkephalin as well as dopamine D2 and A_{2A} adenosine receptors (A_{2A}Rs). Expression and function of A_{2A}Rs are altered in HD but, despite being an important modulator of the striato-pallidal function, the subsequent pathophysiological consequence of such changes remains unclear. Whether blockade of A_{2A}Rs is of therapeutic interest in HD remains ill-defined. We aimed therefore to determine the pathophysiological consequences of genetic deletion of A_{2A}Rs in HD by crossing A_{2A}R knockout mice with the N171-82Q HD transgenic model. Our data demonstrate that knockout of A_{2A}Rs moderately but significantly worsens motor performances and survival of N171-82Q mice and leads to a decrease in striatal enkephalin expression. These results support that early and chronic blockade of A_{2A}Rs might not be beneficial in HD (Mievis et al. 2011).

Adenosine triphosphate has previously been shown to induce semi-mature human monocyte-derived dendritic cells (DC) through the $P2Y_{11}$ receptor. We showed that in mice, ATP and adenosine inhibited the production of IL-12p70 by bone marrow-derived DC (BMDC). In the absence of $P2Y_{11}$ receptor in mouse, the effects of adenine nucleotides on mouse DCs are mediated by their degradation product, adenosine, acting on the A_{2B} receptor (Ben Addi et al. 2008).

4. In vivo function of the CB_1 cannabinoid receptor

The endocannabinoid system is involved in the addictive processes induced by different drugs of abuse. We have tested the role of the CB_1 receptor in the pharmacological effects of 3,4-methylenedioxymethamphetamine (MDMA), a popular recreational drug. Acute MDMA administration increased locomotor activity, body temperature, and anxiogenic-like responses in wild-type mice, but these responses were lower or abolished in CB_1 knockout animals. MDMA produced similar conditioned place preference and increased dopamine extracellular levels in the nucleus accumbens

in both genotypes. However, CB_1 knockout mice failed to self-administer MDMA at any of the doses used. These results indicate that CB_1 receptors play an important role in the acute prototypical effects of MDMA and are essential in the acquisition of an operant behavior to self-administer this drug (Touriño et al. 2008).

Experimental evidence indicates that endogenous cannabinoid mechanisms play important roles in nociceptive information processing in various areas of the nervous system including the spinal cord. Although it is well documented that the CB_1 receptor is strongly expressed in the superficial spinal dorsal horn, its cellular distribution is poorly defined, hampering the interpretation of the effect of cannabinoids on pain processing spinal neural circuits. We investigated therefore the cellular distribution of CB_1 in laminae I and II of the rodent spinal dorsal horn by immunocytochemistry. Axonal varicosities revealed a strong immunoreactivity for CB_1, but no CB_1 expression was observed on dendrites and perikarya of neurons. Investigating the co-localization of CB_1 with markers of peptidergic and non-peptidergic primary afferents, and axon terminals of putative glutamatergic and GABAergic spinal neurons, we found that nearly half of the peptidergic (immunoreactive for calcitonin gene-related peptide) and more than 20% of the non-peptidergic (binding isolectin B4) nociceptive primary afferents, more than one-third and approximately 20% of the axon terminals of putative glutamatergic (immunoreactive for vesicular glutamate transporter 2) and GABAergic (immunoreactive for glutamic acid decarboxylase; GAD65 and/or GAD67) spinal interneurons, respectively, were positively stained for CB_1. In addition to axon terminals, almost half of the astrocytic (immunoreactive for glial fibrillary acidic protein) and nearly 80% of microglial (immunoreactive for CD11b) profiles were also immunolabeled for CB_1. These findings suggest that the activity-dependent release of endogenous cannabinoids activates a complex signaling mechanism in pain processing spinal neural circuits into which both neurons and glial cells may contribute (Hegyi et al. 2009).

Serotonergic and endocannabinoid systems are important substrates for the control of emotional behaviour and growing evidence show an involvement in the pathophysiology of mood disorders. We showed that the absence of the activity of the CB_1 cannabinoid receptor impaired serotonergic negative feedback in mice. Thus, in vivo microdialysis experiments revealed increased basal 5-HT extracellular levels and attenuated fluoxetine-induced increase of 5-HT extracellular levels in the prefrontal cortex of CB_1 knockout compared with wild-type mice. These observations could be related to the significant reduction in the 5-HT transporter binding site density detected in frontal cortex and hippocampus of CB_1 knockout mice. The lack of CB1 receptor also altered some 5-HT receptors related to the 5-HT feedback. Extracellular recordings in the dorsal raphe nucleus (DRN) revealed that the genetic and pharmacological blockade of CB_1 receptor induced a $5\text{-}HT_{1A}$ autoreceptor functional desensitization. In situ hybridization studies showed a reduction in the expression of the $5\text{-}HT_{2C}$ receptor within several brain areas related to the control of the emotional responses, such as the DRN, the nucleus accumbens and the paraventricular nucleus of the hypothalamus, whereas an over-expression was observed in the CA3 area of the ventral hippocampus. These results reveal that the lack of CB_1 receptor induces a facilitation of the activity of serotonergic neurons in the DRN by altering different components of the 5-HT feedback as well as an increase in 5-HT extracellular levels in the prefrontal cortex in mice (Aso et al. 2009).

We have investigated further the involvement of the CB_1 receptor in the responses to stress. Stress is known to cause damage and atrophy of neurons in the hippocampus by deregulating the expression of neurotrophic factors that promote neuronal plasticity. The endocannabinoid system is involved in neuroprotection at both cellular and emotional levels. We showed that CB1 knockout mice exhibit an

increased response to stress, including increased despair behavior and corticosterone levels in the tail suspension test, and decreased brain derived neurotrophic factor (BDNF) levels in the hippocampus. Local administration of BDNF in the hippocampus reversed the increased despair behavior of CB_1 knockout mice, confirming the role played by BDNF in the emotional impairment of these mice. No differences were found in the levels of other neurotrophic factors, NGF and NT-3, or the activity of the BDNF receptor and transcription factor CREB. These results suggest that the lack of CB_1 receptor results in an enhanced response to stress and deficiency in neuronal plasticity by decreasing BDNF levels in the hippocampus, leading to impairment in the responses to emotional disturbances (Aso et al. 2008).

The CB_1 cannabinoid receptor has also been implicated in the control of fear and anxiety. We investigated the effects of genetic and pharmacological blockade of the CB_1 cannabinoid receptor on the behaviour of CD1 mice using three different ethological models of fear and anxiety (elevated T-maze and plus-maze and open field test of emotionality). We also measured tissue levels of noradrenalin (NA), dopamine (DA), serotonin (5-HT) and their metabolites in several forebrain regions, to examine the relationship between CB_1 receptor manipulation and monoaminergic neurotransmission. The CB_1 receptor antagonist SR141617A (rimonabant) modulated anxiety in a dose-dependent manner. At a dose of 3 mg/kg i.p., the compound consistently increased anxiety parameters in the three different anxiety tests applied, while a lower dosage of 1 mg/kg had no such effect. The neurochemical evaluation of the mice administered 3mg/kg SR141617A revealed increases in the concentrations of DOPAC and 5-HIAA in the dorsal striatum, elevated DA levels in the hippocampus and reduced dopamine turnover in the septum. Furthermore, these animals had a higher HVA/DA turnover in the frontal cortex. CB_1 receptor knockout mice as well as mice treated with the selective CB_1 receptor antagonist AM251 did not display any significant alterations in anxiety-related behaviour as measured with the elevated plus-maze and open field test of emotionality, respectively. Our findings support the general idea of a SR141617A-sensitive receptive site that is different from the 'classical' CB_1 receptor and that has a pivotal role in the regulation of different psychological functions. Under physiological conditions this receptive site seems to be involved in the control of anxiolysis (Thiemann et al. 2009).

The role of the CB_1 receptor in serotonin release in the hippocampus was investigated. Mouse hippocampal slices were preincubated with [^{3}H]serotonin and superfused with medium containing a serotonin reuptake inhibitor (citalopram hydrobromide). The cannabinoid receptor agonist WIN55,212-2 did not affect the resting [^{3}H]5-HT release, but decreased the evoked [^{3}H]5-HT efflux in wild-type mice. This effect was abolished by the selective CB_1 antagonists SR141716 and AM251. The inhibitory effect of WIN55,212-2 was also completely absent in hippocampal slices derived from CB_1 knockout mice. Selective degeneration of fine serotonergic axons by the neurotoxin parachloramphetamine reduced the uptake and evoked release of [^{3}H]5-HT, and eliminated the effect of WIN55,212-2. These data suggest that a subpopulation of non-synaptic serotonergic afferents express CB_1 receptors and activation of these CB_1 receptors leads to a decrease in 5-HT release (Balázsa et al. 2008).

The effect of WIN 55,212-2 were also investigated on excitatory postsynaptic currents (EPSCs) evoked by stimulation of Schaffer collaterals in CA1 pyramidal cells. WIN 55,212-2 reduced the amplitude of EPSCs in a dose-dependent manner. In rats and mice, this cannabinoid ligand inhibited excitatory synapses in two steps at the nM and muM concentrations. In CB_1 knockout animals, of under treatment with the CB_1 antagonist AM251, WIN 55,212-2 could still reduce the amplitude of EPSCs at μM but not nM concentrations. The inactive enantiomer, WIN 55,212-3, mimicked the effect of WIN 55,212-2 applied in high doses. The CB_1-independent effect of WIN 55,212-2 at glutamatergic synapses was

abolished by the omega-conotoxin GVIA, but not with the omega-agatoxin IVA. These data suggest that, in the hippocampus, WIN 55,212-2 reduces glutamate release from Schaffer collaterals solely via CB_1 receptors in the nM concentration range, whereas in μM concentrations, WIN 55,212-2 suppresses excitatory transmission by an additional mechanism independent of CB_1, the blockade of N-type voltage-gated Ca^{2+} channels (Németh et al. 2008).

Regulation of Ca^{2+} homeostasis plays a critical role in oligodendrocyte function and survival. Cannabinoid CB_1 and CB_2 receptors have been shown to regulate Ca^{2+} levels and/or K^+ currents in a variety of cell types. We investigated the effect of cannabinoid compounds on the Ca2+ influx elicited in cultured oligodendrocytes by transient membrane depolarization with an elevated extracellular K^+ concentration (50 mM). The CB_1 receptor agonist arachidonoyl-chloro-ethanolamide (ACEA) elicited a concentration-dependent inhibition of depolarization-evoked Ca^{2+} transients in oligodendroglial somata. This activity was mimicked by the CB1/CB2 agonist CP55,940, as well as by the endocannabinoids N-arachidonoyl-ethanolamine (anandamide, AEA) and 2-arachidonoylglycerol (2-AG), whereas the CB2 receptor selective agonist JWH133 was ineffective. The CB_1 receptor antagonist AM251 also reduced the Ca^{2+} response evoked by high extracellular K^+ and did not prevent the inhibition elicited by ACEA. The ability of ACEA and AEA to reduce depolarization-evoked Ca2+ transients was significantly reduced in oligodendrocytes from CB1 receptor knockout mice. Bath application of the inwardly rectifying K^+ channels (Kir channels) blockers $BaCl_2$ and $CsCl_2$ reduced the size of voltage-induced Ca^{2+} influx and partially prevented the inhibitory effect of ACEA. These results indicate that cannabinoids inhibit depolarization-evoked Ca^{2+} transients in oligodendrocytes via CB_1 receptor-independent and -dependent mechanisms that involve the activation of PTX-sensitive $G_{i/o}$ proteins and the blockade of Kir channels (Mato et al. 2009).

We showed that cannabinoid receptor agonists can be immunosuppressive and neuroprotective in models of multiple sclerosis. Immunosuppression was associated with a reduction of myelin-specific T cell responses, central nervous system infiltration and clinical signs. These effects were shown to be largely CB_1-dependent and occurred at doses inducing significant cannabimimetic effects. Lower, non-immunosuppressive doses of cannabinoids slowed the accumulation of nerve loss and disability, but failed to inhibit relapses. These results further highlights the neuroprotective potential of cannabinoids to slow the progression of multiple sclerosis (Croxford et al. 2008).

Activation of the cannabinoid 2 receptor (CB_2) reduces ischemic injury in several organs. However, the mechanisms underlying this protective action are unclear. In a mouse model of ischemic stroke, we showed that the CB_2 agonist JWH-133 decreases the infarct size measured 3 d after onset of ischemia. The neuroprotective effect of JWH-133 was lost in CB_2-deficient mice, confirming the specificity of JWH-133. Analysis of bone marrow chimeric mice revealed that bone marrow-derived cells mediate the CB_2 effect on ischemic brain injury. CB_2 activation reduced the number of neutrophils in the ischemic brain as shown by FACS analysis and by measuring the levels of the neutrophil marker enzyme myeloperoxidase. Indeed, we found in vitro that CB_2 activation inhibits adherence of neutrophils to brain endothelial cells. JWH-133 also interfered with the migration of neutrophils induced by the endogenous chemokine CXCL2 through activation of the MAP kinase p38. This effect on neutrophils is likely responsible for the neuroprotection mediated by JWH-133 because JWH-133 was no longer protective when neutrophils were depleted. In conclusion, our data demonstrate that by activating p38 in neutrophils, CB_2 agonists inhibit neutrophil recruitment to the brain and protect against ischemic brain injury (Murikitani et al. 2010).

5. Characterization of new neuropeptide receptors

Many orphan receptors for which the ligands and function are still unknown are encoded by mammalian genomes. We focus on the characterization of a subset of these receptors expressed in specific brain regions, through the identification of their ligand and the delineation of their function. These include presently over a dozen receptors belonging to various structural families.

We have established cell lines coexpressing $G_{\alpha 16}$, apoaequorin and genes encoding the selected orphan receptors. To be able to monitor the expression at the cell surface of recombinant cells by FACS analysis, we have also fused some of the receptors at their N-terminus to an epitope tag. Finally, $G_{\alpha 16}$ is known to couple most receptors to the phospholipase C-calcium release pathway, but is not the optimal coupling protein for all receptors. The use of a chimeric G protein, made of the $G_{\alpha q}$ backbone, with the last 5 aminoacids originating from the $G_{\alpha i}$ sequence, can be a better partner to couple to calcium mobilization some receptors naturally linked to the G_i pathway. Cell lines coexpressing apoaequorin, the $G_{\alpha qi5}$ chimera, and the receptors of interest were also established.

Finally, in order to have screening assays which do not depend on G protein signalling, we have implemented the TANGO (Invitrogen) and PathHunter (DiscoverX) assays. Both systems are based on the recruitment of beta arrestin. In the TANGO assay, the TEV protease hooked to β-arrestin cleaves a target site engineered in the C-terminus of the receptor, releasing a Gal4-VP16 transcription factor, which activates transcription of a beta lactamase reporter gene. In the PathHunter system, β-arrestin recruitment promotes the complementation of the two β-gal fragments, resulting in the formation of a functional enzyme. These systems have the theoretical advantage of being insensitive to the activation of endogenous receptors of the cell lines.

The cell lines expressing orphan receptors are used for the screening of biological activities in a library of fractions prepared from natural sources. Biological extracts are indeed expected to contain the natural ligands of orphan receptors, more particularly the naturally processed forms of peptides and proteins, containing necessary tertiary structures and post-translational modifications. Extracts, fractionated through a first step of HPLC, were made mainly from pig, rat and mouse tissues. Potential biological activities are purified using various multidimensional chromatographic procedures (reverse phase HPLC, ion exchange, heparin affinity columns) in order to obtain a purity level suitable for mass spectrometry and peptide sequencing (MALDI Q-TOF fragmentation analysis) and identify the primary structure of the peptide or protein.

Starting from a rat colon extract, a first step of fractionation on a strong anion exchange column in Tris/HCl buffer, using a NaCl concentration gradient, resulted in a potential activity peak for one of the orphan receptors tested. The positive fractions were pooled and used to perform a second dimension on a polycationic exchange column in ammonium acetate buffer. In this second step, we confirmed the presence of a specific activity for the receptor eluted between 150 and 230 mM NaCl. The bioactivity has been further purified through several HPLC steps, and a potential candidate has been identified by mass spectrometry. Overexpression in CHO or HEK cell lines of the candidate ligand led to the production of an inactive polypeptide towards the orphan receptor. Size-exclusion chromatographic experiments suggested that the ligand might be bioactive as an oligomer. Moreover, immunodetection experiments on Western blots indicated that the active ligand could present a specific pattern of glycosylation and/or proteolytic cleavage. We are now analysing the potential proteolytic cleavage of the ligand in the presence of pure proteases and colon extracts. We are also optimizing a deglycosylation procedure

for mass spectrometry analysis and bioactivity measurement without any gel electrophoresis step to investigate the requirement of glycosylation for bioactivity. A potential bioactivity has been observed for another orphan receptor, but this will require confirmation in subsequent fractionation steps.

A new knockout model for a brain receptor was obtained. Cohorts of mice were tested in be havioural settings. We have shown a mild motor impairment induced by the inactivation of the receptor gene. These modifications are relatively weak but constitute a set of characteristics of Parkinson disease and psychoaffective disorders.

6. Orphan receptors in other systems

The orphan Leucine-rich repeat G protein-coupled receptor 5 (LGR5/GPR49), a target of Wnt signaling, is a marker of adult intestinal stem cells (SC). However, neither its function in the adults, nor during development of the intestine have been addressed yet. We have investigated the role of LGR5 during ileal development by using LGR5 null/LacZ-NeoR knock-in mice. X-gal staining experiments showed that, after villus morphogenesis, Lgr5 expression becomes restricted to dividing cells clustered in the intervillus region and is more pronounced in the distal small intestine. At day E18.5, LGR5 deficiency leads to premature Paneth cell differentiation in the small intestine without detectable effects on differentiation of other cell lineages, nor on epithelial cell proliferation or migration. Quantitative RT-PCR experiments showed that expression from the LGR5 promoter was upregulated in LGR5-null mice, pointing to the existence of an autoregulatory negative feedback loop in intact animals. This deregulation was associated with overexpression of Wnt target genes in the intervillus epithelium. Transcriptional profiling of mutant mice ileums revealed that LGR5 function is associated with expression of SC and SC niche markers. Together, our data identify LGR5 as a negative regulator of the Wnt pathway in the developing intestine (Garcia et al. 2009).

We have previously shown that Lgr4 knock-out (LGR4KO) male mice are infertile due to a developmental defect of the reproductive tract. Spermatozoa do not reach the epididymis and accumulate at the rete testis and efferent ducts. We have proposed that in LGR4KO, efferent duct might fail to connect resulting in blind-ended tubes that preclude the normal transit of sperm cells. To explore this possibility, we reconstructed the three-dimensional (3D) structure of the organ from serial microphotographs. The resulting model allowed to individualize and follow each efferent duct from the testis up to the epididymis, and to display the spatial distribution of their content. The transit of spermatozoa is indeed blocked in LGR4KO mice but, contrary to the expectation, the ducts connect normally to each other, forming a single tube that flows into the epididymis, as in the wild-type animals. In the KO however, transit of the sperm is abruptly blocked at the same level syncytial-like aggregates appear in the luminal space. The model also allowed calculating, for the first time, morphometric parameters of the mouse efferent duct, such as total volume, surface, radius, and length. These data unambiguously showed that efferent ducts in the mutant mouse are dramatically shortened and less convoluted than in the wild-type animal, providing an explanation to the phenotype observed in LGR4KO. Combined with in situ immunodetection or RNA in situ hybridization, 3D reconstruction of serial histological sections will provide an efficient mean to study expression profiles in organs which do not lend themselves to whole-mount studies (Lambot et al. 2009).

7. Search for partners of olfactory receptors

The structure of olfactory receptors has been known for 20 years, but their functional expression is still a major problem, as mammalian ORs are poorly targeted to the cell surface in heterologous systems. Due to these limitations, only a few mammalian ORs have been characterized functionally to date. One of the most significant improvements reported over the last few years is the demonstration that the transmembrane proteins RTP1, RTP2 (both expressed specifically in olfactory neurons) and REEP1 contribute to the translocation of ORs to the plasma membrane, and promote their functional expression in mammalian cell lines, allowing the design of more reliable functional assays. It is however our hypothesis that additional membrane or soluble proteins are required for the reconstitution of an efficient signalling complex in the knobs of olfactory neuron cilia, complexes that may resemble those found at neuronal synapses, in which receptors, transduction proteins and channels are organized by a number of chaperones and scaffolding proteins. We have started to explore further this hypothesis by using a proteomic approach. We have established a procedure for the homogenization of olfactory mucosa from mouse and the preparation of a fraction enriched in ciliary knobs. This enrichment has been demonstrated by the detection of adenylate cyclase III, Gαolf and CNGA2 proteins, which are part of the signalling machinery proximal to the receptor. In order to purify further the protein complexes containing the receptors, it is necessary to express a specific tagged olfactory receptor in all olfactory neurons. To this end, we have developed a transgenic mice that place the expression of a receptor labelled with a F5-tag under the control of tetracyclin responsive promoter, and bred these mice with a strain expressing two other transgenes (OMP-TTA and Gγ8-TTA), allowing the permanent expression of a given receptor in all olfactory neurons. Such expression is not achieved adequately using less sophisticated approaches, as a result of a complex control of receptor expression in olfactory neurons, involving apparently the coding sequence of the receptors themselves. We first verified the expression of the transgenes using immunofluorescence and, as expected, we observed that, in mice expressing the three transgenes, all olfactory neurons are positive for EGFP as well as for the epitope-tagged olfactory receptor. The comparison with the labelling obtained for adenylate cyclase type III (ACIII) also indicated that the receptor is mainly present in the cilia of olfactory neurons. It is well established that each olfactory neuron only expresses a single type of OR and that all olfactory neurons expressing the same OR project their axons to the same glomerulus in the olfactory bulb. We thus wanted to verify if this was also true in our transgenic mice. To tackle this question, we used RT-PCR to evaluate expression of endogenous OR in olfactory neurons and immunofluorescence to visualize the axon projections in the olfactory bulb. We found that in mice expressing the eugenol receptor in all olfactory neurons, the level of transcripts encoding endogenous ORs was dramatically reduced as compared to wild-type mice, and in most cases we were unable to detect any mRNA coding for endogenous receptors. Thanks to the EGFP reporter, we also observed that in transgenic mice, olfactory neurons project their axons to all glomeruli of the olfactory bulb.

In parallel, we performed setup experiments to try to identify by a proteomic approach OR accessory proteins. While preparing OR-enriched fractions from the transgenic mice by immunoprecipitation, we found that immunodetection of the receptor was more efficient with a specific anti-human eugenol receptor polyclonal antibody than with an anti-Tag monoclonal antibody. We are therefore also developping pull-down experiments to study potential interactions between the C-terminus of the eugenol receptor and accessory proteins. Indeed, as the anti-human eugenol receptor polyclonal recognizes an epitope localized in the C-terminus of the receptor, it is possible that the binding of the antibody would prevent the interaction with endogenous proteins potentially belonging to signalling complexes. We now plan to screen by 2D-DIGE and 2D-LC the proteins over-represented in this fraction, as compared to a total

membrane fraction of the same initial homogenate or as compared to samples prepared from wt mice. Proteins identified by mass spectrometry will be considered as part of signalling complexes and will be tested functionally in reconstituted systems in mammalian cell lines co-expressing ORs, Golf, and the cyclic-nucleotide-gated channel. We will also search for accessory proteins involved in cell surface targeting of OR by studying cellular localisation (using microscopy) of OR in cell lines co-expressing the newly identified accessory proteins and various olfactory receptors.

8. ChemR23

Chemerin is a potent chemotactic factor that was identified recently as the ligand of ChemR23, a G protein-coupled receptor expressed by mononuclear phagocytes, dendritic cells (DCs), and NK cells. Chemerin is synthesized as a secreted precursor, prochemerin, which is poorly active on ChemR23. However, prochemerin can be converted rapidly into a full ChemR23 agonist by proteolytic removal of a carboxy-terminal peptide. This maturation step is mediated by the neutrophil-derived serine proteases elastase and cathepsin G. We have now investigated proteolytic events that negatively control chemerin activity. We demonstrated that neutrophil-derived proteinase 3 (PR3) and mast cell (MC) chymase are involved in the generation of specific chemerin variants, which are inactive, as they do not induce calcium release or DC chemotaxis. Mass spectrometry analysis showed that PR3 specifically converts prochemerin into a chemerin form, lacking the last eight carboxy-terminal amino acids, and is inactive on ChemR23. Whereas PR3 had no effect on bioactive chemerin, MC chymase was shown to abolish chemerin activity by the removal of additional amino acids from its C-terminus. This effect was shown to be specific to bioactive chemerin (chemerin-157 and to a lesser extent, chemerin-156), as MC chymase does not use prochemerin as a substrate. These mechanisms, leading to the production of inactive variants of chemerin, starting from the precursor or the active variants, highlight the complex interplay of proteases regulating the bioactivity of this novel mediator during early innate immune responses (Guillabert et al. 2008).

We contributed to the demonstration that chemerin-expressing plasmacytoid dendritic cells (pDCs) accumulate in white matter lesions and leptomeninges of the brain of multiple sclerosis patients, while chemerin was found in intralesional cerebrovascular endothelial cells. This suggests that the chemerin/ChemR23 system plays a role in the control of pDC recruitment in multiple sclerosis (Lande et al. 2008).

We have characterized chemerin/ChemR23 system in mouse, in terms of pharmacology, structure-function, distribution, and in vivo biological properties. Mouse chemerin is synthesized as an inactive precursor (prochemerin) requiring, as in human, the precise processing of its C-terminus for generating an agonist of ChemR23. Mouse ChemR23 is highly expressed in immature plasmacytoid DCs and at lower levels in myeloid DCs, macrophages, and NK cells. Mouse prochemerin is expressed in most epithelial cells acting as barriers for pathogens but not in leukocytes. Chemerin promotes calcium mobilization and chemotaxis on DCs and macrophages and these functional responses were abrogated in ChemR23 knockout mice. In a mouse model of acute lung inflammation induced by LPS, chemerin displayed potent anti-inflammatory properties, reducing neutrophil infiltration and inflammatory cytokine release in a ChemR23-dependent manner. ChemR23 knockout mice were unresponsive to chemerin and displayed an increased neutrophil infiltrate following LPS challenge. Altogether, the mouse chemerin/ChemR23 system is structurally and functionally conserved between human and mouse, and mouse can therefore be considered as a good model for studying the anti-inflammatory role of this system in the regulation of immune responses and inflammatory diseases (Luangsay et al. 2009).

In collaboration with an Italian group from Brescia, we have also pursued the analysis of the chemerin/ChemR23 system in human inflammatory diseases of the skin and mucosa. Psoriasis is a type I interferon-driven T cell-mediated disease characterized by the recruitment of plasmacytoid dendritic cells (pDC) into the skin. The molecules involved in pDC accumulation in psoriasis lesions are unknown. Chemerin is the only inflammatory chemotactic factor that is directly active on human blood pDC in vitro. We evaluated therefore the role of the chemerin/ChemR23 axis in the recruitment of pDC in psoriasis skin. Prepsoriatic skin adjacent to active lesions and early lesions were characterized by a strong expression of chemerin in the dermis and by the presence of $CD15^+$ neutrophils and $CD123^+$/BDCA-2^+/ $ChemR23^+$ pDC. Conversely, skin from chronic plaques showed low chemerin expression, segregation of neutrophils to epidermal microabscesses, and few pDC in the dermis. Chemerin expression was localized mainly in fibroblasts, mast cells, and endothelial cells. Fibroblasts cultured from skin of psoriatic lesions expressed higher levels of chemerin messenger RNA and protein than fibroblasts from uninvolved psoriatic skin or healthy donors and promoted pDC migration in vitro in a chemerin-dependent manner. Therefore, chemerin expression specifically marks the early phases of evolving skin psoriatic lesions and is temporally strictly associated with pDC. These results support a role for the chemerin/ChemR23 axis in the early phases of psoriasis development (Albanesi et al. 2009).

Finally, we demonstrated the role played by ChemR23 in the physiopathology of viral pneumonia, using the pneumonia virus of mice (PVM)-induced model of acute pneumonia. $ChemR23^{-/-}$ mice displayed higher mortality/morbidity, delayed viral clearance and increased neutrophilic infiltration. We demonstrated in these mice a lower recruitment of pDCs, and as a result, a reduction in type I interferons production. The generation of bioactive chemerin was also shown in the infected lungs. Depletion and adoptive transfer of pDCs, as well as bone marrow transplantation experiments, demonstrated two opposite effects of the chemerin/ChemR23 system. First, the ChemR23-dependent recruitment of pDCs contributes to adaptive immune responses and viral clearance, but enhances also the deleterious inflammatory response. Second, increased morbidity/mortality in $ChemR23^{-/-}$ mice is not due to defective pDC recruitment, but rather to the loss of an anti-inflammatory role of chemerin mediated by non-leukocytic cells. Its important role in the physiopathology of viral pneumonia, suggests that the chemerin/ChemR23 system might be considered as a potential therapeutic target for anti-viral and anti-inflammatory therapies (Bondue et al. 2011).

9. The FPRL2 receptor

Formyl peptide receptors (FPRs) are a small group of seven-transmembrane domain, G protein-coupled receptors that are expressed mainly by mammalian phagocytic leukocytes and are known to be important in host defense and inflammation. The three human FPRs (FPR1, FPR2/ALX, and FPR3) share significant sequence homology and are encoded by clustered genes. Collectively, these receptors bind an extraordinarily numerous and structurally diverse group of agonistic ligands, including N-formyl and nonformyl peptides of different composition, that chemoattract and activate phagocytes. N-formyl peptides, which are encoded in nature only by bacterial and mitochondrial genes and result from obligatory initiation of bacterial and mitochondrial protein synthesis with N-formylmethionine, is the only ligand class common to all three human receptors. Structural and functional studies of the FPRs have produced important information for understanding the general pharmacological principles governing all leukocyte chemoattractant receptors. We provided an overview of the discovery and pharmacological characterization of FPRs, to introduce an International Union of Basic and Clinical Pharmacology (IUPHAR)-recommended nomenclature, and to discuss unmet challenges, including the

mechanisms used by these receptors to bind diverse ligands and mediate different biological functions (Ye et al. 2009).

We have pursued the study of FPRL2/FPR3, fot which a natural ligand (the peptide F2L) was discovered in the laboratory a few years ago. We investigated the detailed functional distribution of FPRL2 in leukocytes by quantitative PCR, flow cytometry, immunohistochemistry, and chemotaxis assays, with the aim of raising hypotheses regarding its potential functions in the human body. We described that FPRL2 is highly expressed and functional in plasmacytoid dendritic cells and up-regulated upon their maturation. FPRL2 is also expressed in eosinophils, which are recruited but do not degranulate in response to F2L. FPRL2 is expressed and functional in macrophages differentiated from monocytes in vitro in different conditions. However, in vivo, only specific subsets of macrophages express the receptor, particularly in the lung, colon, and skin, three organs chronically exposed to pathogens and exogenous aggressions. This distribution and the demonstration of the production of the F2L peptide in mice underline the potential role of FPRL2 in innate immunity and possibly in immune regulation and allergic diseases (Devosse et al. 2009).

We have also investigated which proteases were able to generate the F2L peptide from its precursor HEBP1. Structure-function analysis of F2L identified three amino acids, G3, N7 and S8, as the most important for the interaction of the peptide with FPR3. We have expressed a C-terminally His-tagged form of human HEBP1 in yeast and purified it to homogeneity. The purified protein was used as substrate to identify proteases able to generate bioactive peptides for FPR3-expressing cells. A conditioned medium from macrophages was able to generate bioactive peptides from HEBP1, and this activity was inhibited by pepstatin A. Cathepsin D was identified as the protease responsible for HEBP1 processing, and the bioactive product was identified as F2L. We have therefore determined how F2L, the specific agonist of the FPR3 receptor, is generated from the intracellular protein HEBP1, although it is unknown in which compartment the processing by cathepsin D occurs in vivo (Devosse et al. 2011).

10. Dimerization of G protein coupled receptors

We have pursued the analysis of the functional consequences of the homo- and hetero-dimerization of GPCRs, using chemokine receptors as models.

CCR2/CCR5, CCR2/CXCR4 and CCR5/CXCR4 heterodimers. We reported previously the existence of negative binding cooperativity between the subunits of CCR2/CCR5 and CCR2/CXCR4 heterodimers (El-Asmar et al., 2004, Springael et al., 2006, Sohy et al., 2007). We extended these observations to heterodimers formed by CXCR4 and CCR5 demonstrating that specific agonists and antagonists of one receptor can compete allosterically for the binding of a specific tracer of the other when the two receptors are co-expressed. CCR2, CCR5 and CXCR4 form thus homodimers as well as heterodimers with one another, raising the question of their natural organization at the surface of immune cells expressing these three receptors endogenously. Using Bi-LC BRET assays, we demonstrated that hetero-oligomeric complexes containing simultaneously the three receptors are formed. Importantly, we also showed that specific antagonists of one receptor inhibit the binding of chemokines to the other receptor as a consequence of their heterodimerization, both in recombinant cell lines and primary leukocytes. This resulted in a significant functional cross-inhibition in terms of calcium mobilization and chemotaxis. Using the air pouch model in mice, we established that this trans-inhibition by antagonists has major consequences on the migration of cells in vivo. We showed that the CCR2 and CCR5 antagonist TAK-

779 inhibits both lymphocytes and dendritic cells recruitment into the pouch in response to SDF-1α. This data support the new concept following which small-molecule antagonists can trans-modulate the function of receptors on which they do not bind directly, as the result of their heterodimerization, with important implications on the activities of chemokine receptor antagonists in vivo. We have started to study a larger range of antagonists in order to test whether trans-inhibition is a property shared by all antagonists of these receptors or restricted to some molecules only. From a general point of view, allosteric regulation across GPCR oligomeric interfaces is expected to greatly influence the practice of modern pharmacology. It will likely affect the design of drug discovery programs, which rely mostly on the overexpression of the receptor of interest in a cell line, thereby focusing on homo-oligomers and ignoring the potential effects of other partners.

Heterodimerization of ChemR23 with CXCR4 and CCR7. With the aim of further characterizing functional consequences of chemokine receptors dimerization, we also investigated the dimerization status of ChemR23. Like chemokine receptors, ChemR23 is expressed on leukocytes such as macrophages, dendritic cells as well as on a subset of NK cells. Using BRET, we showed that ChemR23 is able to form heterodimers with chemokine receptors CXCR4 and CCR7 constitutively and that this interaction results in a strong negative binding cooperativity. These results support the view that negative binding cooperativity takes also place across receptors that bind ligands structurally unrelated. We also showed on mouse BMDC expressing endogenously ChemR23 and CXCR4, that chemerin competed for SDF-1α binding and that this cross-inhibitory effect is specifically lost in cells generated from mice invalidated for ChemR23, thus demonstrating the functional relevance of ChemR23/CXCR4 dimerization in primary leukocytes.

Heterodimerization of CCR7 with CXCR4, CCR5 and CCR2. We also investigated the dimerization status of the chemokine receptor CCR7 and showed using BRET that CCR7 forms heterodimers with CCR2, CCR5 and CXCR4 constitutively. Similarly to what we reported for other chemokine heterodimers, we showed that the heterodimerization of CCR7 with CCR2 is associated with a “symmetrical” negative binding cooperativity, the ligand of each receptor being able to compete for the binding of radiolabelled tracer to the other. In contrast, CCR7 heterodimerization with CCR5 is linked to an “asymmetrical” negative binding cooperativity i.e. specific ligands of CCR7 being able to compete for radiolabelled tracer to CCR5 while CCR5-specific ligands being unable to do so for tracer binding to CCR7. Finally, we showed that CCR7 heterodimerization with CXCR4 does not involve binding cooperativity, the ligands of each receptor composing the dimer being unable to compete for binding of radiolabelled tracer to the other. Among all the chemokine heterodimers we tested, this is the first case of receptors for which heterodimerization is not associated with negative binding cooperativity. In contrast, we showed that the functional response of CXCR4 and CCR5 is strongly reduced upon co-expression of CCR7. Expression of CCR7 had no major effect on the EC50 values of the dose-response curves but decreased drastically the maximal response. This decrease of response was however not linked to a reduced expression level of CCR5 or CXCR4 receptor as measured by FACS or saturation binding assay. The molecular mechanism underlying this phenomenon is not known for sure but might involve conformational change of CCR5 and CXCR4 receptors as the result of their interaction with CCR7. Interestingly, this negative effect of CCR7 was not detected in cells coexpressing CCR7 with CCR2 or ChemR23, suggesting that properties of receptors might vary greatly according to the partner with which CCR7 interacts.

We have pursued the analysis of the functional consequences of the homo- and hetero-dimerization of GPCRs, using chemokine receptors as models. Using a combination of luminescence complementation and bioluminescence resonance energy transfer assays, we have demonstrated for the first time the

existence of hetero-oligomeric complexes composed of at least three chemokine receptors (CCR2, CCR5, and CXCR4). We showed in T cells and monocytes that negative binding cooperativity takes place between the binding pockets of these receptors, demonstrating their functional interaction in leukocytes. We also showed that specific antagonists of one receptor (TAK-779 or AMD3100) lead to functional cross-inhibition of the others. Finally, using the air pouch model in mice, we showed that the CCR2 and CCR5 antagonist TAK-779 inhibits cell recruitment promoted by the CXCR4 agonist SDF-1α/CXCL12, demonstrating that cross-inhibition by antagonists also occurs in vivo. Thus, antagonists of the therapeutically important chemokine receptors regulate the functional properties of other receptors to which they do not bind directly with important implications for the use of these agents in vivo (Sohy et al. 2009).

11. Ligand processing and regulation of biological activity

The activity of many GPCR ligands, including chemokines, is regulated at the post-translational level by proteolysis or other types of modifications. We have studied in collaboration with other groups the post-translational modifications of several chemokines affecting their activity on the cognate receptors.

The CC chemokine CCL14a is constitutively expressed in a large variety of tissues and its inactive proform CCL14a(1-74) circulates in high concentrations in plasma. CCL14a(1-74) is converted into CCL14a(9-74) by the proteases urokinase-type plasminogen activator and plasmin and is a highly active agonist for the chemokine receptors CCR1 and CCR5. We have isolated a new CCL14a analog, CCL14a(12-74), from blood filtrate. To elucidate the functional role of the N terminus, a panel of N-terminally truncated CCL14a analogs were tested on the receptors CCR1 to CCR5 and on the human cytomegalovirus (HCMV)-encoded chemokine receptor US28. The rank order of binding affinity to these receptors and of the activation of CCR1 and CCR5-mediated intracellular Ca^{2+} concentration mobilization is CCL14a(6-74)<(7-74)<(8-74)<<(9-74) = (10-74)>>(11-74)>>(12-74). The almost identical affinities of CCL14a(7-74), CCL14a(9-74), and CCL14a(10-74) for the US28 receptor and the inhibition of US28-mediated HIV infection of 293T cells by all of the N-terminally truncated CCL14a analogs support the promiscuous nature of the viral chemokine receptor US28. In high concentrations, CCL14a(12-74) did reveal antagonistic activity on intracellular Ca^{2+} concentration mobilization in CCR1- and CCR5-transfected cells, which suggests that truncation of Tyr^{11} might be of significance for an efficient inactivation of CCL14a. A putative inactivation pathway of CCL14a(9-74) to CCL14a(12-74) may involve the dipeptidase CD26/dipeptidyl peptidase IV (DPPIV), which generates CCL14a(11-74), and the metalloprotease aminopeptidase N (CD13), which displays the capacity to generate CCL14a(12-74) from CCL14a(11-74). Our results suggest that the activity of CCL14a might be regulated by stringent proteolytic activation and inactivation steps (Richter et al. 2009)

We contributed also to studies showing synergistic effects of CC and CXC chemokines in chemotactic assays using monocytes (Gouwy et al. 2008). These synegistic effects likely involve post-receptor signaling cascades. The citrullination of the CXCL12, resulting in the inactivation of the chemokine was reported (Struyf et al. 2009). The cellular receptors for the human CXC chemokine platelet factor-4 variant/CXCL4L1, a potent inhibitor of angiogenesis were also investigated. It was shown that both CXCR3A and CXCR3B are implicated in the chemotactic and vascular effects of CXCL4L1 (Struyf et al. 2011).

12. References

- Albanesi C, Scarponi C, Pallotta S, Daniele R, Bosisio D, Madonna S, Fortugno P, Gonzalvo-Feo S, Franssen JD, Parmentier M, De Pità O, Girolomoni G, Sozzani S (2009). Chemerin expression marks early psoriatic skin lesions and correlates with plasmacytoid dendritic cell recruitment. J Exp Med 206:249-258.
- Aso E, Ozaita A, Valdizán EM, Ledent C, Pazos A, Maldonado R, Valverde O (2008). BDNF impairment in the hippocampus is related to enhanced despair behavior in CB_1 knockout mice. J Neurochem 105:565-572.
- Aso E, Renoir T, Mengod G, Ledent C, Hamon M, Maldonado R, Lanfumey L, Valverde O (2009). Lack of CB1 receptor activity impairs serotonergic negative feedback. J Neurochem 109:935-944.
- Azdad K, Gall D, Woods AS, Ledent C, Ferré S, Schiffmann SN (2009). Dopamine D_2 and adenosine A_{2A} receptors regulate NMDA-mediated excitation in accumbens neurons through A_{2A}-D2 receptor heteromerization. Neuropsychopharmacology 34:972-86.
- Balázsa T, Bíró J, Gullai N, Ledent C, Sperlágh B (2008). CB_1-cannabinoid receptors are involved in the modulation of non-synaptic [^{3}H]serotonin release from the rat hippocampus. Neurochem Int 52:95-102.
- Ben Addi A, Lefort A, Hua X, Libert F, Communi D, Ledent C, Macours P, Tilley SL, Boeynaems JM, Robaye B (2008). Modulation of murine dendritic cell function by adenine nucleotides and adenosine: involvement of the A_{2B} receptor. Eur J Immunol 38:1610-1620.
- Bondue B, Vosters O, De Nadai P, Glineur S, De Henau O, Luangsay S, Van Gool F, Communi D, De Vuyst P, Desmecht D, Parmentier M (2011). ChemR23 dampens lung inflammation and enhances anti-viral immunity in a mouse model of acute viral pneumonia1. J Exp Med (resubmitted).
- Bura SA, Nadal X, Ledent C, Maldonado R, Valverde O (2008). A_{2A} adenosine receptor regulates glia proliferation and pain after peripheral nerve injury. Pain 140:95-103.
- Castañé A, Wells L, Soria G, Hourani S, Ledent C, Kitchen I, Opacka-Juffry J, Maldonado R, Valverde O (2008). Behavioural and biochemical responses to morphine associated with its motivational properties are altered in adenosine A_{2A} receptor knockout mice. Br J Pharmacol 155:757-766.
- Croxford JL, Pryce G, Jackson SJ, Ledent C, Giovannoni G, Pertwee RG, Yamamura T, Baker D (2008). Cannabinoid-mediated neuroprotection, not immunosuppression, may be more relevant to multiple sclerosis. J Neuroimmunol 193:120-129.
- Devosse D, Dutoit R, Migeotte I, Imbault V, Communi D, Salmon I, Parmentier M (2011). Processing of HEBP1 by cathepsin D gives rise to F2L, the agonist of Formyl Peptide Receptor 3. Submitted.
- Devosse T, Guillabert A, D'Haene N, Berton A, De Nadai P, Noel S, Brait M, Franssen JD, Sozzani S, Salmon I, Parmentier M (2009). Formyl peptide receptor-like 2 is expressed and functional in plasmacytoid dendritic cells, tissue-specific macrophage subpopulations, and eosinophils. J Immunol 182:4974-4984.
- El Yacoubi M, Ledent C, Parmentier M, Costentin J, Vaugeois JM (2008). Evidence for the involvement of the adenosine A_{2A} receptor in the lowered susceptibility to pentylenetetrazol-induced seizures produced in mice by long-term treatment with caffeine. Neuropharmacology 55:35-40.
- El Yacoubi M, Ledent C, Parmentier M, Costentin J, Vaugeois JM (2009). Adenosine A_{2A} receptor deficient mice are partially resistant to limbic seizures. Naunyn Schmiedebergs Arch Pharmacol 380:223-232.
- Gaertner H, Lebeau O, Borlat I, Cerini F, Dufour B, Kuenzi G, Melotti A, Fish RJ, Offord R, Springael JY, Parmentier M, Hartley O (2008). Highly potent HIV inhibition: engineering a key anti-HIV structure from PSC-RANTES into MIP-1 /CCL4. Protein Eng Des Sel. 21:65-72.
- Garcia MI, Ghiani M, Lefort A, Libert F, Strollo S, Vassart G (2009). LGR5 deficiency deregulates Wnt signaling and leads to precocious Paneth cell differentiation in the fetal intestine. Dev Biol 331:58-67.
- Gouwy M, Struyf S, Noppen S, Schutyser E, Springael JY, Parmentier M, Proost P, Van Damme J (2008). Synergy between co-produced CC and CXC chemokines in monocyte chemotaxis through receptor mediated events. Mol Pharmacol 74:485-495.
- Guillabert A, Wittamer V, Bondue B, Godot V, Imbault V, Parmentier M, Communi D (2008). Role of neutrophil proteinase 3 and mast cell chymase in chemerin proteolytic regulation. J Leukoc Biol 84:1530-1538.
- Hegyi Z, Kis G, Holló K, Ledent C, Antal M (2009). Neuronal and glial localization of the cannabinoid-1 receptor in the superficial spinal dorsal horn of the rodent spinal cord. Eur J Neurosci 30:251-262.
- Houchi H, Warnault V, Barbier E, Dubois C, Pierrefiche O, Ledent C, Daoust M, Naassila M (2008). Involvement of A_{2A} receptors in anxiolytic, locomotor and motivational properties of ethanol in mice. Genes Brain Behav 7:887-898.
- Hussey MJ, Clarke GD, Ledent C, Kitchen I, Hourani SM (2010). Genetic deletion of the adenosine A_{2A} receptor in mice reduces the changes in spinal cord NMDA receptor binding and glucose uptake caused by a nociceptive stimulus. Neurosci Lett 479:297-301.
- Khelashvili G, Dorff K, Shan J, Camacho-Artacho M, Skrabanek L, Vroling B, Bouvier M, Devi LA, George SR, Javitch JA, Lohse MJ, Milligan G, Neubig RR, Palczewski K, Parmentier M, Pin JP, Vriend G, Campagne F, Filizola M (2010). GPCR-OKB: the G Protein Coupled Receptor Oligomer Knowledge Base. Bioinformatics 26:1804-1805.

- Lambot MA, Mendive F, Laurent P, Van Schoore G, Noël JC, Vanderhaeghen P, Vassart G (2009). Three-dimensional reconstruction of efferent ducts in wild-type and Lgr4 knock-out mice. Anat Rec 292:595-603.
- Lande R, Gafa V, Serafini B, Giacomini E, Visconti A, Remoli ME, Severa M, Parmentier M, Ristori G, Salvetti M, Aloisi F, Coccia EM (2008). Plasmacytoid dendritic cells in multiple sclerosis: intracerebral recruitment and impaired maturation in response to interferon-beta. J Neuropathol Exp Neurol 67:388-401.
- Luangsay S, Wittamer V, Bondue B, De Henau O, Rouger L, Brait M, Franssen JD, de Nadai P, HuauxF, Parmentier M (2009). Mouse ChemR23 is expressed in dendritic cell subsets and macrophages, and mediates an anti-inflammatory activity of chemerin in a lung disease model. J Immunol 183: 6489-6499.
- Mato S, Alberdi E, Ledent C, Watanabe M, Matute C (2009). CB_1 cannabinoid receptor-dependent and -independent inhibition of depolarization-induced calcium influx in oligodendrocytes. Glia 57:295-306.
- Mievis S, Blum D, Ledent C (2011). A_{2A} receptor knockout worsens survival and motor behaviour in a transgenic mouse model of Huntington's disease. Neurobiol Dis 41:570-576.
- Murikinati S, Jüttler E, Keinert T, Ridder DA, Muhammad S, Waibler Z, Ledent C, Zimmer A, Kalinke U, Schwaninger M (2010). Activation of cannabinoid 2 receptors protects against cerebral ischemia by inhibiting neutrophil recruitment. FASEB J 24:788-798.
- Németh B, Ledent C, Freund TF, Hájos N (2008). CB1 receptor-dependent and -independent inhibition of excitatory postsynaptic currents in the hippocampus by WIN 55,212-2. Neuropharmacology 54:51-57.
- Richter R, Casarosa P, Ständker L, Münch J, Springael JY, Nijmeijer S, Forssmann WG, Vischer HF, Vakili J, Detheux M, Parmentier M, Leurs R, Smit MJ (2009). Significance of N-Terminal Proteolysis of CCL14a to Activity on the Chemokine Receptors CCR1 and CCR5 and the Human Cytomegalovirus-Encoded Chemokine Receptor US28. J Immunol 183:1229-1237.
- Safhi MM, Rutherford C, Ledent C, Sands WA, Palmer TM (2010). Priming of signal transducer and activator of transcription proteins for cytokine-triggered polyubiquitylation and degradation by the A_{2A} adenosine receptor. Mol Pharmacol 77:968-978.
- Sohy D, Yano H, de Nadai P, Urizar E, Guillabert A, Javitch JA, Parmentier M, Springael JY (2009). Hetero-oligomerization of CCR2, CCR5 and CXCR4 and the protean effects of "selective"-antagonists. J Biol Chem 284:31270-31279.
- Struyf S, Noppen S, Loos T, Mortier A, Gouwy M, Verbeke H, Huskens D, Luangsay S, Parmentier M, Geboes K, Schols D, Van Damme J, Proost P (2009). Citrullination of CXCL12 Differentially Reduces CXCR4 and CXCR7 Binding with Loss of Inflammatory and Anti-HIV-1 Activity via CXCR4. J Immunol 182:666-674.
- Struyf S, Noppen S, Loos T, Mortier A, Gouwy M, Verbeke H, Huskens D, Luangsay S, Parmentier M, Geboes K, Schols D, Van Damme J, Proost P (2009). Citrullination of CXCL12 Differentially Reduces CXCR4 and CXCR7 Binding with Loss of Inflammatory and Anti-HIV-1 Activity via CXCR4. J Immunol 182:666-674.
- Struyf S, Salogni L, Burdick MD, Vandercappellen J, Gouwy M, Noppen S, Proost P, Opdenakker G, Parmentier M, Gerard C, Sozzani S, Strieter RM, Van Damme J (2011). Angiostatic and chemotactic activities of the CXC chemokine CXCL4L1 (platelet factor-4 variant) are mediated by CXCR3. Blood 117:480-488.
- Tebano MT, Martire A, Chiodi V, Pepponi R, Ferrante A, Domenici MR, Frank C, Chen JF, Ledent C, Popoli P (2009). Adenosine A_{2A} receptors enable the synaptic effects of cannabinoid CB1 receptors in the rodent striatum. J Neurochem 110:1921-1930.
- Thiemann G, Watt CA, Ledent C, Molleman A, Hasenöhrl RU (2009). Modulation of anxiety by acute blockade and genetic deletion of the CB_1 cannabinoid receptor in mice together with biogenic amine changes in the forebrain. Behav Brain Res 200:60-67.
- Touriño C, Ledent C, Maldonado R, Valverde O (2008). CB1 cannabinoid receptor modulates 3,4-methylenedioxymeth-amphetamine acute responses and reinforcement. Biol Psychiatry 63:1030-1038.
- Valverde O, Célérier E, Baranyi M, Vanderhaeghen P, Maldonado R, Sperlagh B, Vassart G, Ledent C (2009). GPR3 receptor, a novel actor in the emotional-like responses. PLoS One 4(3):e4704.
- Vassart G (2010). An in vivo demonstration of functional G protein-coupled receptor dimers. Proc Natl Acad Sci USA 107:1819-1820.
- Ye RD, Boulay F, Wang JM, Dahlgren C, Gerard C, Parmentier M, Serhan CN, Murphy AP (2009). International Union of Basic and Clinical Pharmacology. LXXIII. Nomenclature for the Formyl Peptide Receptor (FPR) Family. Pharmacol Rev 61:119-161.

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Role of protein synthesis in late long-term potentiation (L-LTP)

GENERAL BACKGROUND

Nowadays, scientific thinking about memory is dominated by two basic concepts. First, since 1953, after hippocampus had been removed (for untreatable seizures), from a patient called H.M, it became clear that explicit memory depended on hippocampus and has two temporally distinct phases: a short-term memory (STM) preserved in H.M. and a long-term memory (LTM) that H.M. was not anymore able to acquire. Second, neuroscientists agree on the fact that memories are stored in the brain thanks to changes in the strength of synaptic connections between the neurons. In other words synaptic plasticity underlies memory.

Research on synaptic plasticity has largely benefited from the development of an in vitro model. In the CA1 region of hippocampal slices (0.4 mm) artificially maintained alive in vitro, stimulation of Schaffer collaterals by delivering one or several trains of high-frequency stimulation induced an immediate and prolonged increase in synaptic strength called long-term potentiation (LTP). A single train triggers an early LTP (E-LTP) that lasts less than 3 h whereas several trains (usually 3 or 4) cause a long-lasting LTP (L-LTP) that persists more than 4 h. The mechanism of E-LTP has been revealed. Basically, a Ca^{++} influx through the NMDA receptors activates enzymes (CaMKII in particular) that trigger, in turn, incorporation of extra AMPA receptors in the postsynaptic membrane.

In contrast, very few is known about the mechanisms of long-term memory (LTM) and long-lasting LTP (L-LTP). Due to the fact, that both are blocked when protein-synthesis inhibitors are administered around induction, it has been assumed for more than 30 years that LTM and L-LTM are dependent on a de novo protein synthesis at the moment of induction. However, until now, no one has been identified with certainty.

PROTEOMICS APPROACH

1. General aim

The primary goal of the project was to identify the proteins whose amount increased in the CA1 region of hippocampal slices during the late phase of L-LTP or in the dentate gyrus of the hippocampus during contextual fear conditioning, a form of LTM dependent on the hippocampus.

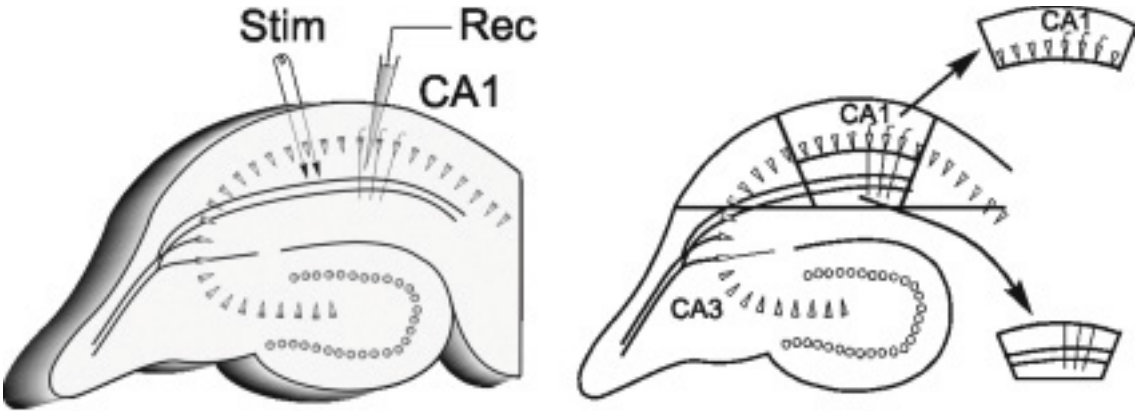
The basic idea was to use techniques of proteomics to compare the relative amounts of several hundred of proteins from samples where L-LTP or LTM has been induced on one hand and from control samples on the other hand.

The basic principle of the technique is a differential stable isotopic labeling (C12 and C13) associated with a proteomics technique, the two-dimensional high pressure liquid chromatography (HPLC) coupled with electrospray tandem mass spectrometry (2D-LC/MS-MS). In the quantitative 2D-LC/MS-MS technique, the proteins extracted from either sample (control or treated) are coupled with succinimide which reacts with the terminal NH2 and the NH2 of the lysine residues. Succinimide is labeled with C13 for one

sample and is natural (C12) for the other. The two labeled protein pools are then mixed. The mixture obtained is submitted to trypsin and endoglu digestion. The resulting peptides are then separated by two-dimensional high pressure liquid chromatography and, after electrospray ionization, by mass spectrometry (MS). A same lysine-containing peptide will give birth to a pair of ionized peptides, the one labeled with C13 being slightly heavier than the other labeled with C12. The ratio of the amounts of the two versions of the peptide gives the ratio of the amounts of the mother-proteins in the two hippocampal samples. Moreover, immediately after their separation, the peptides are fragmented by collision-induced dissociation. These fragments are then separated by MS (tandem mass spectrometry, MS-MS). This procedure allows "immediate" sequencing of the peptides and hence the identification of the mother-protein: the resultant spectral data are indeed automatically processed to search a genome sequence database for protein identification.

2. In vitro experiments

A robust long-lasting LTP (4 h) was induced in the CA1 region of hippocampal slices by application of chemicals (IBMX, forskolin) causing an increase in the endogeneous cAMP. Four hours after induction, the CA1 region from each hippocampal slice was removed using a razor blade under a dissecting microscope. The CA1 dendritic region was further separated from the somatic region. One hundred and eighty slices were used (90 treated and 90 control). Out of the dendritic regions, the synaptoneurosomal fraction was isolated for further biochemical analysis.



3. In vitro results

Three biological replicates were performed, each of them treated technically twice. More than two hundred proteins were detected both in the synaptoneurosomes and in the somas. Surprisingly, given our premises, all of them were found statistically unchanged.

4. In vivo experiments

Contextual fear conditioning was performed in a chamber with grid floor connected to electric output device. The training consisted of a single trial whereby after a 60 s baseline period, rats were exposed to footshock. They were then returned to their home-cages. After 24 hours, contextual fear conditioning was assessed by returning the rats to the conditioning chamber. Their recollection of the condition situation was assessed by their "freezing" behavior. Rats were then killed. Hippocampus were dissected and the dentate gyri were removed. Three biological replicates were done. Among the three hundred proteins identified, only one had its amount increased 24 h after conditioning (but by more than 50%). This is BASP1 protein (paper in preparation).

L-LTP AND PROTEIN SYNTHESIS

1. Somatic versus dendritic protein synthesis

Protein synthesis has been considered as a mechanism playing a role in L-LTP because anisomycin, a protein-synthesis inhibitor, blocks it. However, two hypotheses are in competition. According to one, after strong activation of a synapse, a message is send to the nucleus. As a result, transcription of genes occurs. The corresponding mRNAs are translated in the soma and the resulting proteins are dispatched to the activated synapses marked by a "tag". According to the other hypothesis, mRNAs are permanently present throughout the dendrites and a strong synapse activation triggers a local dendritic protein synthesis. The fact that we were able to elicit an L-LTP for 8 hours in CA1 dendrites isolated from their somas favored the second hypothesis (NeuroReport, 2010).

2. When does the needed protein synthesis take place?

It has been repeatedly reported that, in order to trigger an L-LTP, protein synthesis must occur only around induction. The situation must, in fact, be more complicated. Indeed, we found that anisomycin was more effective in inhibiting the late phase of the L-LTP triggered by four trains when applied during the whole experiment rather than only around LTP induction, as is often done (NeuroReport, 2009).

3. Role of CaMKII autophosphorylation in L-LTP

It was known that in transgenic mice where autophosphorylation of CaMKII was prevented by a mutation of threonin 286, E-LTP could not be triggered by a single tetanus. We recently demonstrated, using a multiple train stimulation protocol, that the late phase of L-LTP was also dependent on the autophosphorylation of CaMKII (Molecular Brain, 2011).

LTP AND ALZHEIMER'S DISEASE

Alzheimer's disease is characterized by a devastating deterioration of cognitive functions and memory. It was thus interesting in investigate the relationship between LTP and proteins whose deregulation if known to play a role in Alzheimer's disease.

1. Amyloid Peptide Precursor (APP)

APP [V717I] is a mutation of APP known to induce early onset familial Alzheimer disease (EOFAD) in man. In transgenic mice carrying this APP [V717I] mutation, LTP is known for a long time to be impaired. We pharmacologically isolated the part of the field potential due to the current flowing through NMDA receptors by addition of 20 µM CNQX in the medium. This allowed us to reveal a pronounced decrease in NMDA receptors (NMDAR) responses in CA1 of APP [V717I] transgenic mice (Neurobiology of Aging, 2009).

2. Presenilin 1 (PS1)

Mutations of presenilin 1 (PS1 [A246E] for instance) are also known to lead to early onset familial Alzheimer disease. We investigated LTP and the NMDAR response in transgenic mice with a postnatal neuron-specific deficiency of PS1 [PS1(n -/-)] and in transgenic mice expressing the postnatal neuron-specific mutation PS1[A246E]. The initial phase of LTP was decreased in PS1 (n-/-) whereas a weak tetanic stimulation induced stronger LTP in the hippocampus of PS1[A246E] as opposed to PS1 [wt] mice. NMDAR responses were decreased in CA1 of PS1(n-/-) mice, as opposed to increased NMDAR mediated responses in CA1 of mutant PS1 mice (Neurobiology of Aging, 2008).

3. Glycogen Synthase Kinase-3 (GSK3)

Glycogen synthase kinase-3 (GSK3) is a proline-directed serine/threonine kinase, originally identified as a regulator of glycogen metabolism. Its isoform GSK3ß is inactivated by phosphorylation at S9 by various protein kinases. Accumulating evidence implicates deregulation of GSK3ß as a pathological event in Alzheimer's disease. We investigated L-LTP in transgenic mice expressing the phosphorylation defective, constitutively active GSK3ß [S9A]. We found that the late phase of L-LTP was impaired in these mice (Neurobiology of Disease, 2009).

PUBLICATIONS WITH ACKNOWLEDGMENTS TO THE FMRE

1. Dewachter, I., Ris, L., Croes, S., Borghgraef, P., Devijver, H., Voets, T., Nilius, B., Godaux, E. and Van Leuven, F. Modulation of synaptic plasticity and Tau phosphorylation by wild-type and mutant presenilin1. Neurobiology of Aging 29(5) (2008) 639-652.
2. Ris, L., Villers, A. and Godaux, E. Synaptic capture-mediated L-LTP is strongly dependent on mRNA translation. NeuroReport 20 (2009) 1572-1576.
3. Dewachter, I., Filipkowski, R.K., Priller, C., Ris, L., Neyton, J., Croes, S., Terwel, D., Gysemans, M., Devijver, H., Borghgraef, P., Godaux, E., Kaczmarek, L., Herms, J. and Van Leuven, F. Deregulation of NMDA-receptor function and down-stream signaling in APP[V717I] transgenic mice. Neurobiology of Aging 30(2) (2009) 241-256.
4. Dewachter, I., Ris, L., Jaworski, T., Seymour, C.M., Kremer, A., Borghraef, P., De Vijver, H., Godaux, E. and Van Leuven, F. GSK3ß, a centre-staged kinase in neuropsychiatric disorders, modulates long term memory by inhibitory phosphorylation at Serine-9. Neurobiology of Disease 35(2) (2009) 193-200.
5. Villers, A., Godaux, E. and Ris, L. Late phase of L-LTP elicited in isolated CA1 dendrites cannot be transferred by synaptic capture. NeuroReport 21 (2010) 210-215.
6. Irvine, E.E., Danhiez, A., Radwansko, K., Nassim, C., Lucchesi, W., Godaux, E., Ris, L. and Giese, K.P. Properties of contextual memory formed in the absence of αCaMKII autophosphorylation. Molecular Brain (in Progress).

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Roles of Specific Neuronal Populations in Functions and Disorders of Basal Ganglia

The basal ganglia system constitutes with the cerebral cortex an interconnected neural network involved in adaptive control of behaviour. The basal ganglia have a tremendous importance in human diseases as they are centrally affected in Parkinson's disease, Huntington's disease, schizophrenia or drug addiction. The striatum, the major input structure of this system is made up several neuronal populations including two efferent medium-size spiny neurons (MSN) sub-populations characterised by their outputs, either substantia nigra *pars reticulata* or globus pallidus (GP); as well as four classes of interneurons. The two populations of MSN, striatonigral and striatopallidal neurons, expressing dopamine D_1 (D_1R) or D_2 (D_2R) receptors, respectively, give rise to the direct and indirect pathways of the basal ganglia circuitry, respectively.

The major aims of our research group is to understand the differential functional properties of the two MSN populations, to determine the distinct roles of these neurons and hence of direct and indirect pathways as well as those of specific striatal interneuron sub-classes in basal ganglia physiology and their distinct involvement in basal ganglia pathologies, through the development of animal models bearing cellular or molecular 'lesions' by conditional transgenesis.

The main achievements obtained on the 2008-2010 period thanks to the support from FMRE/GSKE are summarized below.

1. Elucidation of the striatopallidal neuron's functions by gene targeting

To study of the specific roles of striatopallidal and striatonigral neurons, our first aim was to generate conditional transgenic mice. We have previously generated mice strains expressing the Cre recombinase under the control of the A_{2A} receptor promoter inserted in a BAC (bacterial artificial chromosome) to direct gene expression specifically in striatopallidal neurons (A_{2A}r-Cre) (de Kerchove d'Exaerde et al., 2006). In order to demonstrate the specificity of Cre expression in the striatopallidal neurons, these mice have been crossed with reporter strains expressing either ß-galactosidase (Rosa26-LacZ) (Soriano 1999) or an analogue of GFP (Z/EG mice) (Novak et al., 2000). Co-localisation experiments using anti-enkephalin (Enk) antibody and LacZ activity detection showed that >80% of the LacZ neurons are also Enk+. The expression of eGFP was shown to be restricted in a subpopulation of striatal neurons which were never identified as striatonigral by retrograde labelling and who co-expressed D_2 and A_{2A} receptors as demonstrated by single cell RT-PCR. Therefore, altogether, these results demonstrated that we have generated strains of transgenic mice with a specific Cre expression in the striatopallidal neurons.

This final characterization of this striatopallidal neuron-specific Cre strain was part of the results published in Durieux et al., 2009.

1.a Conditional and selective ablation of striatopallidal neurons

As a first attempt to gain insight on the roles of striatopallidal neurons and thanks to the generation of this specific A_{2A}r-Cre mice strain, we used the strategy of conditional and selective ablation of striatopallidal neurons by crossing these mice with mice allowing the conditional expression of the diphtheria toxin receptor (rosa26-lox-stop-lox-DTR mice). Through the stereotaxic injection of diphtheria toxin, this allowed the specific ablation of striatopallidal neurons in the full striatum or selectively in sub-sectors of the striatum as the accumbens nucleus (Durieux et al., 2009). Since D_1R–striatonigral and D_2R–striatopallidal neurons are intermingled and morphologically indistinguishable they could not

be functionally dissociated with classical techniques as chemical lesions or surgery. Therefore our mice strain was the first genetic model allowing selective ablation of these striatopallidal cells. We first demonstrated the very high selectivity and high efficacy of the ablation since neither markers (i.e. substance P or D_1R) of striatonigral neurons was decreased nor the density of the four classes of interneurons were affected whilst the specific markers (i.e. enkephalin, $A_{2A}r$ or D_2R) of striatopallidal neurons were decreased by more than 90%. Importantly, by using Tyrosine Hydroxylase mRNA in situ hybridization, Dopamine Transporter binding and in vivo microdialysis, we found neither modifications in cell body or terminal dopaminergic markers nor differences in basal and amphetamine–induced dopamine overflow, indicating therefore that D_2R–striatopallidal neuron ablation does not induce major modifications in striatal dopaminergic function. As a first result demonstrating an alteration in the basal ganglia network consecutive to this ablation, we found an increase in GAD67 mRNA in the GP confirming that the D_2R–striatopallidal neurons exert an inhibitory control on GP GABA neuron activity (Durieux et al., 2009).
By using this strategy and examining the spontaneous locomotor activity of mice bearing a striatopallidal neuron loss in the entire striatum bilaterally, we showed that such ablation induced a marked (about 400%) and persistent (up to 40 days after injections) hyperlocomotion detected in open field video tracking (Durieux et al., 2009). It is worth to mention that this locomotor hyperactivity did not notably perturb food and water intakes as well as the circadian day-night cycle.
Since the ventral striatum is the key neuronal substrate for drug reinforcement and the roles of striatopallidal neurons in this area were completely unknown, we realized a restricted ablation of striatopallidal neurons in the nucleus accumbens. Surprisingly and unexpectedly, such ablation increased the preference for amphetamine in a conditioned place preference paradigm designed to model drug reward and reinforcement in rodents (Durieux et al., 2009). Moreover, in this condition, this preference was even maintained much longer (Durieux et al., 2009).
Altogether, our results provide direct experimental evidence that D_2R–striatopallidal neurons are critical for both the control of motor behaviour and drug reinforcement. They validated the hypothesis that these neurons exert inhibitory effect on the motor activity and demonstrated an unexpected involvement of these striatopallidal neurons in limiting the drug-reinforcement and motivational processes (Durieux et al., 2009).

The superior portion of the striatum is not only involved in the planning of new motor tasks but also in motor learning. However, the specific involvement of striatopallidal and striatonigral neurons in motor learning was not fully understand. We used our $A_{2A}r$-Cre mice as well as a Drd1a-Cre mice strain (Gong et al., 2007) targeting striatonigral neurons to reveal the specific functions of these neurons in both motor control and skill learning (Durieux et al., submitted, 2010). Complementary to the results described above on D_2R-striatopallidal neuron ablated mice, we showed that D_1R-striatonigral or D_2R-striatopallidal neurons exert opposite control over motor activity since ablation of striatonigral neurons leads to a dramatic and persistent locomotor hypoactivity. Motor skill learning requires repetitive training during which performance typically shows initial fast improvements, followed by slower ameliorations to reach a plateau that represent progressive skill automatisation (Luft et al., 2005). We showed that during a motor skill task on an accelerated rotarod, D_2R-striatopallidal neurons were required only for the initial learning and not for the later phase whereas animals lacking D_1R-striatonigral neurons were fully unable to acquire the task (Durieux et al., submitted, 2010). Moreover, by ablating each specific subpopulation after an extensive training, we demonstrated that D_2R-striatopallidal neurons can be ablated without any perturbation of the learned task whereas D_1R-striatonigral neurons are still needed to perform this well-known task (Durieux et al., submitted, 2010). Furthermore, it was recently demonstrated that different subparts of the striatum are engaged in different phases of the motor learning (Yim et al., 2009). Again,

the specific involvement in motor learning phases of striatopallidal and striatonigral neurons in the different striatal sectors is completely unknown. We have successfully designed new protocols allowing to ablate these different neuron populations restrictedly in the internal or external parts of caudate-putamen in our different transgenic lines. Preliminary behavioural data on locomotor activity and motor learning showed major differences between these groups of mice.

1.b Specific inactivation of NR1 and cdk5 in striatopallidal neurons

The NMDA receptors seem to play a key role in reward and drug addiction (ventral striatum) and in motor learning (caudate-putamen). Moreover, NMDA receptors are supposed to be involved in synaptic plasticity at the cortico-striatal and cortico-accumbal synapses (LTP and LTD). Synaptic plasticity in these areas is strongly suggested to be the basis for motor learning and addiction. An important question is therefore to understand the specific roles of this receptor in each striatal subpopulation by the selective inactivation of this gene in these populations of neurons. The NR1 floxed ($NR1^{f/f}$) mice allowing a conditional inactivation of NR1 by the Cre recombinase have been double-crossed with our "striatopallidal-Cre" - $A_{2A}R$-Cre mice to obtain homozygous mice. The characterization of $A_{2A}R$-Cre/+ $NR1^{f/f}$ mice showed a selective decrease in NMDA receptor binding in the caudate-putamen and accumbens nucleus as compared to the cerebral cortex. Behavioural analysis showed that mice deficient in NMDA receptors in D_2R-striatopallidal neurons exhibit a hyperlocomotor activity similar to the one observed in mice without striatopallidal neurons (see above point 1.a). Moreover, evaluation in their ability to learn specific motor tasks by using two different tests showed that they exhibited a clear deficit in motor learning. The roles of these receptors in striatopallidal neurons in drug reinforcement have been also evaluated and preliminary results need to be confirmed. On the other hand, electrophysiological characterization of the glutamatergic cortico-striatal synaptic transmission in brain slices from these mice is still running.

Cdk5 is a kinase that is involved in intracellular signalling cascades leading to neuroadaptation and synaptic plasticity and in drug addiction (Bibb et al., 2001; Takahashi et al., 2005). Since its role in specific striatal populations and its involvement in the normal motor learning process or in striatal neuroadaptation in motor diseases are undetermined, we have similarly produced $A_{2A}R$-Cre/+ $cdk5^{f/f}$ mice in order to specifically inactivate this gene in D_2R-striatopallidal neurons. By using immunocytochemical co-detection of cdk5 and enkephalin as a marker of striatopallidal neurons, we demonstrated that the cdk5 locus recombination occurs in a proportion of these neurons. Indeed, co-labelling was detected in 100% of striatopallidal neurons in control mice and in 72,3 % in $A_{2A}R$-Cre/+ $cdk5^{f/f}$ mice meaning that cdk5 gene was inactivated in 27,7% of striatopallidal neurons. A series of behavioural testing for spontaneous locomotor activity (open field), skill learning (accelerated rotarod, runway test) and anxiety behaviour (elevated plus maze) has been realized and did not show significant difference between control mice and mice deficient in cdk5 in D_2R-striatopallidal neurons. Furthermore, to test for the potential involvement of this kinase in D_2R-striatopallidal neurons in drug reinforcement, a drug sensitization protocol was conducted. This showed that both genotypes exhibited a similar acute increase in locomotor activity following the first ampheatmine injection and also exhibited a similar sensitization at the fifth day of chronic treatment. Altogether, these preliminary results could suggest that recombination in about 30% of striatopallidal neurons is not sufficient to lead to a behavioural phenotype. To overcome this problem, we are developing a new strain, cdk5 Δ/flox where only one copy of cdk5 has to be recombined.

1.c Gene profiling of striatonigral and striatopallidal neurons and characterization of striatopallidal neuron-specific genes

The striatopallidal-GFP mice (see point 1.a) have been used as a tool to specifically isolate this population of striatal neurons by using dissociation and sorting by FACS and to specifically record them by using the patch clamp technique. We succeed in isolating green (striatopallidal) and red (striatonigral) neurons from individual mice with a very good rate of enrichment. In this frame, our aim was to establish the gene expression profiles of these different populations by microarrays. RNA amplification and identification was performed to validate the differential expression of some known genes (i.e. enkephalin, substance P, A_{2A} receptor) showing that we have an enrichment of more than 90%. We have identified a series of genes, which showed high differential expression in the striatopallidal neurons with ratio up to 100:1. This differential gene expression has been validated by using different techniques for a dozen of genes. Among these genes, we have selected genes as RGS5, IGFBP7, GuaCy13A, Adk that exhibit both a high differential expression and a putative physiological relevance for further analysis using different knock down strategies. One of these, RGS5, is a member of the large "Regulator of G Protein Signalling" family. It negatively regulates some G proteins and inhibit the signalling of Gαi, Gαq and Gαo and has been shown to be striatally up-regulated by an acute amphetamine treatment (Burchett et al., 1999). The qPCR analysis shows that RGS5 was 33 times more expressed in striatopallidal neurons than in striatonigral neurons (Ena et al. unpublished data). We obtained full RGS5 knock-out mice through collaboration and performed the behavioural study of these mice. We did not find any alteration in spontaneous locomotor activity or anxiety-like behaviour. However, motor skill learning tested on a accelerated rotarod showed significant alteration in the early phase of learning in RGS5 KO mice as compared to wild type. To test for the potential involvement of striatal RGS5 in drug reinforcement processes, drug sensitization and drug conditioned place preference protocols were performed and did not show any significant difference (Ena et al. unpublished data). It is worth to mention that looking at the expression of others RGS of the B/R4 family, we detected a 50% increase in RGS16 expression in the striatum of RGS5 KO mice that could compensate for the RGS5 function.

Other genes showing a high differential expression in D_2R-striatopallidal neurons and a putative physiological relevance have been selected. A strategy allowing a striatal or striatopallidal neuron knock down using lentivirus-mediated small harpin RNA has been developed for some of these genes. By using this strategy, we were able to demonstrate a deficit to learn a motor task on accelerating rotarod in animal devoid of one of them in striatopallidal neurons. We have therefore identified a new gene that was not previously suspected to be specifically expressed in D_2R-striatopallidal neurons and have demonstrated its central role in striatopallidal neurons for motor skill learning (Ena et al. unpublished data).

1.d Conditional targeting of nitrergic neurons

Besides the two populations of striatal efferent neurons (MSN) are four classes of interneurons. Among theses, the nitrergic, synthesizing NO, neurons have been suggested to play important roles. To gain insight to these functions, we had the objective to selectively ablated these neurons from the striatum and, hence, have started the construction of line of mice expressing the Cre recombinase under the control of the neuronal NO synthetase (nNOS) promoter (nNOS-Cre) by using the BAC strategy. Very succinctly, following steps of this construction have been realized: cloning of the Cre-recombinase in pbsk vector, cloning of the Cre-recombinase-3XSTOP-frt-Néo-frt construct in the BAC RP24-164C18, micro-injection of the recombined BAC. Five founders have been obtained and have been crossed with reporter mice Rosa26-LacZ in order to identify the sites of recombination events. Unfortunately, the neuroanatomical characterization of these five lines of mice by co-detection of Lac-Z and Neuropeptide Y (NPY, a marker of nitrergic neurons) showed that there was no full co-expression of both markers

since some Lac-Z-positive neurons were not NPY-positive and some NPY-positive neurons were not Lac-Z-positive. This means that our transgenic strains do not fully recapitulate the nitric oxide synthase distribution pattern and, hence, do not allow to target these nitrergic neurons.

2. Regulation of striatal neurons excitability and corticostriatal synaptic transmission

Striatal neurons (MSN) excitability and corticostriatal synaptic transmission are tightly regulated both by a large series of neurotransmitters among which dopamine plays central role in learning rules and adenosine is a major neuromodulator, as well as through specific intrinsic somato-dendritic conductances that shape their responses. We previously studied the modulation of the glutamatergic corticostriatal transmission and plasticity by A_{2A} receptor (D'Alcantara et al., 2001 and reviewed in Schiffmann et al., 2007; Ferré et al., 2008).

2a. Regulation of striatal neurons excitability by D_2 and A_{2A} receptors

We have studied the mechanisms of the modulation exerted by D_2 and A_{2A} receptors on the MSN excitability (Azdad et al., 2009a). By using the perforated patch configuration of the patch clamp technique in combination with peptide occlusion protocols, we showed that dopamine D_2 receptor activation abolished the NMDA-induced down- to up-state transitions and hence striatal neurons excitability. Peptide occlusion showed that this effect occurs through a pathway involving a specific subtype of calcium channels (CaV1.3). This D_2R-mediated effect is fully reversed by co-stimulation of A_{2A} receptor although activation of the A_{2A} receptor is unable to modify the down- to up-state transitions (Azdad et al., 2009a). Such effect was shown to be specific of the striatopallidal neuron population since it was only detected on GFP-expressing neurons in D_2R-GFP mice. This suggested that the action of A_{2A} receptor activation was completely or partially due to an intramembrane interaction such as D_2-A_{2A} heteromerization rather than to activation of an intracellular cascade. Experiments have been performed to test this hypothesis by using occlusion by specific competitive peptides blocking A_{2A}-D_2 heteromerization. We showed that these peptides fully blocked the ability of A_{2A} receptor activation to counteract the D_2 effect demonstrating the involvement of D_2-A_{2A} heteromerization in this modulation. Not only our results demonstrated a strong D_2R-A_{2A}R antagonistic regulation of MSN excitability but they also supported for the first time in a physiological condition, the functional relevance of this heteromerization (Azdad et al., 2009a; Ferré et al., 2008). In the same line, we have also showed that MSN intrinsic excitability is antagonistically regulated by D_2 and A_{2A} receptors through an additional mechanism involving the modulation of an inactivating A-type potassium current, I_A (Azdad et al., unpublished data).

2b. Homeostatic regulation of striatal neurons excitability in case of dopamine depletion

Dopamine has been previously shown to exert a variety of electrophysiological effects in MSN including the modulation of intrinsic conductances (i.e. see above) and the involvement in different types of corticostriatal synaptic plasticity. However, despite series of available data the effects resulting from dopamine depletion on the MSN intrinsic excitability remained incompletely documented and puzzling. To gain insight on these effects, we studied the alterations in MSN excitability and corticostriatal synaptic transmission in hypodopaminergic conditions mimicking Parkinson's disease (Azdad et al., 2009b). We showed, by performing perforated patch clamp recordings on brain slices, that dopamine depletion leads to an increase in MSN intrinsic excitability through the decrease of an inactivating A-type potassium current, I_A. Despite the large decrease in their excitatory synaptic inputs determined by the decreased dendritic spines density and the increase in minimal current to evoke the first EPSP, this increase

in intrinsic excitability resulted in an enhanced responsiveness to their remaining synapses, allowing them to fire similarly or more efficiently following input stimulation than in control condition (Azdad et al., 2009b). Therefore, this increase in intrinsic excitability through the regulation of I_A represents a form of homeostatic plasticity allowing neurons to compensate for perturbations in synaptic transmission and to promote stability in firing. Such homeostatic plasticity has been demonstrated in a variety of physiological conditions such as memory storage or activity-dependent development in order to adjust synaptic strengths and/or intrinsic excitability to promote stability but its existence and nature in pathological conditions were mostly unknown. Our observations (Azdad et al., 2009b) showed that this homeostatic ability to maintain firing rates within functional range also occurs in pathological conditions, allowing stabilizing neural computation within affected neuronal networks.

2c. Neuronal excitability of striatal fast-spiking interneurons deficient in parvalbumin

The striatal fast-spiking interneurons (FSI) maintain high frequency trains of action potentials and represent a powerful feedforward inhibition on striatal MSN or projection neurons, orchestrating their fine spike timing. Interestingly, recent studies have provided evidence on possible implications of FSI in Tourette syndrome and parkinsonism. These GABAergic interneurons express high level of the calcium binding protein parvalbumin (PV) that is supposed to play a key role in the regulation of intracellular calcium dynamics We investigated the role of PV on the electroresponsiveness of FSI by using the perforated patch configuration of the patch-clamp technique on control (PV-GFP) mice and PV-defcient (PV-GFP/PV$^{-/-}$) mice. We showed that the intrinsic FSI excitability was significantly increased in PV$^{-/-}$ mice and that the maximal spiking frequencies are also higher in FSI deficient in PV. Altogether, these results showed that the absence in PV lead to an increased intrinsic excitability of the FSI. We are also analysing the cortical synaptic input to FSI to evaluate whether their altered excitability would lead to dysfunctional response to this afference. Finally, in order to evaluate the consequences of these alterations on the striatal microcircuit, double patch experiments are also performed to analyse the synaptic transmission and short term plasticity of the FSI to MSN connection. Preliminary results suggest that absence of PV in FSI results in alterations in short term plasticity at the FSI to MSN synapse.

3. Stem cell graft in models of Huntington's disease

We have studied the migration and homing processes of neural stem cells (NSC) and mesenchymal stem cells (MSC) following their intracerebral grafts in vivo, in a rat model of Huntington's disease. We showed that both stem cells (NSC and MSC) exhibit a migration pattern significantly different in the degenerative condition as compared to intact brain (Bantubungi et al., 2008). Our results demonstrated that the host environment determine the final localisation of grated cells and hence their homing. We have identified a molecular mechanism involved in this homing process as the "Stem Cell Factor (SCF) – Kit receptor" system (Bantubungi et al., 2008). Indeed, we demonstrated that SCF expression is highly upregulated in the lesioned striatum as compared to the intact side and showed that NSC and MSC express the Kit receptor. Different technical approaches in vitro demonstrated that this SCF-Kit, ligand-receptor, system is functional in both cell types since recombinant SCF as well as protein extract from the lesioned striatum, increased phosphorylation of Kit and Erk, increased cell proliferation and cell migration. In vivo studies using either a blocking Kit antibody or Kit-deficient mice are currently running in order to demonstrate in situ the functional role of the SCF – Kit receptor system in these migration and homing processes.

4. Collaborations based on expertise developed under the frame of this program.

- We characterized the distribution of the basal ganglia-specific synaptic protein SV2C by showing that it is highly expressed in dopaminergic neurons, in striatal cholinergic interneurons and, at a moderate level, in both MSN subpopulations (Dardou et al., 2010).
- We contributed to the characterization of an in vivo mouse model of maturation of “neocortex isolé” by showing that the excitability of pyramidal neurons in these mutant mice, measured in vitro in patch clamp experiments, was dramatically decreased (Zhou et al., 2010).
- We took part in the characterization of cortical neurons generated from embryonic stem cells by showing an intrinsic excitability and functional synaptic transmission in these cells (Gaspard et al., 2008).
- We participated to the functional characterization of the phosphoinositide 5-phosphatase Inpp5e deficiency who lead to brain development disorder due to the absence of this enzyme in the primary cilium (Jacoby et al., 2009).

References

- Azdad K, Gall D, Woods A., Ferré S. and Schiffmann S.N. Dopamine D_2 and adenosine A_{2A} receptors regulate membrane plateau potential induction in striatal neurons through A_{2A}-D_2 receptor heteromerization. Neuropsychopharmacology, 34: 972-986, 2009a.
- Azdad K., Chavez M., Bishop D.P., Wetzelear P., Marescau B., De Deyn P.P., Gall D. and Schiffmann S.N. Homesotatic plasticity of striatal neurons intrinsic excitability following dopamine depletion. PLoS ONE 4(9): e6908, 2009b.
- Bantubungi K, Blum D, Cuvelier L, Wislet-Gendebien S, Rogister B, Brouillet E and Schiffmann S.N.: Stem Cell Factor and mesenchymal and neural stem cell transplantation in a rat model of Huntington's disease. Mol. Cell. Neurosci., 37, 454-470, 2008.
- Bibb, J.A., Chen, J., Taylor, J.R., Svenningsson, P., Nishi, A., Snyder, G.L., Yan, Z., Sagawa, Z.K., Ouimet, C.C., Nairn, A.C., Nestler, E.J., and Greengard, P. Effects of chronic exposure to cocaine are regulated by the neuronal protein Cdk5. Nature 410, 376-380, 2001.
- Burchett SA, Bannon MJ, Granneman JG. RGS mRNA expression in rat striatum: modulation by dopamine receptors and effects of repeated amphetamine administration. J Neurochem 72:1529-1533, 1999.
- D'Alcantara, P., Ledent, C., Swillens, S., and Schiffmann, S.N. Inactivation of adenosine A(2A) receptor impairs long term potentiation in the accumbens nucleus without altering basal synaptic transmission. Neuroscience 107, 455-464, 2001.
- Dardou D., Dassesse D., Cuvelier L., Deprez T., De Ryck M. and S.N. Schiffmann. Distribution of SV2C mRNA and protein expression in the mouse brain with a particular emphasis on the basal ganglia system. Brain Res., 1367, 130-145, 2010.
- de Kerchove d'Exaerde, A., Bearzatto, B., Ena, S., Houtteman, D., Cuvelier, L., Monlezun, S., and Schiffmann, S.N. Targeting of Cre-mediated recombination to striatopallidal neurons using BAC transgenesis, Soc. Neurosci. Abstract 450.28, 2006.
- Durieux P.F., Bearzatto B, Guiduccci S., Buch T, Waisman A, Zoli, M., Schiffmann S.N. and de Kerchove d'Exaerde A: Striatopallidal neurons inhibit both locomotor and drug reward processes. Nature Neuroscience, 12: 393-395, 2009 (Note: S.N. Schiffmann and A. de Kerchove d'Exaerde contributed equally to this study).
- Ferré S. , Quiroz C., Woods A.S., Cunha R., Popoli P., Ciruela F., Lluis C., Franco R., Azdad K. and Schiffmann S. N. An Update on Adenosine A2A-Dopamine D2 Receptor Interactions: Implications for the Function of G Protein-Coupled Receptors. Current Pharmaceutical Design, 14, 1468-1474, 2008.
- Gaspard N, Bouschet T., Hourez R, Dimidschtein J., Naeije G, VAn den ameele J, Espuny-Camacho I., Herpeol A., Passante L, S.N. Schiffmann, Gaillard A., Vanderhaeghen P. An intrinsic mechanism of corticogenesis from embryonic stem cells. Nature, 455: 351-358., 2008.
- Gong, S., Doughty, M., Harbaugh, C.R., Cummins, A., Hatten, M.E., Heintz, N., and Gerfen, C.R. (2007). Targeting Cre recombinase to specific neuron populations with bacterial artificial chromosome constructs. J Neurosci. 27, 9817-9823.
- Jacoby M., Cox J. J., Gayral S, Hampshire D. J., Ayub M., Blockmans M., Pernot E., Kisseleva M. V., Compère P., Schiffmann S. N., Gergely F., Riley J. H., Pérez-Morga D., Woods C. G. and Schurmans S. INPP5E mutations cause primary cilium signalling defects, cilial instability and ciliopathies in man and mouse. Nature Genetics, 41: 1027-1031, 2009.
- Luft, A.R., and Buitrago, M.M. (2005). Stages of motor skill learning. Mol. Neurobiol. 32, 205–216.
- Novak, A., Guo, C., Yang, W., Nagy, A., and Lobe, C.G. Z/EG, a double reporter mouse line that expresses enhanced green fluorescent protein upon Cre-mediated excision. Genesis 28, 147-55, 2000.
- Schiffmann S.N., Fisone G., Moresco R., Cunha R., Ferré S.: Adenosine A2A receptors and basal ganglia physiology. Prog. Neurobiol., 83: 277-292, 2007.
- Soriano, P. Generalized lacZ expression with the ROSA26 Cre reporter strain. Nat Genet. 21, 70-71, 1999.
- Takahashi, S., Ohshima, T., Cho, A., Sreenath, T., Iadarola, M.J., Pant, H.C., Kim, Y., Nairn, A.C., Brady, R.O., Greengard, P., and Kulkarni, A.B. Increased activity of cyclin-dependent kinase 5 leads to attenuation of cocaine-mediated dopamine signaling. Proc.Natl.Acad.Sci.U.S.A 102, 1737-1742, 2005.
- Yim HH et al., Dynamic reorganization of striatal circuits during the acquisition and consolidation of a skill. Nature Neuroscience 12, 333-341, 2009.
- Zhou L., D. Gall, Y. Qu, C. Prigogine, G. Cheron, F. Tissir, S.N. Schiffmann and A.M. Goffinet. Maturation of "neocortex isolé" in vivo in mice. J. Neurosci., 30: 7928-7939, 2010.

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- Azdad K, Gall D, Woods A., Ferré S. and Schiffmann S.N. Dopamine D_2 and adenosine A_{2A} receptors regulate membrane plateau potential induction in striatal neurons through A_{2A}-D_2 receptor heteromerization. Neuropsychopharmacology, 34: 972-986, 2009a.
- Azdad K., Chavez M., Bishop D.P., Wetzelear P., Marescau B., De Deyn P.P., Gall D. and Schiffmann S.N. Homesotatic plasticity of striatal neurons intrinsic excitability following dopamine depletion. PLoS ONE 4(9): e6908, 2009b.
- Bantubungi K, Blum D, Cuvelier L, Wislet-Gendebien S, Rogister B, Brouillet E and Schiffmann S.N.: Stem Cell Factor and mesenchymal and neural stem cell transplantation in a rat model of Huntington's disease. Mol. Cell. Neurosci., 37, 454-470, 2008.
- Dardou D., Dassesse D., Cuvelier L., Deprez T., De Ryck M. and S.N. Schiffmann. Distribution of SV2C mRNA and protein expression in the mouse brain with a particular emphasis on the basal ganglia system. Brain Res., 1367, 130-145, 2010.
- Durieux P.F., Bearzatto B, Guiduccci S., Buch T, Waisman A, Zoli, M., Schiffmann S.N. and de Kerchove d'Exaerde A: Striatopallidal neurons inhibit both locomotor and drug reward processes. Nature Neuroscience, 12: 393-395, 2009 (Note: S.N. Schiffmann and A. de Kerchove d'Exaerde contributed equally to this study).
- Ferré S. , Quiroz C., Woods A.S., Cunha R., Popoli P., Ciruela F., Lluis C., Franco R., Azdad K. and Schiffmann S. N. An Update on Adenosine A2A-Dopamine D2 Receptor Interactions: Implications for the Function of G Protein-Coupled Receptors. Current Pharmaceutical Design, 14, 1468-1474, 2008.
- Gaspard N, Bouschet T., Hourez R, Dimidschtein J., Naeije G, VAn den ameele J, Espuny-Camacho I., Herpeol A., Passante L, S.N. Schiffmann, Gaillard A., Vanderhaeghen P. An intrinsic mechanism of corticogenesis from embryonic stem cells. Nature, 455: 351-358., 2008.
- Jacoby M., Cox J. J., Gayral S, Hampshire D. J., Ayub M., Blockmans M., Pernot E., Kisseleva M. V., Compère P., Schiffmann S. N., Gergely F., Riley J. H., Pérez-Morga D., Woods C. G. and Schurmans S. INPP5E mutations cause primary cilium signalling defects, cilial instability and ciliopathies in man and mouse. Nature Genetics, 41: 1027-1031, 2009.
- Zhou L., D. Gall, Y. Qu, C. Prigogine, G. Cheron, F. Tissir, S.N. Schiffmann and A.M. Goffinet. Maturation of "neocortex isolé" in vivo in mice. J. Neurosci., 30: 7928-7939, 2010.

Final report of the research group of

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Molecular genetics and biology of Charcot-Marie-Tooth neuropathies

1. Research report:

Charcot-Marie-Tooth (CMT) disease is the most common hereditary neuromuscular disorder with a prevalence of 1 in 2500 (this represents ~4400 CMT patients in Belgium). CMT results in progressive weakness and wasting of foot and hand muscles, and distal sensory loss. Most patients need walking aids and some become wheelchair dependent even at a young age. The disease is caused by a length-dependent degeneration of the peripheral nerves. Currently, over 800 different pathogenic mutations in >40 disease-associated genes have been identified for CMT and closely related diseases. Half of these genes are involved in the myelination and maintenance of the peripheral nerve, and were therefore obvious candidate genes for CMT.

From 2008 to 2010 we obtained funding from the GSKE to study the molecular genetics and biology of CMT. In the past 3 years, we studied ubiquitously expressed genes, with very basic tasks in the cell, that nevertheless cause specific peripheral nerve degeneration. We implemented innovative molecular approaches to find novel disease causing genes, and developed strategies to study the “not-obvious” CMT genes we recently identified. We aimed to make the transition of classic molecular genetic strategies to more integrated functional studies, and to understand the effect of mutations causing degeneration of the peripheral nervous system (PNS). This required the development and use of cellular and animal models for CMT. We achieved this with a team of researchers having interests in high throughput molecular genetics, development of small model organisms, knowledge of neurobiology and with ambitions to contribute to the development of therapeutic approaches. Our team was in the privilege position to have a permanent cross link with clinical researchers, so our lab findings were continually correlated with what is found in patients. This translational research line will result in the identification of novel genes and targets for therapeutic intervention. In addition, our model organisms were developed to be usable in the screening of drug candidates.

Our main achievements were the identification of mutations in 3 novel disease associated genes: the copper transporter *ATP7A* for the X-linked type distal motor neuropathy (Kennerson et al. 2010), the *FAM134B* gene coding an uncharacterized protein of the trans-Golgi-network for a recessive ulcero-mutilating sensory neuropathy (Kurth et al. 2009), and very recently the *SPTLC2* gene coding a subunit for the serine palmitoyltransferase for hereditary sensory and autonomic neuropathy (Rotthier et al. 2010). The latter *SPTLC2* gene resulted in a patent application that we filed in September 2010. We performed genotype-phenotype correlations in large cohorts of patients affected with hereditary sensory and autonomic neuropathies (HSAN; Rotthier and Baets et al. 2009) and distal hereditary motor neuropathies (distal HMN; Dierick et al. 2008). In addition, we defined the phenotypic spectrum of dynamin (*DNM2*) and frabin (*FGD4*) mutations in CMT neuropathies (Claeys et al. 2009 and Fabrizi et al. 2009). We also determined that dominant mutations in the cation channel *TRPV4* cause an unusual spectrum of peripheral neuropathies (Zimon et al. 2010). We modeled dominant mutations in the tyrosyl-tRNA synthetase *YARS* in *Drosophila* with the aim to study the pathomechanisms of dominant intermediate CMT neuropathy (Storkebaum and Gonçalves et al. 2009). We found that increased monomerization of mutant small heat shock protein (*HSPB1*) leads to protein hyperactivity in distal HMN and axonal CMT neuropathy (Almeida-Souza et al. 2010). And we recently showed that mutant HSPB8

causes motor neuron specific neurite degeneration in axonal CMT neuropathy (Irobi et al. 2010). Other relevant publications in frame of the GSKE project can be found in our publication list. Finally, two PhD students successfully graduated during this GSKE project in December 2010: Annelies Rotthier and Ricardo Gonçalves.

2. Research Activities:

Publication List

Articles in International Journals – Acknowledging GSKE

- Burgunder,J-M., Schöls,L., Baets,J., Andersen,P.M., Gasser,T., Szolnoki,Z., Fontaine,B., Van Broeckhoven,C., Di Donato,S., **De Jonghe,P.**, Lynch,A., Mariotti,C., Spinazzola,A., Tabrizi,S.J., Tallaksen,C., Zeviani,M., Harbo,H.F., Finsterer,J.: EFNS guidelines for the molecular diagnosis of neurogenetic disorders: motoneuron, peripheral nerve and muscle disorders. European Journal of Neurology (2010) Epub: 25-Mar-2010 (PMID: 20500522) (I.F.: 2.732)
- Rotthier,A., Auer-Grumbach,M., Janssens,K., Baets,J., Penno,A., Almeida-Souza,L., Van Hoof,K., Jacobs,A., De Vriendt,E., Schlotter-Weigel,B., Löscher,W., Vondracek,P., Seeman,P., **De Jonghe,P.**, Van Dijck,P., Jordanova,A., Hornemann,T., Timmerman,V.: Mutations in the SPTLC2 subunit of serine palmitoyltransferase cause hereditary sensory and autonomic neuropathy type I. American Journal of Human Genetics 87(4): 513-522 (2010) (PMID: 20920666) (I.F.: 12.303)
- Appenzeller,S., Schirmacher,A., Halfter,H., Bäumer,S., Pendziwiat,M., **Timmerman,V.**, **De Jonghe,P.**, Fekete,K., Stögbauer,F., Lüdemann,P., Hund-Georgiadis,M., Quabius,E.S., Ringelstein,B., Kuhlenbäumer,G.: Autosomal-dominant striatal degenerationis caused by a mutation in the phosphodiesterase 8B gene. American Journal of Human Genetics 86(1): 83-87 (2010) (PMID: 20085714) (I.F.: 10.153)
- Kennerson,M., Nicholson,G., Kowalski,B., Chu,S., Takata,R., Speck-Martins,C., Baets,J., de almeida Souza,L., Fischer,D.F., **Timmerman,V.**, Kaler,S., Tang,J., Llanos,R., Taylor,P., Mercer,J., Bird,T., **De Jonghe,P.**, Feely,S.M.E., Shy,M., Garbern,J.: Missense mutations in the copper transporter gene ATP7A cause X-linked distal hereditary motor neuropathy. American Journal of Human Genetics 86(3): 343-352 (2010) (PMID: 20170900) (I.F.: 10.153)
- Zimon,M.*, Baets,J.*, Auer-Grumbach,M., Berciano,J., Garcia,A., Lopez-Laso,E., Merlini,L., Hilton-Jones,D., McEntagart,M., Barisic,N., Barisic,N., Boltshauser,E., Shaw,C.E., Landouré,G., Ludlow,C.L., Gaudet,R., Houlden,H., Reilly,M., Fischbeck,K., Sumner,C.J., **Timmerman,V.**, Jordanova,A., **De Jonghe,P.**: Dominant mutations in the cation channel gene transient receptor potential vanilloid 4 cause an unusual spectrum of neuropathies. Brain 133(Pt 6): 1798-1809 (2010) (PMID: 20460441) (I.F.: 9.603) * equal contribution for the first authors.
- Irobi,J., de almeida Souza,L., Asselbergh,B., De Winter,V., Goethals,S., Dierick,I., Krishnan,J., Timmermans,J-P., Robberecht,W., **De Jonghe,P.**, Van Den Bosch,L., Janssens,S., **Timmerman,V.**: Mutant HSPB8 causes motor neuron specific neurite degeneration. Human Molecular Genetics 19(16): 3254-3265 (2010) (PMID: 20538880) (I.F.: 7.386)
- Baets,J., Deconinck,T, Smets,K., Goossens,D., Van Den Berg,P., Dahan,K., Schmedding,E., Santens,P., Rasic,V.M., Van Damme,P., Robberecht,W., De Meirleir,L., Michiels,B., Del-Favero,J., Jordanova,A., **De Jonghe,P.**: Mutations in SACS cause atypical and late-onset forms of ARSACS. Neurology 75(13): 1181-1187 (2010) (PMID: 20876471) (I.F.: 7.043)
- Almeida-Souza,L., Goethals,S., De Winter,V., Dierick,I., Gallardo,R., Van Durme,J., Irobi,J., Gettemans,J., Rousseau,F., Schymkowitz,J., **Timmerman,V.**, Janssens,S.: Increased monomerization of mutant HSPB1 leads to protein hyperactivity in CMT neuropathy. Journal of Biological Chemistry 285-17: 12778-12786 (2010) (PMID: 20178975) (I.F.: 5.52)
- Kabzinska,D., Strugalska-Cynowska,H., Kostera-Pruszczyk,A., Ryniewicz,B., Posmyk,R., Midro,A., Seeman,P., Báranková,L., Zimon,M., Baets,J., **Timmerman,V.**, Guergueltcheva,V., Tournev,I., Sarafov,S., **De Jonghe,P.**, Jordanova,A., Hausmanowa-Petrusewicz,I., Kochañski,A.: L239F founder mutation in GDAP1 is associated with a mild Charcot-Marie-Tooth type 4C4 (CMT4C4) phenotype. Neurogenetics 11(3): 357-366 (2010) (PMID: 20232219) (I.F.: 3.0)
- Rotthier,A*., Baets,J.*, De Vriendt,E., Jacobs,A., Auer-Grumbach,M., Levy,N., Bonello-Palot,N., Kilic,S.S., Weis,J., Nascimento,A., Swinkels,M., Kruyt,M., Jordanova,A., **De Jonghe,P.**, **Timmerman,V.**: Genes for hereditary sensory and autonomic neuropathies: a genotype-phenotype correlation. Brain 132(Pt 10): 2699-2711 (2009) (PMID: 19651702) (I.F.: 9.603) * equal contribution for the first authors.
- Claeys,K., Züchner,S., Kennerson,M., Berciano,J., García,A., Verhoeven,K., Storey,E., Merory,J., Bienfait,H.M.E., Lammens,M., Nelis,E., Baets,J., De Vriendt,E., Berneman,Z., De Veuster,I., Vance,J., Nicholson,G., **Timmerman,V.**, **De Jonghe,P.**: Phenotypic spectrum of dynamin 2 mutations in Charcot-Marie-Tooth neuropathy. Brain 132(Pt7): 1741-1752 (2009) (PMID: 19502294) (I.F.: 9.603)
- Storkebaum,E., Gonçalves,R., Godenschwege,T., Nangle,L., Mejia,M., Bosmans,I., Ooms,T., Jacobs,A., Van Dijck,P., Yang,X-L, Schimmel,P., Norga,K., **Timmerman,V.**, Callaerts,P., Jordanova,A.: Dominant mutations in the tyrosyl-tRNA synthetase gene recapitulate in Drosophilafeatures of human Charcot-Marie-Tooth neuropathy. Proceedings of the

National Academy of Sciences of the USA 106(28): 11782-11787 (2009) (PMID: 19561293) (I.F.: 9.38)
- Fabrizi,G.-M., Taioli,F., Ferrari,S., Bertolasi,L., Casarotto,M., Rizzuto,N., Deconinck,T, **Timmerman,V.**, **De Jonghe,P.**: Further evidence that mutations in FRABIN/FGD4 cause Charcot-Marie-Tooth disease type 4H. Neurology 72(13): 1160-1164 (2009) (PMID: 19332693) (I.F.: 7.043)
- Sebnem,S., Ozturk,R., Sarisozen,B., Rotthier,A., Baets,J., **Timmerman,V.**: Humoral immunodeficiency in congenital insensitivity to pain with anhidrosis. Neurogenetics 10:161-165 (2009) (PMID: 19089473) (I.F.: 4.281)
- Hornemann,T., Penno,A., Richard,S., Nicholson,G., van Dijk,F.S., Rotthier,A., **Timmerman,V.**, von Eckardstein,A.: A systematic comparison of all mutations in hereditary sensory neuropathy type I (HSAN I) reveals that the G387A mutation is not disease associated. Neurogenetics 10(2): 135-143 (2009) (PMID: 19132419) (I.F.: 3.0)
- Kilic,S.S., Ozturk,R., Sarisozen,B., Rotthier,A., Baets,J., **Timmerman,V.**: Humoral immunodeficiency in congenital insensitivity to pain with anhidrosis. Neurogenetics 10(2): 161-165 (2009) (PMID: 19089473) (I.F.: 3.0)
- Baets,J., Dierick,I., Ceuterick-de Groote,C., Van Den Ende,J., Martin,J-J., Geens,K., Robberecht,W., Nelis,E., **Timmerman,V.**, **De Jonghe,P.**: Peripheral neuropathy and 46XY gonadal dysgenesis: a heterogeneous entity. Neuromuscular Disorders 19(2): 172-175 (2009) (PMID: 19167223) (I.F.: 2.932)
- Gallardo,E., García,A., Ramòn,C., Maraví,E., Infante,J., Gastón,I., Alonso,A., Combarros,O., **De Jonghe,P.**, Berciano,J.: Charcot-Marie-Tooth disease type 2J with MPZ Thr124Met mutation: Clinico-electrophysiological and MRI study of a family. Journal of Neurology 256: 2061-2071 (2009) (PMID: 19629567) (I.F.: 2.536)
- Dierick,I.*, Baets,J.*, Irobi,J., Jacobs,A., De Vriendt,E., Deconinck,T, Merlini,L., Van den Bergh,P., Milic-Rasic,V., Robberecht,W., Fischer,D.F., Juntas Morales,R., Mitrovic,Z., Seeman,P., Mazanec,R., Kochañski,A., Jordanova,A., Auer-Grumbach,M., Helderman-van den Enden,A.T.J.M., Wokke,J.J.H., Nelis,E., **De Jonghe,P.**, **Timmerman,V.**: Relative contribution of mutations in genes for autosomal dominant distal hereditary motor neuropathies: a genotype - phenotype correlation study. Brain 131(Pt 5): 1217-1227 (2008) (PMID: 18325928) (I.F.: 9.603) * equal contribution for the first authors.
- Gallardo,E., Claeys,K.G., Nelis,E., García,A., Canga,A., Combarros,O., **Timmerman,V.**, **De Jonghe,P.**, Berciano,J.: Magnetic resonance imaging findings of leg musculature in Charcot-Marie-Tooth disease type 2 due to dynamin 2 mutation. Journal of Neurology 255(7): 986-992 (2008) (PMID: 18560793) (I.F.: 2.536)
- Barisic,N., Claeys,K., Sirotkovic-Skerlev,M., Löfgren,A., Nelis,E., **De Jonghe,P.**, **Timmerman,V.**: Charcot-Marie-Tooth Disease: A Clinico-genetic Confrontation. Annals of Human Genetics 72: 416-441 (2008) (PMID: 18215208) (I.F.: 2.195)
- Auer-Grumbach,M., Fischer,C., Papic,L., John,E., Plecko,B., Bittner,R.E., Bernert,G., Pieber,T., Miltenberger,G., Schwartz,R., Windpassinger,C., Grill,F., **Timmerman,V.**, Speicher,M., Janecke,A.R.: Two Novel Mutations in the GDAP1 and PRX Genes in Early Onset Charcot-Marie-Tooth Syndrome. Neuropediatrics 39(1): 33-38 (2008) (PMID: 18504680) (I.F.: 1.216)

Articles in National Journals and Books – Acknowledging GSKE

- Irobi,J.: A molecular genetic update of inherited distal motor neuropathies. Verhandelingen - Koninklijk academie voor geneeskunde van België 70(1): 25-46 (2008)

PhD THESES

Name	Promoters	Title	Date of public defense
Annelies Rotthier	V. Timmerman	Genetic and functional characterization of mutations in two subunits of serine palmitoyltransferase and genotype-phenotype correlations for hereditary sensory and autonomic neuropathies	08/12/2010
Ricardo Gonçalves	V. Timmerman P. Callaerts A. Jordanova	Towards a better understanding of DI-CMTC, a contribution from Drosophila	21/12/2010

MSc THESES

Name	Promoters	Title	Academic Year
Kristof Van Avondt	S. Janssens	De rol van de aangeboren immuunrespons in acute perifere neuropathieën	2008
Quinten Verelst	I. Dierick	Mutatie-analyse van genen voor CMT: GDAP1 en SETX	2008
Kim Van Hoorenbeeck & Laetitia Yperzeele	P. De Jonghe	Het klinisch spectrum van erfelijke polyneuropathieën.	2008
Ben De Clerck	S. Janssens	Optimalisatie van de Proximity Ligation Assay (PLA) methode als uitleessysteem voor TLR activering	2009
Jascha Vervoort	A. Jordanova & V. Timmerman	Effect van DI-CMTC mutaties in YARS op celgroei en eiwitinteracties	2009
Veerle Smits	S. Janssens	Expressie van Nod-like receptors in het perifere en het centrale zenuwstelsel	2009
Yves Dondelinger	S. Janssens	Zoektocht naar nieuwe pathomechanismen voor CMT-geassocieerde HSPB1 en HSPB8 mutanten	2009
Jonas Busseniers	J. Irobi	Onderzoek naar het effect van mutante small heat shock proteïnen 8 en 1 op het neurofilament netwerk	2010
Evelien Fredrickx	V. Timmerman A. Bolino	Characterisation of the Mtmr2/Fig4 double knockout mouse model	2010
Kristien Peeters	A. Jordanova P. De Jonghe	Genetische koppelingsanalyse en identifivatie van nieuwe FLNC mutaties in families met distale myopathie	2010

PROFESSIONAL BSc THESES

Name	Supervisor / Co-supervisor	Title	Academic Year
Kristien Peeters	V. Timmerman	Fijnmappen van de locus voor de ziekte van Charcot-Marie-Tooth type 2G	2008
Yannick Waumans	A. Jordanova	Moleculaire karakterisatie van dominant intermediaire vorm van de ziekte van Charcot-Marie-Tooth type D: op zoek naar een gemenschappelijke voorouder	2008
Josephine Roberts	A. Jordanova J. Baets	Mutatieanalyse van PRX bij vroeg beginnende vormen van de ziekte van Charcot-Marie-Tooth	2009

Gillis Elisabeth	J. Irobi	Het effect van mutant HSPB8 en HSPB1 in endogene neurofilament assemblage	2010

PATENTS

Patent on mutations in SPTLC2 gene associated with sensory neuropathy: application n°GB1015581.0 (filed on 17/09/10).

PRIZES & AWARDS

Prizes

- Baets, J.: Pfizer Wetenschappelijke Prijs van de Faculteit Geneeskunde 2009, 29/09/2009
- Almeida-Souza, L.: EMBO Poster Prize. Awarded for outstanding poster presentation ath the EMBO/FEBS Lecture Course on 'The cytoskeleton in development and pathology, Djurhamn, Sweden, June 19-24 (2010)
- Timmerman, V. & De Jonghe, P.: Prize Baron van Gysel by the Medical Foundation Queen Elisabeth (2010)

Meeting Fellowships & Travel Awards

- Irobi, J.: Fellowship to attend an Advanced Course Symposium and Practical Training on "Lentiviral vectors: concepts, practice, hope and reality", University Evry Val d'Essonne, Evry, France, June 29-July 5 (2008)
- Gonçalves, R.: Anacor Travel Fellowship to attend the International Conference on aminoacyl-tRNA synthetases, Les Pensières, Veyrier du Lac, France, September 7-11 (2008)
- Janssens, S.: Travel Fellowship from the Fund for Scientific Research – Flanders (FWO) to attend the 9th International Congress of Neuroimmunologyn, Fort Worth, Texas, USA:, October 26-30 (2008)
- Rotthier, A.: Travel Fellowship of the Peripheral Nerve Society (PNS) to attend the 2009 Biennial PNS Meeting , Wurzburg, Germany, July 4-8, 2009
- Baets, J.: FWO travel grant to attend the 2010 Peripheral Nerve Society Meeting, Sydney, Australia, July 5 – 7 (2010)
- Baets, J.: PNS Fellowship Award to attend the 2010 Peripheral Nerve Society Meeting, Sydney, Australia, July 5 – 7 (2010)
- Zimon, M.: PNS Fellowship Award to attend the 2010 Peripheral Nerve Society Meeting, Sydney, Australia, July 5 – 7 (2010)

PRESENTATIONS

Chair and Organizational Activities - International

- Jordanova, A.: Member of the organising committee. 38th EAMDA annual conference, Sofia, Bulgaria, September 13 (2008)
- Jordanova, A.: Chair. TREAT-NMD network meeting. 38th EAMDA annual conference, Sofia, Bulgaria, September 13 (2008) Timmerman,V.: Peripheral Nerve Society Meeting: Chair Session 6 – Hereditary Neuropathies, Würzburg, Germany, July 4-8 (2009)
- Timmerman,V.: Third International Charcot-Marie-Tooth Consortium Meeting: Organizer, Antwerp, Belgium, July 9-11 (2009)
- Timmerman,V.: Third International Charcot-Marie-Tooth Consortium Meeting: Chair Session 8 – Immune mediated neuropathies and models for peripheral neuropathies, Antwerp, Belgium, July 9-11 (2009)
- De Jonghe, P.: Third International Charcot-Marie-Tooth Consortium Meeting: Organizer, Antwerp, Belgium, July 9-11 (2009)
- De Jonghe, P.: Third International Charcot-Marie-Tooth Consortium Meeting: Chair Session 5 – HSAN and new forms of neuropathies, Antwerp, Belgium, July 9-11 (2009)
- Jordanova, A.: Third International Charcot-Marie-Tooth Consortium Meeting: Chair Session 7 – Pathomechamisms of GDAP1, YARS and GARS mutations, Antwerp, Belgium, July 9-11 (2009)
- Timmerman,V.: Peripheral Nerve Society Meeting: Chair Session 6 – Hereditary Neuropathies, Würzburg, Germany, July 4-8 (2009)
- Baets, J.: chair Poster Session – Peripheral Nerve Society Satellite Meeting, Sydney, Australia, July 5 – 7 (2010)
- Timmerman, V.: European Molecular Quality Network (EMQN): CMT Best Practice Meeting to update the guidelines for diagnostics in CMT. Munich, Germany, December 13-14 (2010)

Invited Lectures - International

- Timmerman, V.: Inaugural International Neuromuscular Conference: 'Are ubiquitously expressed genes good candidates for CMT neuropathies?', UCL Institute of Child Health, London, UK, February 1-2 (2008)
- Timmerman, V.: CMT workshop at the 18th Meeting of the European Neurological Society: 'CMT genes and proteins: what we are learning for understanding peripheral neuropathies?', Nice, France, June 7-11 (2008)

- Timmerman, V.: International Symposium on Rare Diseases: 'Genetic and cell heterogeneity of Charcot-Marie-Tooth disease', Valencia, Spain, November 16-18 (2008)
- Baets, J.: Distal Hereditary Motor Neuropathies. Danish consortium for neuromuscular diseases, Korsør, Denemark, September 1 (2008)
- De Jonghe, P.: Developments in research and future treatment opportunities in CMT (HMSN). 38th EAMDA annual conference, Sofia, Bulgaria, September 13 (2008)
- Timmerman, V.: Animal models for peripheral neuropathies. EU COST B30 meeting on animal models in research on neurodegeneration and neuroplasticity, Antwerp, Belgium, March 29-30 (2009)
- Timmerman,V.: Unraveling the molecular genetics and biology of inherited peripheral neuropathies. International Symposium. Neuromuscular diseases.recent advances and translation to therapy, Madrid, Spain, May 28-29 (2009)
- De Jonghe, P.: Frequency of different types of CMT. The 168th ENMC workshop and second ENMC workshop on Charcot-Marie-Tooth: Clinical trials in Charcot-Marie-Tooth disease, Naarden, The Netherlands, September 18-20 (2009)
- Jordanova, A.: Unraveling the role of aminoacyl-tRNA synthetases in neurodegeneration: comeFly with us.Second International Conference on Aminoacyl-tRNA-Synthetases: From the Genetic Code to Human Diseases and Medicine, San Diego, CA, USA, April 1-5 (2009)
- Jordanova, A.: Role of aminoacyl-tRNA synthetases in the peripheral nervous system: lessons from Drosophila. 11th International Congress on Amino Acids, Peptides and Proteins, Workshop: Aminoacyl-tRNA synthetase in signalling and disease, Vienna, Austria, August 3-7 (2009)
- Irobi, J.: Asselbergh B. Human Disease Genetics, from identification of disease gene to functional approaches. Research Training: Axonal Transport assays in mouse motor neuron cultures, with Prof. Giampietro Schiavo, Cancer Research UK London Research Institute Lincolns Inn Fields Laboratories, 15-17 March (2010)
- Timmerman, V.: Mutant small heat shock proteins and developing their models for peripheral nerve degeneration. European Metabolic Group Meeting, Lisbon, Portugal, July 4-6 (2010)
- Timmerman, V.: Mutant small heat shock proteins and mutant SPT developing their models for peripheral nerve degeneration. Workshop on Disease Genetics, FP7-REGPOT project, Bogazici University, Istanbul October 15-17 (2010).
- Timmerman, V.: small HSP's and CMT. North-American CMT association international workshop on defining therapeutic approaches to CMT2, San Diego, CA, November 10-12 (2010).

Invited Lectures - National

- Timmerman, V.: Nationale Studie- en Contactdag van CMT België v.z.w: 'Overzicht van het CMT onderzoek', Antwerpen, Belgium, November 8 (2008)
- Jordanova, A.: Current trends in human molecular genetics: look to the future but learn from the past. VIB Seminar 2008, Blankenberge, Belgium, March 6 (2008)
- Gonçalves, R.: Introducing flies to Charcot-Marie-Tooth disease, the YARS example. VIB Science Club Neurodegenerative Diseases II, Provinciehuis Leuven, Belgium, October 30 (2009)
- Janssens, S.: Possible involvement of TLRs in JNK expression upon acute neurodegeneration, University College London (Laboratory of Prof. Jessen and Prof. Mirsky), UK, August 6 (2009)
- Timmerman,V.: The molecular variability of inherited peripheral neuropathies, Deutschen Gesellshaft für Muskelkranke. German Society for Muscle Disease in Darmstadt, Darmstadt, Germandy, June 4-7 (2009)
- De Jonghe, P.: Seminar at the University of Kiel, Department of Neurology, Germany, June 10 (2009)
- Jordanova, A.: Unraveling the role of aminoacyl-tRNA synthetases in neurodegeneration: come fly with us. Seminar at the Max-Planck-Institute, University of Göttingen, Germany, February 26-27 (2009)
- Jordanova, A.: Genetic studies in Gypsies – the Bulgarian experience. Seminar at the Department of Medical Genetics, VUB, Brussels, Belgium, April 2 (2009)
- Jordanova, A.: Genetische studies in de zigeunerpopulatie: ervaringen uit Bulgarije ideeën voor België. Integratiecentrum Foyer, Brussels, Belgium, September 9 (2009)
- Timmerman, V.: La Génétique Fondamentale & le Diagnostic. Congres CMT-France, Marseille, France, March 27 (2010)
- De Jonghe, P.: Guidelines for the molecular diagnosis of Charcot-Marie-Tooth disease. 10Th Anniversary Symposium: Progress in Neuromuscular Disorders. Centre de Référence Neuromusculaire UCL Saint-Luc, Belgium, November 20 (2010)

Oral Presentations – Slide Sessions - International

- Holmgren, A.: Mutant HSPB8 and HSPB1 impairs formation of stable neurofilament network, International Symposium on Rare Diseases, Valencia, Spain, November 16-18 (2008)
- Gonçalves, R.: From YARS mutations to a peripheral neuropathy, a flying perspective. International Conference on Aminoacyl-tRNA synthetases: From Basic Mechanisms to System Biology; Les Pensieres, Lac Annecy France; September 7-11 (2008)

- Timmerman, V.: Phenotypic characterisation of Charcot-Marire-Tooth disease type 2 associated to a novel dynamin 2 mutation. 18th Meeting of the European Neurological Society, Nice, France, June 7-11 (2008)
- Almeida Souza, L.: How do CMT-related mutations in HSPB1 affect its biochemical properties? Third International Charcot-Marie-Tooth Consortium Meeting, Antwerp, Belgium, July 9-11: Oral (2009)
- Gonçalves, R.: Towards a better understanding of DI-CMTC, a contribution from Drosophila. Third International Charcot-Marie-Tooth Consortium Meeting, Antwerp, Belgium, July 9-11: Oral (2009)
- Irobi, J.: A targeted research effort to meet the challenges for Water, Food Security and Better Health for Africa, EU-Africa strategic partnership, European commission, Conference Centre "Albert Borchette", Brussels, September 18 (2009)
- Janssens, S.: TLR expression in the peripheral nerve. Journal of the Peripheral Nervous System 14 (Suppl 2): 148-149 (2009), 2009 Biennial PNS Meeting, Wurzburg, Germany, July 4-8 (2009)
- Rotthier, A.: Mutation analysis of genes for hereditary sensory and autonomic neuropathies: Identification of new mutations and a genotype-phenotype correlation study. Journal of the Peripheral Nervous System 14 (Suppl 2): 128-129 (2009), 2009 Biennial PNS Meeting, Wurzburg, Germany, July 4-8 (2009)
- Rotthier, A.: Mutation analysis of genes for hereditary sensory and autonomic neuropathies: Identification of new mutations and a genotype-phenotype correlation study. Annual Scientific IAP P6/43 meeting, Brussels, Belgium, October 26 (2009)
- Janssens, J.: overexpressing mutant human TDP-43 develop a dose-dependent ALS motor neuron phenotype. Models of dementia; the good, the bad and the future, Cambridge, UK, December 15-17 (2010)
- Baets, J.: Genetic spectrum of hereditary peripheral neuropathies with onset in the first year of life. Peripheral Nerve Society Satellite Meeting, Sydney, Australia, July 5 - 7 (2010)
- Zimoń, M.: Zimoń, M., Baets, J., Auer-Grumbach, M., Berciano, J., Garcia, A., Lopez-Laso, E., Merlini, L., Hilton-Jones, D., McEntagart, M., Crosby, A., Barisic, N., Boltshauser, E., Shaw, C.E., Landouré, G., Ludlow, C.L., Gaudet, R., Houlden, H., Reilly, M.M., Fischbeck, K.H., Sumner, C.J., Timmerman, V., Jordanova, A., De Jonghe, P.: "Dominant mutations in the cation channel gene TRPV4 cause an unusual spectrum of neuropathies". Peripheral Nerve Society Satellite Meeting, Sydney, Australia, July 5 – 7 (2010)
- Irobi, J.: Almeida-Souza L, Asselbergh B, De Winter V, Goethals S, Dierick I, Krishnan J, Timmermans JP, Robberecht W, De Jonghe P, Van Den Bosch L, Janssens S, Timmerman V. Mutant HSPB8 causes motor neuron specific neurite degeneration. Joint Meeting of the Belgian-Dutch Neuromuscular Study Club and German Reference Center for Neuromuscular Diseases of the DGNN, Hotel Kasteel Bloemendal, Vaals, The Netherlands, April 23-24 (2010)
- Zimoń, M.: Zimoń, M., Baets, J., Fabrizi, G.M., Jaakkola, E., Timmerman, V., De Jonghe, P, Jordanova, A.: "Rare dominant GDAP1 mutations cause clinically distinct CMT phenotypes". Joint Meeting of the Belgian-Dutch Neuromuscular Study Club and German Reference Center for Neuromuscular Diseases of the DGNN, Hotel Kasteel Bloemendal, Vaals, The Netherlands, April 23-24 (2010)

Poster Presentations - International

- Irobi, J.: Mutant heat shock protein HSPB8 induces aggregation and a pro-apoptotic phenotype in distal motor neuropathy. The 58th Annual Meeting of the American Society of Human Genetics, Philadelphia, Pennsylvania, USA, November 11-15 (2008)
- Irobi, J.: Mutant heat shock protein HSPB8 induces aggregation and a pro-apoptotic phenotype in distal motor neuropathy. Society for Neuroscience, SFN annual meeting, Washington, USA, November 15–19 (2008)
- Irobi, J.: Mutant heat shock protein HSPB8 induces aggregation and a pro-apoptotic phenotype in distal motor neuropathy. Lentiviral vectors: concepts, practice, hope and reality, Advanced Course Symposium and Practical Training. University Evry Val d'Essonne, Evry, France, June 29-July 5 (2008)
- Almeida-Souza, L.: Generation of a human neuronal Flp-in host cell line. Molecular and Cellular Mechanisms of Axon Degeneration, Woods Hole, MA, USA, October 26-28 (2008)
- Gonçalves, R.: DI-CMTC fly – the first Drosophila model for inherited peripheral neuropathy. Society for Neuroscience, Washington, USA, November 15-19 (2008)
- Gonçalves, R.:DI-CMTC fly – the first Drosophila model for inherited peripheral neuropathy. Neurofly meeting, 12th European Drosophila Neurobiology Conference, Wurzburg, Germany, 6-10 September (2008)
- Janssens, S.: Induction of TLR expression in the peripheral nerve. 9th International Congress for Neuroimmunology, Forth Worth, Texas, USA, October 26-30 (2008)
- Timmerman, V.: Mutation analysis of genes for hereditary sensory and autonomic neuropathies: Identification of new mutations and a genotype-phenotype correlation study; Inherited Neuromuscular Diseases: Translation from Pathomechanisms to Therapies; International Symposium on Rare Disease, Valencia Spain, November 16-18 (2008)
- Jordanova, A.: DI-CMTC fly – the first Drosophilla model for inherited peripheral neuropathy. Society for Neuroscience, Washington, USA, November 15-19 (2008)
- Jordanova, I.: Mutation analysis of the SCN1A gene and genotype-phenotype correlations in Bulgarian epilepsy patients, European Human Genetic Conference 2008, Barcelona, Spain, May 31 - June 3 (2008)

- Holmgren, A.: Mutant HSPB8 and HSPB1 impairs formation of stable neurofilament network. Third International Charcot-Marie-Tooth Consortium Meeting, Antwerp, Belgium, July 9-11 : P40 (2009)
- Janssens, S.: Induction of TLR expression in the peripheral nerve upon neurodegeneration. Third International Charcot-Marie-Tooth Consortium Meeting, Antwerp, Belgium, July 9-11 : P51 (2009)
- Rotthier, A.: Mutation analysis of genes for hereditary sensory and autonomic neuropathies: Identification of new mutations and a genotype-phenotype correlation study. Third International Charcot-Marie-Tooth Consortium Meeting, Antwerp, Belgium, July 9-11: P37 (2009)
- Baets, J.: ARSACS in patients initially referred as CMT. Third International Charcot-Marie-Tooth Consortium Meeting, Antwerp, Belgium, July 9-11 : P29 (2009)
- Baets,J.: Genetic spectrum of hereditary peripheral neuropathies with onset in the first year of life. Third International Charcot-Marie-Tooth Consortium Meeting, Antwerp, Belgium, July 9-11 : P28 (2009)
- Zimon, M.: Large scale genetic approach for the molecular characterization of autosomal-recessive Charcot-Marie-Tooth disease. Third International Charcot-Marie-Tooth Consortium Meeting, Antwerp, Belgium, July 9-11 : P48 (2009)
- Zimon, M.: Novel mutations bring novel insight into GDAP1-associated CMT neuropathies. Third International Charcot-Marie-Tooth Consortium Meeting, Antwerp, Belgium, July 9-11 : P47 (2009)
- Rotthier, A.: Genes for Hereditary Sensory Neuropathy. HSAN-I Symposium, Boston, USA. February 18-19 (2010)
- Rotthier, A., Janssens, K., Van Hoof, K., Penno, A., Hornemann, T. & Timmerman, V. (2010). Biochemical characterization of novel mutations in SPTLC1. Gordon glyco- and sphingolipid conference. Ventura, USA, February 7-12 (2010)
- Almeida-Souza, L.: Increased monomerization of mutant HSPB1 leads to protein hyperactivity in CMT neuropathy. Joint Meeting Belgian-Dutch Neuromuscular Study Club and German Reference Center for Neuromuscular Diseases of the DGNN, April 23-24 (2010)
- Timmerman, V.: Mutant HSPB8 causes motor neuron specific neurite degeneration in axonal Charcot-Marie-Tooth disease. Society for Neuroscience, San Diego, November 13-17 (2010)
- Irobi, J.: Almeida-Souza L, Asselbergh B, De Winter V, Goethals S, Dierick I, Krishnan J, Timmermans JP, Robberecht W, De Jonghe P, Van Den Bosch L, Janssens S, Timmerman V. Mutant HSPB8 causes motor neuron specific neurite degeneration. 3rd International Workshop on Molecular and Cellular Mechanisms of Axon Degeneration, Germany, Eibsee, 3-6 October (2010)
- Janssens, S.: Almeida-Souza L, Asselbergh B, d'Ydewalle C, Goethals S; de Winter V, Irobi J, Timmerman E, Gevaert K, Van Den Bosch L, Timmerman V, Janssens S.: HSPB1 mutations causing Charcot-Marie-Tooth neuropathy disturb microtubule dynamics through enhanced interaction to tubulin. 3rd International Workshop on Molecular and Cellular Mechanisms of Axon Degeneration, Eibsee, Germany, October 3-6 (2010)
- Janssens, S.: Ydens E, Goethals S, Timmerman , Janssens S Acute neurodegeneration triggers an alternative macrophage response. 3rd International Workshop on Molecular and Cellular Mechanisms of Axon Degeneration, Eibsee, Germany, October 3-6 (2010).
- Janssens, S.: Goethals S, Ydens E, Jacobs A, Timmerman V and Janssens S. Toll-like receptor induction by neurodegeneration in the peripheral nerve. Cell Symposia: Inflammation and Disease, Lissabon, Portugal, September 26-28 (2010)
- Irobi, J.: Almeida-Souza L, Asselbergh B, De Winter V, Goethals S, Dierick I, Krishnan J, Timmermans JP, Robberecht W, De Jonghe P, Van Den Bosch L, Janssens S, Timmerman V. Mutant HSPB8 causes motor neuron specific neurite degeneration. 18th Euroconference on Apoptosis, 7th training course on concepts and methods in programmed cell death, Ghent, Belgium, Sept. 1-4 (2010)
- Ydens, E.: Goethals Sofie, Ydens Elke, Timmerman Vincent and Janssens Sophie; Expression of TLRs in the peripheral nerve upon neurodegeneration, 1st Conference of the European Research Institute for Integrated Cellular Pathology (ERI-ICP), Paris, France, April 22-23 (2010)
- Ydens, E.: Sofie Goethals, Vincent Timmerman and Sophie Janssens: Acute neurodegeneration triggers an alternative macrophage response, BSCDB Autumn Meeting 2010 Neuroimmunology, Hasselt, Belgium, October 22 (2010)
- Almeida-Souza, L.: Almeida-Souza L, Goethals S, de Winter V, Dierick I, Gallardo R, Van Durme J, Irobi J, Gettemans J, Rousseau F, Schymkowitz J, Timmerman V; Janssens S.: Increased monomerization of mutant HSPB1 leads to protein hyperactivity in CMT neuropathy. VIB seminar 2010, Blankenberge, Belgium, March 4 (2010).
- Almeida-Souza, L.: Almeida-Souza L, Goethals S, de Winter V, Dierick I, Gallardo R, Van Durme J, Irobi J, Gettemans J, Rousseau F, Schymkowitz J, Timmerman V; Janssens S.: Increased monomerization of mutant HSPB1 leads to protein hyperactivity in CMT neuropathy. Second Joint Meeting of the Belgian-Dutch Neuromuscular Study Club and German Reference Center for Neuromuscular Diseases, Vaals, The Netherlands, April 23-24 (2010).
- Almeida-Souza, L.: Almeida-Souza L, Asselbergh B, d'Ydewalle C, Goethals S; de Winter V; Irobi J; Van Den Bosch L, Timmerman V; Janssens S.: CMT-associated HSPB1 mutations affect microtubule stability. Annual Scientific IAP6/43 meeting, KULeuven, Leuven, Belgium, October 25 (2010).

Poster Presentations - National

- Rotthier, A.: Screening for mutations in genes associated with hereditary sensory neuropathies. Annual Scientific IAP Meeting (P6/43), Antwerpen, Belgium, October 15 (2008)
- Dierick, I.: Mutation analysis of HSPB1, HSPB8, BSCL2, GARS, DCTN1, SETX and VAPB in a large cohort of patients with distdal (hereditary) motor neuropathy. VIB seminar 2008, Blankenberge, Belgium, March 6 (2008)
- Dierick, I.: Relative contribution of mutations in genes for autosomal dominant distal hereditary motor neuropathies: a genotype - phenotype correlation study. 8th annual meeting Belgian Society of Human Genetics, Leuven, Belgium, April 5 (2008)
- Irobi, J.: Distal hereditary motor neuropathy caused by mutant HSPB8 reduced cell viability and induced protein aggregation. VIB Seminar 2008, Blankenberge, Belgium, March 6-7 (2008Irobi, J.: Mutant heat shock protein HSPB8 induces aggregation and a pro-apoptotic phenotype in distal motor neuropathy. Lentiviral vectors: concepts, practice, hope and reality, Advanced Course Symposium and Practical Training. University Evry Val d'Essonne, Evry, France, June 29-July 5 (2008)
- Souza, L.: Searching for the pathomechanism of peripheral neuropathies related genes through a differential interaction-based technology: A methodology proposal. VIB seminar 2008, Blankenberge, Belgium, March 6 (2008)
- Baets, J.: Gene and mutation spectrum in dominantly inherited spastic paraplegias 8th annual meeting Belgian Society of Human Genetics, Leuven, Belgium, April 5 (2008)
- Baets, J.: Gene and mutation spectrum in dominantly inherited spastic paraplegias. Annual Scientific IAP Meeting (P6/43), Antwerpen, Belgium, October 15 (2008)
- Rotthier, A.: Mutation analysis of genes for hereditary sensory and autonomic neuropathies: identification of new mutations and a genotype-phenotype correlation study. VIB Seminar 2009, Blankenberge, Belgium, March 12 : P92 (2009)
- Irobi, J.: Mutant heat shock protein HSPB8 induces aggregation and a pro-apoptotic phenotype in distal motor neuropathy. VIB Seminar 2009, Blankenberge, Belgium, March 12: (2009)
- Irobi, J.: Distal hereditary motor neuropathy caused by mutant HSPB8 reduced cell viability and induced protein aggregation. Third International Charcot-Marie-Tooth Consortium Meeting, Antwerp, Belgium, July 9-11 (2009)
- Irobi, J.: Mutant heat shock protein HSPB8 induces aggregation and a pro-apoptotic phenotype in distal motor neuropathy. Annual Scientific IAP P6/43 meeting, University of Brussels (ULB), Brussels, Belgium, October 26 (2009)
- Janssens, S.: Induction of TLR expression in the peripheral nerve upon neurodegeneration. VIB Seminar 2009, Blankenberge, Belgium, March 12 : P72 (2009)
- Baets, J.: Genetic spectrum of hereditary peripheral neuropathies with onset in the first year of life. Annual Scientific IAP6/43 meeting, University of Brussels (ULB), Brussels, Belgium, October 26 (2009)
- Zimon, M.: Novel mutations in GDAP1 causing both dominant and recessive CMT disease. 9th annual meeting of the Belgian Society of Human Genetics: Darwin's 200th Birthday, ULB, Brussels, Belgium, February 13: (2009)
- Zimon, M.: Novel mutations bring novel insight into GDAP1-associated CMT neuropathies. Annual Scientific IAP6/43 meeting, University of Brussels (ULB), Brussels, Belgium, October 26 (2009)
- Ydens, E.: Ydens Elke, Sofie Goethals, Vincent Timmerman and Sophie Janssens: Acute neurodegeneration triggers an alternative macrophage response, Annual Scientific IAP P6/43 meeting, Leuven, Belgium, October 25 (2010)
- **Zimoń**, M.: **Zimoń**, M., Baets, J., Auer-Grumbach, M., Berciano, J., Garcia, A., Lopez-Laso, E., Merlini, L., Hilton-Jones, D., McEntagart, M., Crosby, A., Barisic, N., Boltshauser, E., Shaw, C.E., Landouré, G., Ludlow, C.L., Gaudet, R., Houlden, H., Reilly, M.M., Fischbeck, K.H., Sumner, C.J., Timmerman, V., Jordanova, A., De Jonghe, P.: "Dominant mutations in the cation channel gene TRPV4 cause an unusual spectrum of neuropathies", TRP 2010 - TRP channels, from structure to disease, Leuven, Belgium, September 23-24 (2010)
- **Zimoń**, M.: **Zimoń**, M., Baets, J., Auer-Grumbach, M., Berciano, J., Garcia, A., Lopez-Laso, E., Merlini, L., Hilton-Jones, D., McEntagart, M., Crosby, A., Barisic, N., Boltshauser, E., Shaw, C.E., Landouré, G., Ludlow, C.L., Gaudet, R., Houlden, H., Reilly, M.M., Fischbeck, K.H., Sumner, C.J., Timmerman, V., Jordanova, A., De Jonghe, P.: "Dominant mutations in the cation channel gene TRPV4 cause an unusual spectrum of neuropathies". 10th annual meeting of the Belgian Society of Human Genetics, Gent, February 26th (2010)

Final report of the research group of

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Progranulin in Neurodegenerative Dementia: Genetic, Functional and Neuropathological Characterization

Specific Aims of the Project

1. To evaluate the occurrence of complex *PGRN* null-mutations in a collection of 190 Belgian FTLD patients as well as study the contribution of *PGRN* mutations in FTLD-associated neurodegenerative diseases including PD, ALS and AD.
2. To identify genes modifying the highly variable onset age of FTDU associated with *PGRN* mutations.
3. To construct *Grn* knockout mice and illustrate whether $Grn^{-/+}$ and $Grn^{-/-}$ mice have neuronal loss especially in the basal forebrain or develop behavioral or cognitive abnormalities compared to the wild-type mice or to mice overexpressing human wild-type PGRN. However, because *PGRN* is an important gene expressed in a variety of tissues, the targeting construct utilizes a conditional knockout approach and will be utilized if the constitutive Grn loss is embryonically lethal. Moreover, this approach would also allow tissue- or cell type-specific *Grn* ablation.
4. To develop *PGRN* overexpressing and deficient cellular models such as primary neuronal cortical neurons derived from $Grn^{-/-}$ mice or RNAi-silenced immortalized neuron-like cells (SH-SY5Y, P19, and NTera) and non-neuronal cells (HEK293) and to utilize these models to study overexpressed and/or endogenous PGRN cellular localizations and protein trafficking and turnover. Moreover, these models will also be utilized to study PGRN-mediated cell proliferation or other phenotypic changes as well as altered cell-signaling pathways, inter alia, PI3K and MAPK pathways.

Final Progress Report

Frontotemporal lobar degeneration (FTLD) is a neurodegenerative condition that predominantly affects behavior, social awareness, and language. It is characterized by extensive heterogeneity at the clinical, pathological, and genetic levels. Recognition of these levels of heterogeneity is important for proper disease management. The identification of progranulin and TDP-43 as key proteins in a significant proportion of FTLD patients has provided the impetus for a wealth of studies probing their role in neurodegeneration (Sleegers et al., 2010b)

Specific aim 1 – *GRN* mutation spectrum

Worldwide, 63 heterozygous mutations were identified in 163 families, all leading to loss of functional GRN, implicating a haploinsufficiency mechanism (Gijselinck et al., 2008a). Since whole gene deletions also lead to the loss of a functional allele, we performed systematic quantitative analyses of PGRN in a series of 103 Belgian FTLD patients. We identified in one patient (1%) a genomic deletion that was absent in 267 control individuals. The deleted segment was between 54 and 69 kb in length and comprised PGRN and two centromeric neighboring genes RPIP8 and SLC25A39. The patient presented clinically with typical FTD without additional symptoms, consistent with haploinsufficiency of PGRN being the only gene contributing to the disease phenotype. This study demonstrates that reduced PGRN in absence of mutant protein is sufficient to cause neurodegeneration and that previously reported PGRN mutation frequencies are underestimated. Together with the previously described null mutations in 11 patients, PGRN mutations account for 11.7% of all FTLD patients (Gijselinck et al., 2008b).

Mutations in PGRN might influence susceptibility to a wider range of neurodegenerative diseases including Alzheimer's disease (AD) and Parkinson's disease (PD) (Cruts and Van Broeckhoven, 2008). AD and FTLD are two frequent forms of primary neurodegenerative dementias. Despite distinctive clinical diagnostic criteria for both brain disorders, differential diagnosis is often complicated by overlapping symptomatology. As we learn more about brain pathology and genetic makeup underlying these dementia disorders, evidence is accumulating for a clinical, pathologic, and genetic spectrum of neurodegenerative brain diseases in which AD and FTLD occur along one continuum. This has important implications for molecular diagnostic testing and genetic counseling of patients with dementia (van der Zee et al., 2008). Therefore, we assessed whether PGRN also contributes to genetic risk for AD in an extended Belgian AD patient group (n = 779, onset age 74.7 ± 8.7 years). We performed a mutation analysis of the PGRN coding region and assessed the effect of missense mutations using in silico predictions and protein modeling. We observed seven missense mutations in eight patients (1.3%). Convincing pathogenic evidence was obtained for two missense mutations, Cys139Arg and Pro451Leu, affecting PGRN protein folding and leading to loss of PGRN by degradation of the misfolded protein. In addition, using logistic regression analysis and gene-based haplotype association analysis, we showed that PGRN haplotypes were associated with increased risk for AD. Our data support a role for PGRN in patients with clinically diagnosed AD (Brouwers et al., 2008). Further, we hypothesize that at least some PGRN missense mutations might lead to loss of functional protein. Whether the underlying pathology in our cases proves to be AD, FTLD, or a combination of both must await further investigations. We next screened PGRN exons for mutations in PD patients (n = 255) by direct genomic sequencing. We identified four missense mutations of which Asp33Glu and Arg514Met were absent in 459 control individuals. At this stage and in the absence of functional data, it remains unclear whether Asp33Glu and Arg514Met are biologically relevant to PD pathogenesis in the mutation carriers. Genetic association of PGRN with risk for PD was assessed using single nucleotide polymorphisms (SNPs) across the gene. Single SNP and haplotype analyses did not detect significant associations with PD. Our results therefore do not support a major role for PGRN in the genetic etiology of PD (Nuytemans et al., 2008).

Where the focus of previous studies was on mutations affecting the coding region of the GRN gene, we also investigated the effect of noncoding, regulatory GRN variants. It has been claimed that homozygosity of the SNP rs5848 located in the 3'UTR of GRN increases risk for FTLD. The authors proposed that homozygosity of the T allele of rs5848 increases binding of the microRNA miR-659 which leads to an inhibition of GRN translation. However, the genetic association was only demonstrated in a single cohort. Given that association studies are fraught with problems of replication, we undertook the first replication of this data in three separate European FTLD cohorts representing a total of 467 patients and 1049 controls. No association with FTLD was observed in any individual cohort nor was any observed when the data was combined. Also, we did not identify significant association in the groups of FTLD patients with confirmed TDP43 neuropathology. These data argue that rs5848 is not a risk factor for FTLD (Rollinson et al., 2009).

To evaluate serum PGRN levels as a biomarker for FTLD, we used an ELISA to measure in serum the PGRN protein levels of 6 affected and 8 unaffected carriers from within an extended Belgian founder FTLD-U family segregating the null mutation (IVS1 +5G>C). Further, we measured serum PGRN levels in 2 patients with other null mutations, in 4 patients carrying a predicted pathogenic missense mutation and in 5 patients carrying a benign missense polymorphism, in 9 unaffected non-carrier relatives and in 22 community control individuals. Serum PGRN levels were reduced in both affected and unaffected null mutation carriers compared to non-carriers (p exact <0.0001), and allowed perfect discrimination

between carriers and non-carriers (sensitivity: 1.0, 1-specificity: 0.0). Serum PGRN levels in Cys139Arg and Arg564Cys mutation carriers were significantly lower than in control individuals, but higher than in null mutation carriers, fitting the hypothesis of partial loss-of-function due to these missense mutations. As expected, levels for carriers of benign missense polymorphisms were not significantly different from control individuals. Our results indicate that the serum PGRN level is a reliable biomarker for diagnosis and early detection of FTLD-U caused by GRN null mutations, and provided the first in vivo evidence that at least some missense mutations in GRN may lead to a (partial) loss of PGRN (Sleegers et al., 2009).

As both null mutations and missense mutations in GRN have also been observed in patients with Alzheimer's disease we reviewed the evidence for a role of circulating GRN as a biochemical biomarker in neurodegeneration, with a specific focus on its relevance in AD. We concluded that circulating GRN is a promising, nonintrusive biomarker that warrants screening in both patients with dementia of the Alzheimer type and people with mild cognitive impairment; specifically for, but not limited to, those that have a positive family history of neurodegenerative disease. Once a cure for GRN-related neurodegeneration becomes available, this biomarker will be an important tool in the effort to personalize treatment of dementia (Sleegers et al., 2010a).

Specific aim 2 – FTLD-GRN modifier genes

The high variability in onset age and age-dependent penetrance suggests that the PGRN pathway is highly susceptible to modulating factors that might be exploited to delay the disease processes (Cruts and Van Broeckhoven, 2008). We performed a genome-wide linkage scan for genes modifying the variable onset age in the extended Belgian founder family DR8, segregating the PGRN IVS1+5G>C null mutation. In preliminary studies, we excluded the apolipoprotein E gene (APOE) known to modify onset age in AD (Cruts et al., 2006). Also, we found that serum levels of PGRN in all mutation carriers was about 50% of the levels observed in non-carriers, suggesting that the PGRN protein level produced from the unaffected gene copy is not a determinant of onset age (Sleegers et al., 2009). In the genome-wide STR-based linkage mapping study, in which we treated onset age as a censored quantitative trait we observed that one single quantitative trait locus (QTL) explained up to 91% of genetic variability corresponding to 65% of the total variability in onset age in this family. This QTL is mapped to a 7 Mb region and contains > 100 genes, none of which is associated with FTLD or another neurodegenerative disease.

Specific aim 3 – PGRN *in vivo* models

In patients loss-of-function mutations in GRN are associated with FTLD with intraneuronal ubiquitinated protein accumulations composed primarily of hyperphosphorylated TDP-43 (FTLD-TDP). The mechanism by which GRN deficiency causes TDP-43 pathology and neurodegeneration remains elusive. To explore the role of GRN in vivo, we established Grn knockout mice using a targeted genomic recombination approach based on Cre-LoxP technology. Constitutive Grn homozygous knockout (Grn-/-) mice were born in an expected Mendelian pattern of inheritance and showed no phenotypic alterations compared to heterozygous (Grn+/-) or wild-type (WT) littermates till 7 months of age. However, beginning from 11 months of age, Grn-/- mice showed reduced survival and significantly increased gliosis accompanied by ubiquitin-positive accumulations in the cortex, hippocampus and subcortical regions. The ubiquitinated inclusions did not contain detectable phosphorylated TDP-43, but increased levels of hyperphosphorylated full-length TDP-43 were recovered from detergent-insoluble brain fractions of aged Grn-/- mice. Interestingly, phosphorylated TDP-43 was primarily extracted from the nuclear fraction. Our data suggest that progranulin deficiency in mice leads to reduced survival in adulthood

and increased brain aging accompanied by hyperphosphorylation of TDP-43, thus recapitulating key aspects of FTLD-TDP neuropathology (Wils et al., 2011).

Neuronal cytoplasmic and intranuclear aggregates of RNA-binding protein TDP-43 are a hallmark feature of neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD). ALS and FTLD show a considerable clinical and pathological overlap and occur as both familial and sporadic forms. Though missense mutations in TDP-43 cause rare forms of familial ALS, it is not yet known whether this is due to loss of TDP-43 function or gain of aberrant function. Moreover, the role of wild-type (WT) TDP-43, associated with the majority of familial and sporadic ALS/FTLD patients, is also currently unknown. Generating homozygous and hemizygous WT human TDP-43 transgenic mouse lines, we show here a dose-dependent degeneration of cortical and spinal motor neurons and development of spastic quadriplegia reminiscent of ALS. A dose-dependent degeneration of nonmotor cortical and subcortical neurons characteristic of FTLD was also observed. Neurons in the affected spinal cord and brain regions showed accumulation of TDP-43 nuclear and cytoplasmic aggregates that were both ubiquitinated and phosphorylated as observed in ALS/FTLD patients. Moreover, the characteristic approximately 25-kDa C-terminal fragments (CTFs) were also recovered from nuclear fractions and correlated with disease development and progression in WT TDP-43 mice. These findings suggest that approximately 25-kDa TDP-43 CTFs are noxious to neurons by a gain of aberrant nuclear function (Wils et al., 2010).

Amyloid-beta (Abeta) plaques are pathological hallmarks of AD. In addition, innate inflammatory responses, such as those mediated by microglia, are integral to the pathogenesis of AD. Interestingly, only dense-core plaques and not diffuse plaques are associated with neuritic and inflammatory pathology in AD patients as well as in mouse AD models. However, the precise neuropathological changes that occur in the brain in response to amyloid deposition are largely unknown. To study the molecular mechanism(s) responsible for Abeta-mediated neuropathology, we performed a gene expression analysis on laser-microdissected brain tissue of Tg2576 and APPPS1 mice that are characterized by different types of amyloid plaques and genetic backgrounds. Data were validated by image and biochemical analyses on different ages of Tg2576, APPPS1, and Abeta42-depositing BRI-Abeta42 mice. Consistent with an important role of inflammatory responses in AD, we identified progranulin (mouse Grn; human GRN) as one of the top ten up-regulated molecules in Tg2576 (approximately 8-fold increased) and APPPS1 (approximately 2-fold increased) mice compared to littermate controls, and among the eight significantly up-regulated molecules common to both mouse models. In addition, Grn levels correlated significantly with amyloid load, especially the dense-core plaque pathology ($p < 0.001$). We further showed that Grn is up-regulated in microglia and neurons and neurites around dense-core plaques, but not in astrocytes or oligodendrocytes, as has been shown in AD patients. Our data therefore support the ongoing use of these mouse models in drug trials, especially those with anti-inflammatory compounds. Moreover, the correlation of Grn with increasing disease severity in AD mouse models prompts human studies exploring the viability of GRN as a disease biomarker. Because loss of GRN has recently been shown to cause frontotemporal dementia and serves as a risk factor for AD, the strong GRN reactivity around dense-core plaques is consistent with an important role of this factor in AD pathogenesis (Pereson et al., 2009).

Specific aim 4 – PGRN *in vitro* model systems

The mechanism by which GRN deficiency leads to neurodegeneration remains largely unknown. In primary cortical neurons derived from Grn knockout (Grn-/-) mice, we found that Grn-deficiency causes significantly reduced neuronal survival and increased caspase-mediated apoptosis, which was not

observed in primary mouse embryonic fibroblasts derived from Grn-/- mice. Also, neurons derived from Grn-/- mice showed an increased amount of pTDP-43 accumulations. Furthermore, proteasomal inhibition with MG132 caused increased caspase-mediated TDP-43 fragmentation and accumulation of detergent-insoluble 35- and 25-kDa C-terminal fragments in Grn-/- neurons and mouse embryonic fibroblasts. Interestingly, full-length TDP-43 also accumulated in the detergent-insoluble fraction, and caspase-inhibition prevented MG132-induced generation of TDP-43 C-terminal fragments but did not block the pathological conversion of full-length TDP-43 from soluble to insoluble species. These data suggest that GRN functions as a survival factor for cortical neurons and GRN-deficiency causes increased susceptibility to cellular stress. This leads to increased aggregation and accumulation of full-length TDP-43 along with its C-terminal derivatives by both caspase-dependent and independent mechanisms (Kleinberger et al., 2010).

Publications

- Brouwers N, Bettens K, Gijselinck I, Engelborghs S, Pickut BA, Van Miegroet H, Montoya AG, Mattheijssens M, Peeters K, De Deyn PP, Cruts M, Sleegers K, Van Broeckhoven C. 2010. Contribution of TARDBP to Alzheimer's disease genetic etiology. J Alzheimers Dis 21:423-430.
- Brouwers N, Sleegers K, Engelborghs S, Maurer-Stroh S, Gijselinck I, van der Zee J, Pickut BA, Van den Broeck M, Mattheijssens M, Peeters K, Schymkowitz J, Rousseau F, Martin JJ, Cruts M, De Deyn PP, Van Broeckhoven C. 2008. Genetic variability in progranulin contributes to risk for clinically diagnosed Alzheimer disease. Neurology 71:656-664.
- Cruts M, Van Broeckhoven C. 2008. Loss of progranulin function in frontotemporal lobar degeneration. Trends Genet 24:186-194.
- Gijselinck I, Van Broeckhoven C, Cruts M. 2008a. Granulin mutations associated with frontotemporal lobar degeneration and related disorders: An update. Hum Mutat 29:1373-1386.
- Gijselinck I, van der Zee J, Engelborghs S, Goossens D, Peeters K, Mattheijssens M, Corsmit E, Del-favero J, De Deyn PP, Van Broeckhoven C, Cruts M. 2008b. Progranulin locus deletion in frontotemporal dementia. Hum Mutat 29:53-58.
- Kleinberger G, Wils H, Ponsaerts P, Joris G, Timmermans JP, Van Broeckhoven C, Kumar-Singh S. 2010. Increased caspase activation and decreased TDP-43 solubility in progranulin knockout cortical cultures. J Neurochem 115:735-747.
- Nuytemans K, Pals P, Sleegers K, Engelborghs S, Corsmit E, Peeters K, Pickut B, Mattheijssens M, Cras P, De Deyn PP, Theuns J, Van Broeckhoven C. 2008. Progranulin variability has no major role in Parkinson disease genetic etiology. Neurology 71:1147-1151.
- Pereson S, Wils H, Kleinberger G, McGowan E, Vandewoestyne M, Van Broeck B, Joris G, Cuijt I, Deforce D, Hutton M, Van Broeckhoven C, Kumar-Singh S. 2009. Progranulin expression correlates with dense-core amyloid plaque burden in Alzheimer disease mouse models. J Pathol 219:173-181.
- Pirici D, Van Cauwenberghe C, Van Broeckhoven C, Kumar-Singh S. 2009. Fractal analysis of amyloid plaques in Alzheimer's disease patients and mouse models. Neurobiol Aging.
- Rollinson S, Rohrer JD, van der Zee J, Sleegers K, Mead S, Engelborghs S, Collinge J, De Deyn PP, Mann DM, Van Broeckhoven C, Pickering-Brown SM. 2009. No association of PGRN 3'UTR rs5848 in frontotemporal lobar degeneration. Neurobiol Aging.
- Sleegers K, Brouwers N, Van BC. 2010a. Role of progranulin as a biomarker for Alzheimer's disease. Biomark Med 4:37-50.
- Sleegers K, Brouwers N, Van Damme P, Engelborghs S, Gijselinck I, van der Zee J, Peeters K, Mattheijssens M, Cruts M, Vandenberghe R, De Deyn PP, Robberecht W, Van Broeckhoven C. 2009. Serum biomarker for progranulin-associated frontotemporal lobar degeneration. Ann Neurol 65:603-609.
- Sleegers K, Cruts M, Van Broeckhoven C. 2010b. Molecular pathways of frontotemporal lobar degeneration. Annu Rev Neurosci 33:71-88.
- Sleegers K, Kumar-Singh S, Cruts M, Van Broeckhoven C. 2008. Molecular pathogenesis of frontotemporal lobar degeneration: basic science seminar in neurology. Arch Neurol 65:700-704.
- van der Zee J, Sleegers K, Van Broeckhoven C. 2008. Invited article: the Alzheimer disease-frontotemporal lobar degeneration spectrum. Neurology 71:1191-1197.
- Wils H, Kleinberger G, Janssens J, Pereson S, Joris G, Cuijt I, Smits V, Ceuterick-de Groote C, Van Broeckhoven C, Kumar-Singh S. 2010. TDP-43 transgenic mice develop spastic paralysis and neuronal inclusions characteristic of ALS and frontotemporal lobar degeneration. Proc Natl Acad Sci U S A 107:3858-3863.
- Wils H, Kleinberger G, Pereson S, Janssens J, Van Dam D, Smits V, Joris G, Cuijt I, De Deyn PP, Van Broeckhoven C, Kumar-Singh S. 2011. Increase mortality and FTLD-TDP-associated neuropathology in aged progranulin knockout mice.

Theses

PhD

Name Date	Supervisor Co-supervisor	Title
I. Gijselinck 08/09/2008	**C. Van Broeckhoven** M. Cruts	'Molecular genomics of tau-negative, ubiquitin-positive frontotemporal lobar degeneration'
N. Brouwers 12/12/2008	**C. Van Broeckhoven** K. Sleegers	'Molecular genetic analysis of Alzheimer disease'
D. Pirici 20/03/2009	**S. Kumar-Singh** **C. Van Broeckhoven**	Molecular mechanisms of extracellular and intracellular proteinopathy in Alzheimer's disease and frontotemporal dementia
H. Wils 07/12/2010	**C. Van Broeckhoven** **S. Kumar-Singh**	Development and characterization of novel mouse models for frontotemporal lobar degeneration and amyotrophic lateral sclerosis

MSc Theses

Name Date	Supervisor Co-supervisor	Title
C. Van Cauwenberghe 2007-2008	**S. Kumar-Singh** G. Kleinberger	Inleiding tot immunocytochemie en western blotting technieken voor FTLD Introduction to immunocytochemistry and western blotting techniques in FTLD
T. Van Langenhove 2007-2008	**C. Van Broeckhoven** J. van der Zee	Genetic analysis for the valosin containing protein gene in Belgian frontotemporal lobar degeneration patients and clinico-pathological characterization of mutation carriers
J. Janssens 2008-2009	**S. Kumar-Singh** H. Wils	Biochemical and neuropathological characterization of a novel human TDP-43 overexpression mouse model
Q. Verelst 2008-2009	**M. Cruts** **I. Gijselinck**	Identification of genetic defect in a locus for frontotemporal lobe degeneration
S. Philtjens 2008-2009	**P. Stinissen** **M. Cruts** I. Gijselinck	Pathway-based genetic analyses to detect novel genes associated with frontotemporal lobar degeneration
J. Busseniers 2009-2010	**C. Van Broeckhoven** G. Kleinberger	Investigation of apoptosis in progranulin knockdown cell models
S. Vanbeginne 2009-2010	**K.Sleegers** **N. Brouwers**	Characterization and identification of granulin missense mutations in patients with Alzheimer dementia

Professional BSc Theses

Name Date	Supervisor Co-supervisor	Title
J. Brys 2007-2008	**C. Van Broeckhoven** J. van der Zee	Mutatiescreening van het microtubuli geassocieerde proteïne tau (MAPT) en progranuline gen (PGRN) in Alzheimer en frontotemporale dementie patiënten Mutation screening of the microtubule associated protein tau (MAPT) and progranulin gene (PGRN) in Alzheimer and frontotemporal dementia patients.
J. De Ren 2008-2009	**M. Cruts** **I. Gijselinck**	Search of novel genetic factors for frontotemporal lobe degeneration by mutation analysis of functional candidate genes
L. Van Hoeck 2009-2010	**T. Van Langenhove**	Mutatieanalyse van h et optineurine gen in amyotrofische laterale sclerose patiënten en van het microtubule-associated proteint au gen in frontaalkwabdementie patiënten

Honors, Prizes & Awards

Honors

- **Christine Van Broeckhoven**: Chevalier dans l'Ordre de la Légion d'Honneur, France, 06/02/2009
- **Christine Van Broeckhoven**: Distinguished Lecturer in Neuroscience and Aging at the National Institute on Aging IRP in Baltimore, USA, 16/09/2008
- **Christine Van Broeckhoven**: European Ambassador for Creativity and Innovation for Belgium, 2009
- **Julie van der Zee**: Certificate of Honor of the Foundation for Alzheimer Research (SAO/FRMA), Belgium, 2009
- **Kristel Sleegers**: Certificate of Honor of the Foundation for Alzheimer Research (SAO/FRMA), Belgium, 2009
- **Marc Cruts**: Certificate of Honor of the Foundation for Alzheimer Research (SAO/FRMA), Belgium, 2009
- **Samir Kumar-Singh:** Certificate of Honor of the Foundation for Alzheimer Research (SAO/FRMA), Belgium, 2009

Prizes

- **Kristel Sleegers & Marc Cruts**: Nationale Alzheimer Liga, De Santkin Prijs, 21/09/2009
- **Marc Cruts & Samir Kumar-Singh**: Koning Boudewijn Stichting, De Marie-Thérèse De Lava Prijs 2009, 26/11/2009
- Awards
- **Ilse Gijselinck**: Travel Award of the AD/PD 2009 organizers to attend the 9th International Conference on AD/PD 2009, Prague, Czech Republic, March 11-15, 2009
- **Julie van der Zee:** ICAD Travel Fellowship for the International Conference on Alzheimer's disease (ICAD), Honolulu, Hawaii, USA, 10-15/07/2010
- **Julie van der Zee:** Travel Fellowship to attend the 11th International Conference on Alzheimer's disease (ICAD), Chicago, Illinois, USA, 26-31/07/2008
- **Julie van der Zee**: Travel Fellowship to attend the 6th International Conference on FTD, Rotterdam, The Netherlands, 3-5/09/2008
- **Karen Nuytemans**: Fellowship from the Fund for Scientific Research – Flanders (FWO) to attend the Cold Spring Harbor Laboratory Course 'Eukaryotic gene expression', Cold Spring Harbor, NY, USA, 16/07- 05/08/2008
- **Kristel Sleegers**: Travel Fellowship to attend the 6th International Conference on FTD, Rotterdam, The Netherlands, 3-5/09/2008
- **Nathalie Brouwers:** Young Scientist Travel Award – Pfizer Scholar: to attend the 'Human Genome Meeting – HGM 2010', Montpellier, France, 18-21/05/2010
- **Sandra Pereson**: Travel Award of the Alzheimer's Association to attend the 12th Alzheimer's Association International Conference on Alzheimer's Disease (ICAD) 2009, Vienna, Austria, 11-16/07/2009
- **Sandra Pereson**: Travel Fellowship of company tebu-bio nv to attend the 11th International Conference on Alzheimer's Disease (ICAD), Chicago, Illinois, USA, 26-31/07/2008

Activities & Presentations

Chair and Organizational Activities

International

- **Christine Van Broeckhoven:** 11th International Congress on Alzheimer's disease: **Chair**: Session: Disease mechanisms: Tau, Tauopathies2, Chicago, 26-31/07/2008
- **Christine Van Broeckhoven:** 12th International Congress of Parkinson's disease and Movement Disorders: **Chair**: Session: Frontotemporal lobar degeneration, Chicago, USA, 22-26/06/2008
- **Christine Van Broeckhoven:** 6th International Conference on Frontotemporal Dementias, **International Advisory Board,** Rotterdam, the Netherlands, 3-5/09/2008
- **Christine Van Broeckhoven:** 6th International Conference on Frontotemporal Dementias, **Chair**: Session: PGRN gene/ mutations**,** Rotterdam, The Netherlands, 3-5/09/2008
- **Christine Van Broeckhoven:** 7th International Conference on Frontotemporal Dementias. **International Advisory Committee**, Indianapolis, Indiana, USA, October 6-8, 2010
- **Christine Van Broeckhoven:** International Conference on Alzheimer's disease and related disorders (ICAD), **Co-Chair** of the Scientific Program Committee, Vienna, Austria, 11-16/07/2009
- **Christine Van Broeckhoven:** International Conference on Alzheimer's disease and related disorders (ICAD), **Symposium Chair**, Vienna, Austria, 11-16/07/2009
- **Christine Van Broeckhoven:** International Conference on Alzheimer's disease and related disorders (ICAD). **Co-Chair** of the Scientific Program Committee, Honolulu, Hawaii, USA, July 10-15, 2010
- **Christine Van Broeckhoven:** International Conference on Alzheimer's disease and related disorders (ICAD). **Co-Chair** of the Symposium Session 'Genetics', Honolulu, Hawaii, USA, July 10-15, 2010
- **Kristel Sleegers**: 6th European Meeting on Molecular Diagnostics, **Symposium Chair**, Scheveningen, the Netherlands, 21-23/10/2009
- **Kristel Sleegers**: Capita Selecta on Complex Disease Analysis 2010 – First Edition. **Organizer**, Leuven, Belgium, August 25-27, 2010
- **Kristel Sleegers:** Capita Selecta on Complex Disease Analysis 2010 – First Edition. Leuven, Belgium, August 25-27, 2010 **Chair** of the session "Case Studies", Aug 27, 2010
- **Samir Kumar-Singh:** International Congress of Vascular Behavioral and Cognitive Disorders (Vas-Cog), **Symposium Chair** of plenary session II: Animal models and VCI, Singapore, 14-16/01/2009
- **Samir Kumar-Singh:** Representative from University of Antwerp on Board of the Cytron Consortium, University of Leiden, The Netherlands, 2009

National

- **Samir Kumar-Singh**: Annual IAP P6/43 Meeting, **Co-Chair** Thematic Workshop: 'Molecular morphology and neuropathology', Brussels, Belgium, 26/10/2009
- **Samir Kumar-Singh**: VIB Science Club Neurodegenerative Diseases II, **Symposium Chair**, Leuven, 30/10/2009

Invited Lectures

International

- **Christine Van Broeckhoven:** Instituutdag Universiteit Maastricht: Progranulin and neurodegeneration: a new pathway to treatment? Maastricht, The Netherlands, 11/04/2008
- **Christine Van Broeckhoven:** 12th International Congress of Parkinson's disease and Movement Disorders: 'Genetics of frontotemporal lobar degenerations', Chicago, USA, 22-26/06/2008
- **Christine Van Broeckhoven:** Seminar at the National Institute on Aging, Biomedical Research Center, Laboratory of Neurosciences: 'A genetic approach to neurodegenerative dementias', Baltimore, USA, 16/09/2008
- **Christine Van Broeckhoven**: Neurogenetics of dementia. Medizinisch Genetisches Zentrum Symposium Neurogenetik im Erwachsenenalter, Munich, Germany, 27/06/2009
- **Christine Van Broeckhoven:** Exploring the brain: from function to dysfunction: molecular genetics of neurodegenerative dementias. The Amsterdam International Medical Summer School 20th Edition, Amsterdam, the Netherlands, 17/07/2009
- **Christine Van Broeckhoven:** Genetics of familial frontotemporal degeneration, 7th International Conference on Frontotemporal Dementias, Indianapolis, Indiana, USA, October 6-8, 2010
- **Christine Van Broeckhoven:** GEOPD meeting 2008: 'Progranulin and neurodegenerative diseases', Trondheim, Norway, 9-11/06/2008
- **Christine Van Broeckhoven:** NIH Symposium on Progranulin and the TDP-43opathies: 'PGRN in familial and sporadic FTD', Washington DC, USA, 4-5/02/2008
- **Christine Van Broeckhoven:** Progranulin associated neurodegeneration: new insights in frontal temporal dementia and

related disorders, The 10th Eibsee Meeting on Cellular and Molecular Mechanisms of Axon Degeneration: Keynote Lecture, Eibsee, Germany, October 27-30, 2010
- **Christine Van Broeckhoven:** Recent developments in the genetics of neurodegenerative brain disorders, Erasmus Summer School 2010, The Netherlands, August 19, 2010
- **Christine Van Broeckhoven:** Scientific Research in a changing society, Thinking Ahead – Higher Education in Antwerp, Belgian EU Pavilion, Shanghai World Expo, China, June 8, 2010
- **Christine Van Broeckhoven:** Seminar at the national Institute on Aging, Biomedical Research Center, Laboratory of Neurosciences: 'A genetic approach to neurodegenerative dementias', Bethesda, USA, 16/09/2008
- **Christine Van Broeckhoven:** Session 5 – Genetics, The Herrenhausen Symposium on Neurodegeneration, Kloster Seeon, Seeon, Germany, May 25-28, 2010
- **Christine Van Broeckhoven:** Sustainable brain power: R&D in Life Sciences, Seminar Belgium – Flanders: - World leader in chemistry and life sciences, Belgian EU Pavilion, Shanghai World Expo, China, 9/06/2010
- **Christine Van Broeckhoven:** The role of progranulin (PGRN) in brain function and neurodegenerative disease. 9th International Conference on Alzheimer's & Parkinson's Diseases: AD/PD 2009, Prague, Czech Republic, 11-15/03/2009
- **Julie van der Zee:** Novel insights into the genetic profile of FTD. Dementia update "Focus on FTD" Amstelveen, The Netherlands, 27/05/2010
- **Kristel Sleegers**: Serum Progranulin Levels in the Diagnosis of Progranulin-Related Neurodegenerative Diseases. 12th International Conference on Alzheimer's disease (ICAD) Vienna, Austria, 11-16/07/2009
- **Marc Cruts**: The history of molecular genetics of dementia, Institute of Molecular Medicine, Educational Seminar University of Lisbon, Portugal, 7/04/2010
- **Marc Cruts**: Molecular genetics of frontotemporal lobar degeneration, Neurology Lecture Santa Maria University Hospital, Lisbon, Portugal, 8/04/2010
- **Samir Kumar-Singh**: CAA and familial forms of dementia, 4th International Congress of Vascular Behavioural and Cognitive Disorders (Vas-Cog), Singapore, 14-16/01/2009
- **Samir Kumar-Singh**: Pre-Congress Special Symposium of Vascular Behavioral and Cognitive Disorders Congress: 'Molecular pathology of vascular dementia', Singapore, 13/01/2009
- **Samir Kumar-Singh**: Spectrum of parenchymal and vascular amyloidosis, VIII Congress of the Society of Morphology, Craiova, 27-30/05/2009

National

- **Christine Van Broeckhoven**: Biologie van (Alzheimer-) dementie. Minisymposium: (Alzheimer-)dementie en beslissingen bij het levenseinde, UA, Antwerpen, October 3 (2009)
- **Christine Van Broeckhoven**: De ontdekking van progranuline in dementie. Studium Generale in de Biomedische Wetenschappen – UA, Antwerpen, Belgium, April 27 (2009)
- **Christine Van Broeckhoven:** Seminarie KULeuven: Progranulin and neurodegeneration: a new pathway to treatment? Leuven, Belgium, June 20 (2008)
- **Christine Van Broeckhoven**: The role of progranulin in the genetic etiology of frontotemporal lobe degeneration and related disorders. Seminar Universiteit Luik, Luik, Belgium, April 22 (2009)
- **Gernot Kleinberger:** A cell culture model for progranulin deficiency. Annual Scientific IAP P6/43 Meeting, Leuven, Belgium, 25/10/2010
- **Kristel Sleegers.** Nieuwe inzichten in de genetica van dementie. Science Club Dementia, Department Neurology and Memory Clinic ZNA Middelheim/Hoge Beuken, Antwerp, Belgium, 22/10/2010
- **Kristel Sleegers:** Alzheimer dementie. Het Labyrint, Praatcafé Dementie Brugge. Brugge, Belgium, May 19 (2008)
- **Kristel Sleegers**: Molecular Genetics of Frontotemporal Dementia. 15th Annual Meeting of the German Society of Neurogenetics, Homburg/Saar, Germany, October 8-10 (2009)
- **Kristel Sleegers**: Serum Progranulin Levels in the Diagnosis of Progranulin-related Neurodegenerative Diseases. Focus Conferences – Spring conference Focus Diagnostica. Anderlecht, Belgium, April 21 (2009)
- **Kristel Sleegers:** The genetics of movement disorders with dementia. Belgian Neurological Society Symposium: 'Movement Disorders: Genotype and Phenotype', Brussels, Belgium, May 17 (2008)
- **Marc Cruts:** The Alzheimer Disease & Frontotemporal Dementia Mutation Database. Human Variome Project Meeting 2009 – Spotlight on Neurogenetics, Honolulu Hawai, October 19 (2009)
- **Samir Kumar-Singh**: A Novel ALS Mouse Model with TDP-43 Neuronal Inclusions. VIB Science Club Neurodegenerative Diseases II, Provinciehuis Leuven, Belgium, October 30 (2009)
- **Samir Kumar-Singh**: CAA associated with familial forms of dementia and mouse models. VIB Seminar 2009, Blankenberge, Belgium, March 12 (2009)
- **Samir Kumar-Singh:** Molecular neuropathology of dementias, University of Medicine and Pharmacy Craiova, DOLJ Medical Association, Craiova, May 26 (2009)
- **Samir Kumar-Singh:** Mouse models of FTLD and ALS, Maastricht Neuroscience Seminar Series, University of Maastricht,

The Netherlands, November 17 (2009)
- **Samir Kumar-Singh:** Plaques and vascular Aß in Alzheimer's disease, van Leeuwenhoek Lecture, University of Leiden, The Netherlands, January 30 (2009)
- **Samir Kumar-Singh**: Wild-type human TDP-43 overexpression in transgenic mice causes motor neuron degeneration. Annual IAP P6/43 Meeting, Brussels, Belgium, October 26 (2009)

Societal activities

- **Christine Van Broeckhoven:** 50+ Beurs Antwerpen: 'Geheugen en dementie', Antwerpen, Belgium, April 25 (2008)
- **Christine Van Broeckhoven:** 80 jaar FWO: Kennismakers – Dag van de onderzoeker: Panelgesprek 'Popularisering van de wetenschap', Brussel, Belgium, October 23 (2008)
- **Christine Van Broeckhoven:** ActUA - Spectrum Plus reeks: De rol van de groeistof progranuline in frontaalkwabdementie. Nieuwe diagnostische en therapeutische mogelijkheden, UA, Antwerpen, April 30 (2008)
- **Christine Van Broeckhoven:** Algemeen Psychiatrisch Ziekenhuis (APZ) – Sint Niklaas: Midzomeravond 'Lithafeest': De ziekte van Alzheimer: genezen, behandelen of voorkomen? Sinaai, Belgium, June 20 (2008)
- **Christine Van Broeckhoven:** Alzheimer en dementie, Club 18/8 vzw, Edegem, Belgium, June 16, 2010
- **Christine Van Broeckhoven:** Alzheimer en dementie, Grijze Geuzen Antwerpen, Belgium, Antwerpen, March 1, 2010
- **Christine Van Broeckhoven:** Alzheimer en dementie, Molenheide WZC & Curieus Wijnegem, Belgium, Wijnegem, October 11, 2010
- **Christine Van Broeckhoven:** Alzheimer en dementie, Praatcafé dementie Aalst, Aalst, Belgium, September 16, 2010
- **Christine Van Broeckhoven:** Alzheimer en dementie, Sociaal Huis OCMW Sint-Truiden, Sint-Truiden, Belgium, October 4, 2010
- **Christine Van Broeckhoven:** Alzheimer en dementie, Stichting vrienden van de Openbare Bibliotheek Huizen, Huizen, The Netherlands, March 31, 2010
- **Christine Van Broeckhoven:** Alzheimer en dementie, VIVA SVV Kontich (Socialistische vrouwenvereniging), Kontich, Belgium, February 2, 2010
- **Christine Van Broeckhoven:** Alzheimer en dementie, Viva-SVV en Zij-kant, Hasselt, Belgium, December 15, 2010
- **Christine Van Broeckhoven:** Alzheimer en dementie, WZC Sint-Elisabeth, Woon- en Zorgcentrum Sint-Elisabeth, Eeklo, Belgium, December 14, 2010
- **Christine Van Broeckhoven:** Alzheimer en dementie, WZC Ter Vlierbeke, Woonzorghuis Ter Vlierbeke, Kessel-Lo, Belgium, November 26, 2010
- **Christine Van Broeckhoven:** Alzheimer Research, Universiteit Gent – Lescyclus 'Verouderingsbiologie', Gent, Belgium, March 26, 2010
- **Christine Van Broeckhoven**: Alzheimer, vergrijzing en emancipatie. Openbare Bibliotheek Huizen-Laren-Blaricum, Huizen, The Netherlands, October 28 (2009)
- **Christine Van Broeckhoven:** Avicenna, DirectieCollege: Pionieren in Alzheimer, Amersfoort, The Netherlands, May 5 (2008)
- **Christine Van Broeckhoven:** Belgian Neurological Society – Neuroprotection in dementia: 'Progranulin and neurodegeneration: a new pathway to treatment?, La Hulpe, Brussels, Belgium, December 13 (2008)
- **Christine Van Broeckhoven:** Bibliotheek Anderlecht: **'**Dementie, waar staat de wetenschap nu', Anderlecht, Belgium, November 12 (2008)
- **Christine Van Broeckhoven:** Bibliotheek Kortrijk – Boterhammen in de Bib: Mijn verhaal naar aanleiding van mijn boek Brein en Branie, Kortrijk, Belgium, January 28 (2008)
- **Christine Van Broeckhoven:** Bibliotheek Tielt – Boterhammen in de Bib: Mijn verhaal naar aanleiding van mijn boek Brein en Branie: een pionier in Alzheimer, Tielt, Belgium, February 1 (2008)
- **Christine Van Broeckhoven:** Bibliotheek Tremelo: Geheugen en dementie, Tremelo, Belgium, October 13 (2008)
- **Christine Van Broeckhoven:** Bibliotheek van Kortenberg & Curieus Afdeling Kortenberg: Alzheimer en dementie; Kortenberg, Belgium, May 14 (2008)
- **Christine Van Broeckhoven**: Brein en branie – een pionier in Alzheimer. Vormingplus regio Mechelen & cultuurcentrum De Mol in Lier, Lier, Belgium, May 13 (2009)
- **Christine Van Broeckhoven:** Brein en Branie, Gezinsbond Gewest Antwerpen, Mortsel, Belgium, March 29, 2010
- **Christine Van Broeckhoven**: Brein en Branie. De Uil van Minverva, Bilzen, September 30 (2009)
- **Christine Van Broeckhoven**: Brein en branie. Gezinsbond, Antwerpen, Belgium, May 18 (2009)
- **Christine Van Broeckhoven:** Business Professional Women (BPW) – Kortrijk: '"*Brein en Branie"* over Alzheimer', Kortrijk, Belgium, November 5 (2008)
- **Christine Van Broeckhoven:** Conferentieavonden -ten huize van': De ziekte van Alzheimer, Wemmel, Belgium, March 7 (2008)
- **Christine Van Broeckhoven:** Cultuurcentrum 'Poorthuis', Voordrachtenreeks 'Planeet Utopia': Geheugen en dementie, Peer, Belgium, February 12 (2008)
- **Christine Van Broeckhoven:** Curieus, Westmalle: De ziekte van Alzheimer en dementie, February 20 (2008)

- **Christine Van Broeckhoven**: De Mens voorbij? 20ste Gentse Feestendebatten, Gent, Belgium, July 25 (2009)
- **Christine Van Broeckhoven**: De ziekte van Alzheimer - Recente wetenschappelijke ontdekkingen. VormingPlus & de Vlaamse Alzheimer Liga, Scheldewinkede-Oosterzele, September 25 (2009)
- **Christine Van Broeckhoven:** De ziekte van Alzheimer, Markant – Artemis Brugge: Causerie, Zeebrugge, Belgium, April 28, 2010
- **Christine Van Broeckhoven:** De ziekte van Alzheimer, Volkshogeschool Elcker-ik, Antwerpen, Belgium, June 2, 2010
- **Christine Van Broeckhoven**: De ziekte van Alzheimer. Buitenbeentjes 2009 - Bibliotheken van het Waasland: Thema – Mens-en-kennis, Sint-Gillis-Waas, Belgium, October 12 (2009)
- **Christine Van Broeckhoven**: De ziekte van Alzheimer. Causerie Koninklijk Atheneum Sint-Truiden, Sint-Truiden, Belgium October 27 (2009)
- **Christine Van Broeckhoven**: Debat: Vrouw in de wetenschap. 20 jaar Medical Women Association Belgium, Brussel, Belgium, November 14 (2009)
- **Christine Van Broeckhoven:** Dementie en Alzheimer, Lommel Creatief & Curieus, Lommel, Belgium, January 27, 2010
- **Christine Van Broeckhoven**: Dementie en Alzheimer. Het Paleis (in samenwerking met Vlaams-Nederlands huis de Buren), Antwerpen, Belgium, December 15 (2009)
- **Christine Van Broeckhoven**: Dementie. Gemeentelijke Ouderenadviesraad – Gemeente Steenokkerzeel, Steenokkerzeel, Belgium, October 14 (2009)
- **Christine Van Broeckhoven:** Dementie... waar staat de wetenschap vandaag, Het Kompas - Praatcafé Dementie Haacht, Haacht, Belgium, March 23, 2010
- **Christine Van Broeckhoven:** Dienstencentrum 'BinnenHof': Geheugen en dementie, Neerpelt, Belgium, May 26 (2008)
- **Christine Van Broeckhoven:** Dienstencentrum 'De Vlaskapelle': Geheugen en dementie, Bissegem, Belgium, February 11 (2008)
- **Christine Van Broeckhoven**: Geheugen en Alzheimerdementie, hoe draagt erfelijkheid bij? Praatcafé 'Dementie' Bree – Bocholt – Meeuwen-Gruitrode, Meeuwen-Gruitrode, Belgium, June 2 (2009)
- **Christine Van Broeckhoven:** Geheugen en dementie, Club Montgomery, Belgium, Brussel, February 22, 2010
- **Christine Van Broeckhoven:** Geheugen en dementie, Curieus Wijnegem, Belgium, Wijnegem, February 23, 2010
- **Christine Van Broeckhoven:** Geheugen en dementie, Dienstencentra, Sociaal Huis Oostende, Oostende, Belgium, April 12, 2010
- **Christine Van Broeckhoven:** Geheugen en dementie, Familiegroep Alzheimer - regio Oostende (in samenwerking met AD Liga), Oostende, Belgium, April 12, 2010
- **Christine Van Broeckhoven:** Geheugen en dementie, Gemeentelijke Seniorenraad Essen (S.O.V. – Essen), Essen, Belgium, April 26, 2010
- **Christine Van Broeckhoven**: Geheugen en dementie, Lions Club Antwerp Airport, Schilde, Belgium, June 15 (2009)
- **Christine Van Broeckhoven:** Geheugen en dementie, Markante Dialogen Gent, Belgium, Gent, March 8, 2010
- **Christine Van Broeckhoven**: Geheugen en dementie. Bibliotheek De Eendracht te Dessel, Dessel, Belgium, November 30 (2009)
- **Christine Van Broeckhoven:** Gemeentelijke Openbare Bibliotheek Edegem: 'Op de koffie bij … Christine Van Broeckhoven', Edegem, Belgium, December 5 (2008)
- **Christine Van Broeckhoven:** Gemeentelijke seniorenadviesraad van Boechout: Geheugen en dementie, Boechout, Belgium, March 12 (2008)
- **Christine Van Broeckhoven:** Genetica van de ziekte van Alzheimer – Wat hebben we geleerd?, Vlaamse Alzheimer Liga – Regio Gent, Gent, Belgium, October 15, 2010
- **Christine Van Broeckhoven**: Genetica van de ziekte van Alzheimer, Zorg-Saam – Ik verlies elke dag (Studiedag over dementie), Wijgmaal, Belgium, October 9 (2009)
- **Christine Van Broeckhoven**: Genetica van de ziekte van Alzheimer. Vormingplus – Volkshogeschool Gent-Eeklo, Oosterzele, Belgium, February 27 (2009)
- **Christine Van Broeckhoven**: Genetica van de ziekte van Alzheimer. Bibliotheek Ter Elst te Gistel, Gistel, Belgium, November 18 (2009)
- **Christine Van Broeckhoven:** Gentse apothekers en biologen (GAB): De ziekte van Alzheimer: genezen, behandelen of voorkomen?, Gent, Belgium, April 22 (2008)
- **Christine Van Broeckhoven:** Het voorkomen van jongdementie: de cijfers & Conclusies en beleidsaanbevelingen, Mini-symposium 'Een gezicht geven aan jongdementie', Belgium, Vlaams Parlement, Brussel, February 23, 2010
- **Christine Van Broeckhoven:** Humanistisch vrijzinnig verbond waasland (HVV Waasland): Debat: Westerf feminisme versus Islamitisch feminisme, St. Niklaas, Belgium, February 22 (2008)
- **Christine Van Broeckhoven:** Is the brain the most sexy part of the body?, Opening 'De Singel': Jan Fabre & Christine Van Broeckhoven – Discussion, Antwerpen, Belgium, October 2, 2010
- **Christine Van Broeckhoven:** Jaarlijks Congress van het Verbond der Vlaamse Academici: Betere academici: de maatschappelijke roeping van de academicus, Leuven, Belgium, April 26 (2008)

- **Christine Van Broeckhoven**: Jong-Dement. Euregionale bijeenkomst 'Jong-Dement' van politici/beleidsmakers, Provinciehuis Maastricht, The Netherlands, November 25 (2009)
- **Christine Van Broeckhoven**: Jongdementie. Werelddag Dementie, Turnhout, Belgium, September 19 (2009)
- **Christine Van Broeckhoven:** Kiwanis Eeklo-Meetjesland: 'De ziekte van Alzheimer en het geheugen', Lembeke, Belgium, March 21 (2008)
- **Christine Van Broeckhoven:** Lezing Lodewijk De Raet: Leeftijdingen – Ontmoetingsnamiddagen: 'Geheugen en dementie', Cultureel Ontmoetingscentrum Sint Andries, Antwerpen, Belgium, September 25 (2008)
- **Christine Van Broeckhoven:** Lions – Antwerpen Zuid: Onderzoek naar Alzheimer, Antwerpen, Belgium, January 29 (2008)
- **Christine Van Broeckhoven:** Lustrumviering, Professional Women's Association: Geheugen en dementie, Brasschaat, Belgium, January 25 (2008)
- **Christine Van Broeckhoven**: Molecular genetics of frontotemporal lobar neurodegeneration. 10ème Réunion Francophone sur la maladie d'Alzheimer et les syndromes apparentés, Nantes, France, October 20-22 (2009)
- **Christine Van Broeckhoven:** Nederlandse Vereniging Neurologie Verpleegkundigen (NVNV): Erfelijkheid en dementie, Amsterdam,The Netherlands, February 15 (2008)
- **Christine Van Broeckhoven:** OKRA – Mechelen: Geheugen en dementie, Mechelen, Belgium, January 21 (2008)
- **Christine Van Broeckhoven:** Onderzoek in Evolutie, Werelddag dementie 2010 – 25 jaar Alzheimer Liga … 'dementie in beweging', Leuven, Belgium, September 18, 2010
- **Christine Van Broeckhoven:** Onderzoek naar de ziekte van Alzheimer & De ziekte van Alzheimer, SAO & Sociaal Huis Oostende, Oostende, Belgium, February 5, 2010
- **Christine Van Broeckhoven**: Over dementie: Een gesprek met Professor Christine Van Broeckhoven. Dementie Café Grimbergen, Grimbergen, Belgium, February 18 (2009)
- **Christine Van Broeckhoven:** Praatcafé 'Dementie' Dilbeek: Dementie, waar staat de wetenschap nu? Itterbeek, Belgium, March 17 (2008)
- **Christine Van Broeckhoven:** Praatcafé 'Dementie', Oostmalle 'Dwaallicht': Waar staat de wetenschap?, Oostmalle, Belgium, March 18 (2008)
- **Christine Van Broeckhoven:** Première "Verdwaald in het Geheugenpaleis" – Panelgesprek, Antwerpen, Belgium, September 14, 2010
- **Christine Van Broeckhoven:** Privacy & Research conference, Brussels, Belgium, November 23, 2010
- **Christine Van Broeckhoven:** Quercus (Stichting vrienden van de Geriatrie Zeeland): Wetenschap en dementie: hoop of hype?, Middelburg, The Netherlands, May 23 (2008)
- **Christine Van Broeckhoven:** Soroptimist Club Hasselt: 'Geheugen en dementie', Diepenbeek, Belgium, November 26 (2008)
- **Christine Van Broeckhoven**: Stand van zaken van de wetenschap mbt de dementieproblematiek. Vlaamse Alzheimer Liga & Familiegroep in Asse, Asse, Belgium, November 23 (2009)
- **Christine Van Broeckhoven**: The failing brain, Opening BRAI²N (Brain Research center Antwerp for Innovative and Interdisciplinary Neuromodulation), UZA, Antwerpen, Belgium, June 19 (2009)
- **Christine Van Broeckhoven:** Tribute symposium Josée Leysen "Women in Neuroscience": Genetics of neurodegenerative brain diseases, Beerse, Belgium, May 13 (2008)
- **Christine Van Broeckhoven**: Vergrijzing en dementie. Pluralistische Vereniging van Senioren, Hof van Aragon, Lier, October 5 (2009)
- **Christine Van Broeckhoven:** Vergrijzing, geheugen en dementie, Curieus Zwalm, Zwalm, Belgium, September 9, 2010
- **Christine Van Broeckhoven:** Vergrijzing, geheugen en dementie, Museum Dr. Guislain Gent & IPSOC-Bijscholing Kortrijk: Twee daags Symposium 'Uit het geheugen - over weten en vergeten', Kortrijk, Belgium, February 4, 2010
- **Christine Van Broeckhoven:** Vergrijzing, geheugen en dementie, Wetenschappelijk Interdisciplinair Seminarie (WIS) & The Friday Seminar (FriS) – Department of Mathematics and Computer Science of the University of Antwerp, Antwerpen, Belgium, April 28, 2010
- **Christine Van Broeckhoven**: Vergrijzing, geheugen en dementie. Rotary Meise-Bouchout, Wolvertem, Belgium, September 2 (2009)
- **Christine Van Broeckhoven**: Vergrijzing, geheugen en dementie. Soroptimist Club Brussel Iris, Brussel, September 16 (2009)
- **Christine Van Broeckhoven:** Vergrijzing, Sp.a Aartselaar, Belgium, Aartselaar, March 22, 2010
- **Christine Van Broeckhoven:** Vlaamse AlzheimerLiga: Dementie en wetenschap, Turnhout, Belgium, January 22 (2008)
- **Christine Van Broeckhoven:** Vormingplug Gent – Eeklo: Vorming in het teken van de Werelddag Dementie: De genetica van de ziekte van Alzheimer, recente wetenschappelijke ontdekkingen', Gent, Belgium, September 24 (2008)
- **Christine Van Broeckhoven:** Vrijzinnig Centrum Oostkamp 'De Molensteen': Debat: Euthanasie bij dementie – pro en contra, Oostkamp, Belgium, February 28 (2008)
- **Christine Van Broeckhoven:** Wereldag dementia 'Samen Dementie Draagbaar Maken': 'Openingstoespraak', Gent, Belgium, September 21 (2008)

- **Christine Van Broeckhoven**: Wetenschap en dementie: hoop of hype? Vitamine Q – Staden, Staden, Belgium, November 13 (2009)
- **Christine Van Broeckhoven:** Wetenschappelijk Onderzoek naar Alzheimer dementie, Tweedekansonderwijs – Brugge, Sint-Michiels Brugge, Belgium, October 22, 2010
- **Christine Van Broeckhoven:** Wetenschapscafe Antwerpen: 'Het onderzoek naar hersenziekten zoals de ziekte van Alzheimer', Antwerpen, Belgium, November 18 (2008)
- **Christine Van Broeckhoven:** WISE-netwerk – Technische Universiteit Eindhoven: 'Vrouwen in de Wetenschap', Eindhoven, The Netherlands, December 8 (2008)
- **Christine Van Broeckhoven:** Ziekte van Alzheimer, Openbare Bibliotheek Erme-Mere, Erpe-Mere, Belgium, January 18, 2010

Oral Presentations and Slide Sessions

International

- **Hans Wils**: Biochemical characterization of FTLD-TDP overexpression and knockout mouse models. Alzheimer & Dementia 5 (4-Suppl 1): 154 (O4-03-03) (2009), 12th International Conference on Alzheimer's disease (ICAD) Vienna, Austria, July 11-16 (2009)
- **Hans Wils:** Generation of FTLD-U mouse models: conditional Grn knockout and wild-type and mutant Htdp-43 overexpressing mice. 6th International Conference on Frontotemporal Dementias, Rotterdam, the Netherlands, September 3-5 (2008)
- **Ilse Gijselinck:** Genomic study of the chromosome 9 locus linked with FTLD and ALS, HUGOs 14th Human Genome Meeting, Montpellier, France, 18-21/05/2010
- **Ilse Gijselinck:** Identification of a novel chromosomal locus in a Belgian FTLD-MND family. Human Genome Meeting - HGM 2008, Hyderabad, India, September 27-30 (2008)
- **Ilse Gijselinck:** Linkage to chromosome 9p21 in a Belgian frontotemporal lobar degeneration family with motor neuron disease. Neurodegenerative Diseases 6(S1): 1100 (2009), 9th International Conference AD/PD - Alzheimer's and Parkinson's Diseases: Advances, Concepts and New Challenges, Prague, Czech Republic, March 11–15 (2009)
- **Jonathal Janssens:** Mice overexpressing mutant human TDP-43 develop a dose-dependent ALS motor neuron phenotype. http://www.biochemistry.org/Portals/0/Conferences/abstracts/SA120/SA120P004.pdf, Biochemical Society Focused Meeting - Models of Dementia: the good, the bad and the future, Cambridge, UK, 15–17/12/2010
- **Julie van der Zee**: A multigenerational family with inherited pathologically confirmed Creutzfeldt-Jakob disease unexplained by PRNP. Alzheimer & Dementia 5 (4-Suppl 1): 164 (O4-06-08) (2009), 12th International Conference on Alzheimer's disease (ICAD) Vienna, Austria, July 11-16 (2009)
- **Julie van der Zee**: CHMP2B C-truncating mutations in frontotemporal lobar degeneration. 6th International Conference on Frontotemporal Dementias, Rotterdam, the Netherlands, September 3-5 (2008)
- **Julie van der Zee:** TMEM106B is Associated with Frontotemporal Lobar Degeneration in a Flanders-Belgian Cohort of Clinically Diagnosed Patients. 7th International Conference on Frontotemporal Dementias - Hot Topic session, Indianapolis, IN, USA, 6-8/10/2010
- **Julie van der Zee:** TMEM106B the first common risk factor for FTLD: replication in a clinically diagnosed cohort of FTLD patients. Alzheimer's Association International Conference on Alzheimer's disease, Honolulu, HI, USA, 10-15/07/2010
- **Julie van der Zee:** VCP mutation in frontotemporal lobar degeneration with frequent TDP-43-positive intranuclear inclusions. 11th International Conference on Alzheimer's disease (ICAD), Chicago, Illinois, USA, July 26-31 (2008)
- **Kristel Sleegers**: PGRN mutations in AD, ALS and PD. 6th International Conference on Frontotemporal Dementias, Rotterdam, The Netherlands, September 3-5 (2008)
- **Kristel Sleegers**: Serum progranulin is a noninvasive biomarker for frontotemporal lobar degeneration. Neurodegenerative Diseases 6(S1): 1021 (2009), 9th International Conference AD/PD - Alzheimer's and Parkinson's Diseases: Advances, Concepts and New Challenges, Prague, Czech Republic, March 11–15 (2009)
- **Samir Kumar-Singh**: Fractal Analysis of amyloid plaques in Alzheimer's disease patients and mouse models. Alzheimer & Dementia 5 (4-Suppl 1): 107 (O2-03-01 (2009), 12th International Conference on Alzheimer's disease (ICAD) Vienna, Austria, July 11-16 (2009)
- **Samir Kumar-Singh:** Overexpression of wild-type TDP-43 Leads To Motor Neuron Degeneration and ALS-like phenotype in germline transgenic mice (Hot Session Symposium II): 12th International Conference on Alzheimer's Disease (ICAD) Vienna, Austria, July 11-16 (2009)
- **Samir Kumar-Singh:** Relationship between plaques and vascular beta-amyloid. 11th International Conference on Alzheimer's disease (ICAD), Chicago, Illinois, USA, July 26-31 (2008)
- **Sandra Pereson**: Progranulin correlates with dense-core plaque burden in Alzheimer's disease mouse models. Alzheimer & Dementia 5 (4-Suppl 1): 83 (O1-03-07) (2009), 12th International Conference on Alzheimer's disease (ICAD) Vienna, Austria, July 11-16 (2009)

National

- **Samir Kumar-Singh:** Molecular neuropathology of FTLD, Neurology Research Club, Academic Hospital of Antwerpen, June 12, 2009
- **Samir Kumar-Singh:** Molecular Pathology of Alzheimer's disease and frontrotemporal lobar degeneration Onderzoeksdag – Faculteit Farmaceutische, Biomedische en Diergeneeskundige Wetenschappen, October 30 (2009)
- **Sandra Pereson:** Role of progranulin in an Alzheimer disease. Annual Scientific IAP P6/43 meeting, Brussels, Belgium, October 26 (2009)
- **Tim Van Langenhove**: Clinical heterogeneity in two unrelated families linked to the valosin-containing protein p.R159H mutation. 8th Bi-annual Meeting of the Belgian Society for Neuroscience, Liège, Belgium, May 11 (2009)
- **Tim Van Langenhove**: Genetic contribution of FUS to Frontotemporal Lobar Degeneration. Annual Scientific IAP P6/43 meeting, Brussels, Belgium, October 26 (2009)
- **Tim Van Langenhove:** Genetic contribution of FUS to Frontotemporal Lobar Degeneration, VIB Science Club Genetics IV, Leuven, Belgium, 10/05/2010
- **Tim Van Langenhove:** Replication of genome-wide association findings on frontotemporal lobar degeneration in a Flanders-Belgian sample: TMEM106B a first risk factor for frontotemporal lobar degeneration, Capita Selecta in Complex Disease Analysis (CSCDA) meeting, Leuven, Belgium, August 25-27, 2010

Poster Presentations

International

- **Gernot Kleinberger:** Increased caspase activation and decreased TDP-43 solubility in progranulin knockout cortical cultures. Dementia and Geriatric Cognitive Disorders 30(S1): 10 (2010), 46 (P39), 7th International Conference on Frontotemporal Dementias, Indianapolis, United States, 6-8/10/2010
- **Gernot Kleinberger:** Progranulin (GRN) is directly involved in cell proliferation but not in caspase-activation in glial and non-glial cells. 11th International Conference on Alzheimer's disease (ICAD), Chicago, Illinois, USA, July 26-31 (2008)
- **Gernot Kleinberger:** Progranulin has growth modulatory property but is not directly involved in caspase-mediated apoptosis in glial and non-glial cell culture. 6th International Conference on Frontotemporal Dementias, Rotterdam, the Netherlands, September 3-5 (2008)
- **Gernot Kleinberger**: Survival apoptosis and characterization of TDP-43 in cells derived from progranulin knockout mice. Alzheimer & Dementia 5 (4-Suppl 1): 444-445 (P4-046) (2009), 12th International Conference on Alzheimer's disease (ICAD) Vienna, Austria, July 11-16 (2009)
- **Hans Wils:** Neuropathological and biochemical characterization of GRN mutation carriers and GRN conditional mice. 11th International Conference on Alzheimer's disease (ICAD), Chicago, Illinois, USA, July 26-31 (2008)
- **Ilse Gijselinck:** Genomic study of the chromosome 9 locus linked with FTLD and ALS. Dementia and Geriatric Cognitive Disorders 30(S1): 58 (2010), 7th International Conference on Frontotemporal Dementias, Indianapolis, USA, 6-8/10/2010
- **Ilse Gijselinck:** Genomic study of the chromosome 9 locus linked with FTLD and ALS. HUGOs 14th Human Genome Meeting, Montpellier, France, 18-21/05/2010
- **Ilse Gijselinck:** Identification of a novel chromosomal locus in a Belgian FTLD-MND family. 11th International Conference on Alzheimer's disease (ICAD), Chicago, Illinois, USA, July 26-31 (2008)
- **Ilse Gijselinck:** Identification of a novel chromosomal locus in a Belgian FTLD-MND family. 6th International Conference on Frontotemporal Dementias, Rotterdam, The Netherlands, September 3-5 (2008)
- **Ilse Gijselinck:** Identification of a novel chromosomal locus in a Belgian FTLD-MND family. Human Genome Meeting - HGM 2008, Hyderabad, India, September 27-30 (2008)
- **Jonathan Janssens:** Mice overexpressing mutant human TDP-43 develop a dose-dependent ALS motor neuron phenotype. Biochemical Society Focused Meeting - Models of Dementia: the good, the bad and the future, Cambridge, UK, 15–17/12/2010
- **Julie van der Zee:** TMEM106B is Associated with Frontotemporal Lobar Degeneration in a Flanders-Belgian Cohort of Clinically Diagnosed Patients. 7th International Conference on Frontotemporal Dementias - Hot Topic session, Indianapolis, IN, USA, 6-8/10/2010
- **Kristel Sleegers:** GAB2 and risk for Alzheimer's dementia in a Belgian population. 11th International Conference on Alzheimer's disease (ICAD), Chicago, Illinois, USA, July 26-31 (2008)
- **Kristel Sleegers:** Genetic variability in progranulin and apisodic memory. Biology of Cognition, Chantilly, France, October 16-18 (2008)
- **Nathalie Brouwers:** Genetic variability at the progranulin locus contributes to risk for clinically diagnosed Alzheimer disease. Ipsen Foundation - Intracellular traffic and neurodegenerative disorders, Paris, France, April 28 (2008)
- **Nathalie Brouwers:** Genetic variability at the progranulin locus contributes to risk for Alzheimer's disease. 11th International Conference on Alzheimer's disease (ICAD), Chicago, Illinois, USA, July 26-31 (2008)
- **Nathalie Brouwers:** Genetic variability at the progranulin locus contributes to risk for clinically diagnosed Alzheimer

disease. 6th International Conference on Frontotemporal Dementias, Rotterdam, the Netherlands, September 3-5 (2008)
- **Nathalie Brouwers**: No major role for TARDBP in Alzheimer genetic etiology. Neurodegenerative Diseases 6(S1): 950 (2009), 9th International Conference AD/PD - Alzheimer's and Parkinson's Diseases: Advances, Concepts and New Challenges, Prague, Czech Republic, March 11–15 (2009)
- **Nathalie Brouwers:** Variability at the progranulin locus contributes to risk for Alzheimer disease. XX International Congress of Genetics, Berlin, Germany, July 12-18 (2008)
- **Sandra Pereson:** Elucidating the role of progranulin in the pathogenesis of Alzheimer disease. Biochemical Society Focused Meeting - Models of Dementia: the good, the bad and the future, Cambridge, UK, 15–17/12/2010
- **Sandra Pereson:** Gene expression profiling to identify microvascular changes in Alzheimer's disease mouse models. 6th International Conference on Frontotemporal Dementias, Rotterdam, The Netherlands, September 3-5 (2008)
- **Tim Van Langenhove:** Genetic contribution of OPTN and ATXN2 intermediate-length polyglutamine expansions to frontotemporal lobar degeneration, 7th International Conference on Frontotemporal Dementias, Indianapolis, USA, 6-8/10/2010

National

- **Gernot Kleinberger**: Increased apoptosis in cortical cultures derived from progranulin knockout mice. Annual Scientific IAP P6/43 Meeting, University of Brussels (ULB), Brussels, Belgium, October 26 (2009)
- **Gernot Kleinberger**: Increased apoptosis in cortical cultures derived from progranulin knockout mice. VIB Science Club Neurodegenerative Diseases II, Leuven, Belgium, October 30 (2009)
- **Gernot Kleinberger:** Increased caspase activation and decreased TDP-43 solubility in progranulin knockout cortical cultures. 13th VIB Seminar, Blankenberge, Belgium, 4/03/2010
- **Gernot Kleinberger:** Progranulin (GRN) is directly involved in cell proliferation but not in caspase-activation in glial and non-glial cells. VIB Seminar 2008, Blankenberge, Belgium, March 6 (2008)
- **Hans Wils**: Biochemical characterization of FTLD-TDP overexpression and knockout mouse models. VIB Science Club Neurodegenerative Diseases II, Leuven, Belgium, October 30 (2009)
- **Hans Wils**: Wild-type human TDP-43 overexpression in transgenic mice causes ALS-like motor neuron degeneration with associated neuronal inclusions. Annual Scientific IAP P6/43 Meeting, University of Brussels (ULB), Brussels, Belgium, October 26 (2009)
- **Ilse Gijselinck**: A genome-wide linkage study in a multiplex FTLD-ALS family identifies two loci at chromosomes 9 and 14. Annual Scientific IAP P6/43 Meeting, University of Brussels (ULB), Brussels, Belgium, October 26 (2009)
- **Ilse Gijselinck:** Genomic study of the chromosome 9 locus linked with FTLD and ALS, Annual Scientific IAP P6/43 meeting, Leuven, Belgium, 25/10/2010
- **Nathalie Brouwers**: No major role for TARDBP in Alzheimer genetic etiology. Annual Scientific IAP P6/43 meeting, University of Brussels (ULB), Brussels, Belgium, October 26 (2009)
- **Sandra Pereson**: The role of progranulin in Alzheimer Disease. VIB Science Club Neurodegenerative Diseases II, Leuven, Belgium, October 30 (2009)
- **Tim Van Langenhove**: Genetic contribution of FUS to Frontotemporal Lobar Degeneration. VIB Science Club Neurodegenerative Diseases II, Leuven, Belgium, October 30 (2009)
- **Tim Van Langenhove:** Genetic contribution of OPTN and ATXN2 intermediate-length polyglutamine expansions to frontotemporal lobar degeneration, Annual Scientific IAP P6/43 Meeting, Leuven, Belgium, 25/10/2010

Final report of the research group of

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Functional analysis of novel adhesive and signaling proteins in development and tumorigenesis of neural tissues

1. Overview and mission statement

The **research topics** of the Molecular Cell Biology group at the Department for Molecular Biomedical Research (Ghent University - VIB) in the field of neurosciences were the following:
- functional analysis and manipulation of selected catenin genes in neural tissues;
- structure-function analysis of selected protocadherin genes;
- structure-function analysis of the completely new NBPF gene family with putative tumor suppressor activity in neural tissues.

Briefly, **catenins** are proteins that bind to the cytoplasmic domains of classic cadherins, which are well-known cell-cell adhesion molecules. They often form a physical bridge between cadherins and the cytoskeleton, but they can also be involved in cytoplasmic and intranuclear signaling processes. Catenin molecules we focus on are **αT-catenin**, which is related to the epithelial αE-catenin but is expressed also in the brain, and catenin p120 (**p120ctn**), which is a so-called Armadillo protein that is expressed in numerous isoforms, some of which are predominant in the brain.

Protocadherins are transmembrane proteins that differ in various aspects from classic cadherins. They are expressed predominantly in the brain, but their functions are largely unexplored. We are particularly interested in the delta-protocadherins.

NBPF (Neuroblastoma BreakPoint gene Family) is a new gene family that is presumably involved in **suppression** of **neuroblastoma**, a malignant tumor from undifferentiated neuroectodermal cells derived from the neural crest. We discovered the first member of this gene family at a balanced chromosomal breakpoint in a neuroblastoma patient. This gene family, which now includes 22 members, is intricate structurally and possibly functionally as well. Our current documented hypothesis is that NBPF proteins are candidate tumor suppressors involved in signaling processess in the cytoplasm.

2. Research on Catenins

2.A. αT-catenin: a novel α-catenin with tissue-restricted expression

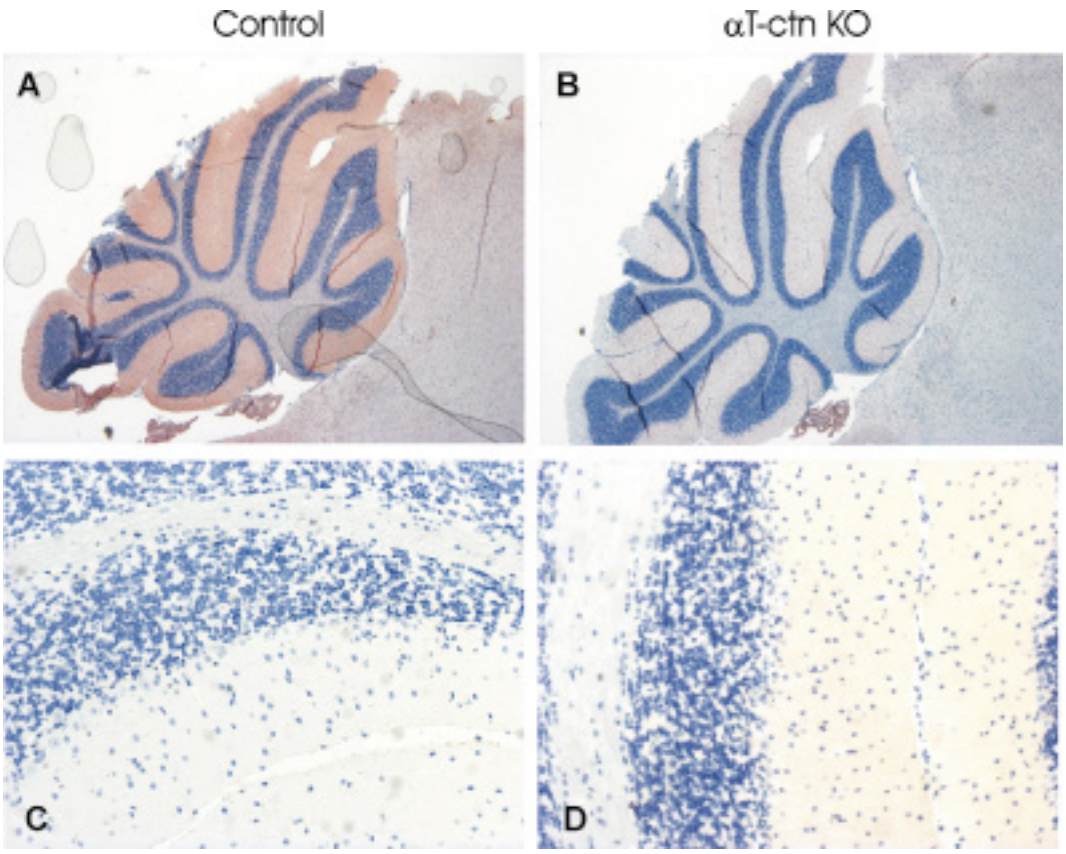
Classic cadherins interact homophilically via their extracellular domains, functioning as physical linkers between adjacent cell membranes. The cytoplasmic region of classic cadherins binds β-catenin, which in turn associates with **α-catenin**. Alpha-catenin is indispensable for cadherin-mediated cell adhesion. In the absence of α-catenin, the intercellular junctions are strongly affected, and in the case of tumors derived from epithelial cells this has severe consequences in the form of increased malignancy (Vermeulen et al., 1999).

Additional complexity exists, as there are three homologous α-catenin proteins: the rather ubiquitously expressed αE-catenin (Herrenknecht et al., 1991; Nagafuchi et al., 1991), neural αN-catenin (Hirano et al., 1992), and **αT-catenin** (Janssens et al., 2001). Loss of αN-catenin affects the stability of dendritic spines and synaptic contacts between neurons (Abe et al., 2004). For αT-catenin, we have demonstrated that it has a restricted expression pattern: from very strong expression in heart tissue, where it is co-

expressed with αE-catenin at intercalated discs, to strong expression in the peritubular myoid cells of testis, and modest expression in skeletal muscle and brain. Using antibodies raised 'in house' we showed localization of αT-catenin at specific cortical layers of the brain and in the molecular layer of the cerebellum. The mRNA of αT-catenin was detected in the granular layer (Vanpoucke et al., 2004) However, the function of αT-catenin in the brain remains poorly understood.

Human αT-catenin is encoded by *CTNNA3* (also called *VR22*). As this gene is positioned within a chromosome-10 region that has been linked to particular cases of familial **Alzheimer's disease** (AD), we assessed in collaboration the possible involvement of *CTNNA3* in this disabling disease. We showed that αT-catenin can inhibit Wnt signaling and meets the criteria for both a positional and a functional candidate for AD susceptibility (Busby et al., 2004). However, none of the *CTNNA3* SNPs in our study appeared to be strongly associated with chromosome-10-linked AD (Busby et al., 2004), whereas another study suggested the contrary (Ertekin-Taner et al., 2003). Whether particular variants or mutations of αT-catenin influence susceptibility to AD remains a matter of debate (Bertram et al., 2007; Martin et al., 2005; Miyashita et al., 2007).

Recently we were able to produce a mouse in which αT-catenin is ablated by conditional gene knock out (KO) (see Fig. 1A and B). We are now studying this mouse anatomically, histologically, physiologically, and behaviorally. This will allow us to prove or disprove whether αT-catenin plays an important role in neural tissues. For instance, the granular layer of the cerebellum of these KO mice shows fewer and poorly organized cell bodies (exemplified in Fig. 1C and D). Previous results from Rotarod testing suggested cerebellum-related movement defects in αT-catenin KO mice (Fig 1E). However, Rotarod testing may not be the most informative and sensitive behavioral assessment to identify primarily cerebellar defects. Indeed, this test does not reliably differentiate among defects in muscle, nerve, upper motor neuron or cerebellar motor control. To determine whether the neuronal acitivity in the brain is changed, we will analyze now the fluid-licking behavior in our mice. Fluid licking is a naturally occurring behavior that is easy to analyze in the home-cage environment (Heck et al., 2008).



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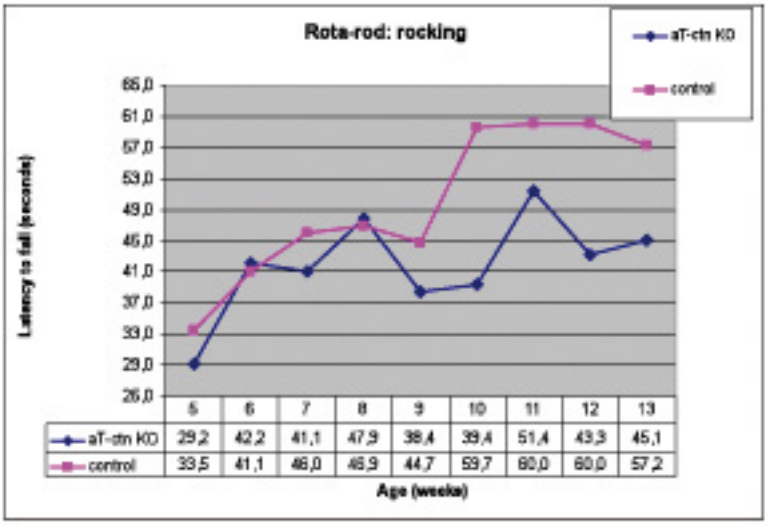

Figure 1: Cerebellar histology and functionality of αT-catenin KO mouse compared to WT mouse. (A, B) Immunohistochemical staining for αT-catenin in molecular layer of the cerebellum is greatly reduced in the αT-catenin KO mouse compared to wild type mouse (both 8 months old). (C, D) Cell density and organization of cell bodies is affected in the granular layer of the cerebellum of KO mouse compared to wild type mouse. (E) Rotarod analysis of motor neuron functioning in wild-type versus αT-catenin KO mice. Eleven control mice and fifteen KO mice were tested on a rotating Rotarod with the same speed at regualar intervals for nine consecutive weeks. The latencies from rotation onset until the mice fell off the rod were measured. Wild-type mice (pink squares) managed to stay longer on the rotating Rotarod than αT-catenin KO mice (blue triangles). Each data point represents the average measurement per mouse category.

2.B. Functional analysis of p120ctn isoforms in the brain

The aim of this part of the project was to study the role of **p120catenin** (p120ctn) isoforms in the brain using a transgenic approach. **Alternative splicing** of the human p120ctn gene *(CTNND1)* gives rise to 48 possible p120ctn isoforms originating from four start codons and four alternatively used exons (Keirsebilck et al., 1998). The alternatively used exon C encodes six amino acids that interrupt a nuclear localization sequence *(NLS)*. This interruption abrogates the p120ctn inhibition of RhoA, suggesting that the p120ctn isoform C acts as a physiological regulator of RhoGTPase activity in the brain. Indeed, RT-PCR and Q-PCR have shown strong expression of p120ctn isoform C in the brain. To assess the *in vivo* function of exon C, we generated p120ctn **exon-C-specific knock-out *(p120 KO-C)* and knock-in *(p120 KI-C)* mice**. Surprisingly, both KO-C/KO-C and KI-C/KI-C mice died as early as the blastocyst stage (3.5 dpc).

We are now analyzing the functionality of p120ctn isoform C beyond the developmental stage. For this purpose we used **p120 fl/fl** mice, in which exons with all four possible start codons are flanked by loxP sites (provided by Dr. A. Reynolds, Nashville, USA) (Davis and Reynolds, 2006). These mice were crossed with **Nestin-Cre mice** (Tronche et al., 1999), resulting in offspring lacking all p120ctn isoforms in the cerebellum and in all cortical layers of the brain. These KO mice showed higher levels of active RhoA in the brain compared to control mice whereas active Rac1 levels were unchanged. Nonetheless, the brain-confined p120ctn knock-out mice turned out to be viable and to develop brains of normal size and overall normal anatomy and histology.

To examine the influence of p120ctn exon C on the brain phenotype, we crossed the p120ctn fl/fl x Nestin-Cre mice with p120 KO-C/wt and p120 KI-C/wt mice. The offspring had a constitutive ablation/insertion of p120ctn exon C in one allele in combination with a brain-specific deletion of all p120ctn isoforms in the other allele. Both KOC/fl;Nestin-Cre and KIC/fl;Nestin-Cre mice were viable. Histological analysis of KOC/fl;Nestin-Cre mice revealed a medial hippocampal abnormality (Fig. 2A) that was not obvious more laterally (Fig. 2B).

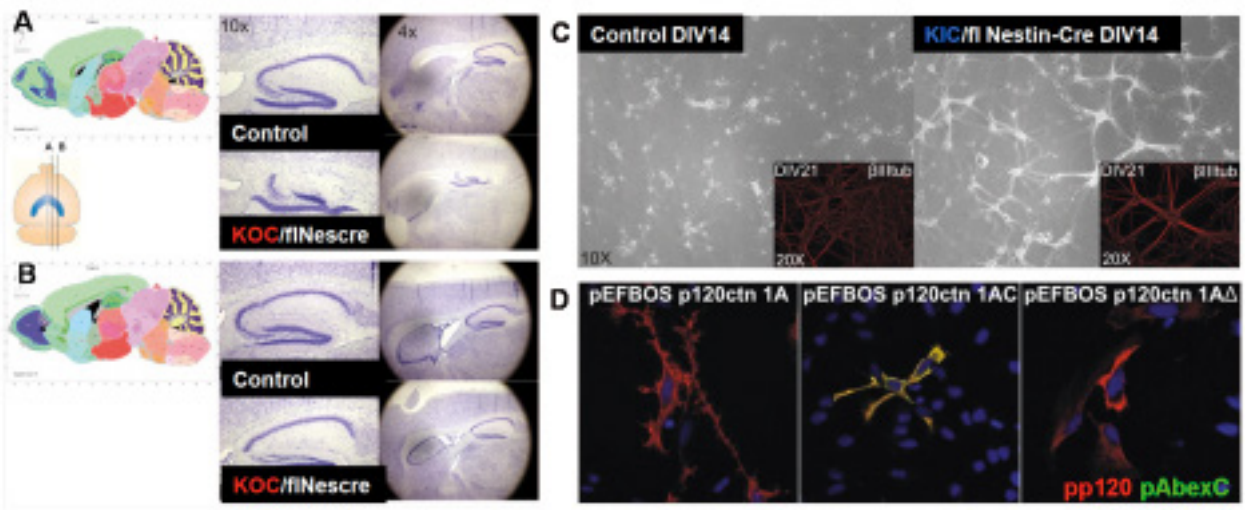

Figure 2. Analysis of p120ctn KOC/fl;Nestin-Cre and KIC/fl;Nestin-Cre mice. KOC/fl;Nestin-Cre mice show a medial (A), but not a lateral (B) hippocampal defect. KIC/fl;Nestin-Cre hippocampal cultures show a fasciculation phenotype (C) compared to control cultures. Inset: staining for neuronal marker βIII tubulin. (D) NIH3T3 cells transfected with p120ctn isoform 1A, p120ctn isoform 1AC or RhoA-uncoupled p120ctn (p120ctn 1AΔ), and stained for all p120ctn isoforms (pp120) and for p120ctn isoform C (pAbexC).

Microcephaly was observed in KIC/fl;Nestin-Cre mice, but this phenotype was not fully penetrant. Neuronal cultures derived from KIC/fl;Nestin-Cre hippocampi showed a predominant fasciculation phenotype compared to control hippocampal cultures (Fig. 2C). These data are in line with our *in vitro* results. Overexpression of p120ctn isoform 1A, which is the most abundant p120ctn isoform in the brain, led to a dendritic branching phenotype (Fig. 2D *left*) (Reynolds et al., 1996). In contrast, p120ctn isoform 1AC expression inhibited this branching phenotype (Fig. 2D *middle*) to a similar extent as a RhoA-uncoupled p120ctn mutant (Fig. 2D *right*). This reflects the ability of p120ctn isoforms to modulate the activity of **RhoGTPases**, which are known to influence normal dendritic spine density and morphology (Anastasiadis and Reynolds, 2000). Abnormal spine morphology is also seen in patients with nonsyndromic mental retardation and cognitive disorders (Govek et al., 2005). We are now investigating the RhoA status in the brains of our different mouse models and trying to dissect how RhoGTPase signaling in hippocampus-derived neuronal cultures is affected by using constitutively active or dominant-negative RhoA mutants, or by using pharmacological inhibitors and activators. Suitable assays will be used to assess potential behavioral alterations in these unique mouse lines. By determining the function of p120ctn and more particularly its isoform C in the brain, we hope to contribute to the understanding of particular human neurological disorders at the molecular level.

2.C. Functional analysis of p120ctn in neural crest cell development

Several cadherins play important roles and display dynamic spatial and temporal expression patterns during development of neural crest cells (NCC) (Taneyhill, 2008). NCC are pluripotent migratory cells arising from the dorsal neural tube via an epithelial-to-mesenchymal transition (Hay, 1995; Thiery, 2003). Disruption of normal cadherin function results in aberrant NCC delamination from the neural fold, migration abnormalities, or defective reaggregation after homing (Borchers et al., 2001; Bronner-Fraser et al., 1992; Luo et al., 2006). It is known that p120ctn plays an important role in regulating the adhesive strength of cadherins by inhibiting their degradation and controlling their level in cell junctions (Davis et al., 2003; Xiao et al., 2003).

The aim of this part of the project was to explore the function of p120ctn during NCC development. For that purpose we crossed p120 $^{fl/fl}$ mice (Davis and Reynolds, 2006) with Wnt1Cre mice (Danielian et al., 1998) to generate knockout mice in which p120ctn is specifically deleted from NCC. We showed that deficiency of p120ctn in NCC results in complex malformations of the ocular anterior segment structures, including corneal opacification, loss of iridocorneal angle and anterior chamber, corneal malformation, and hypoplastic iris and ciliary body. In addition, the mutant mice finally develop glaucoma because of loss of trabecular meshwork and Schlemm's canal. Previous *in vivo* fate mapping experiments revealed that NCC contribute substantially to the eye (Ittner et al., 2005). Expression analysis of p120ctn by immunohistochemistry at the E13.5 and P1 stages in p120$^{fl/fl}$;Wnt1Cre mice showed that its expression in developing ocular mesenchyme cells, corneal stroma and endothelium, and iridoconeal angle was substantially reduced in comparison to wild type mice (Fig. 3A).

To determine if the ocular defects are due to impaired NCC migration or to a differentiation defect, we introduced the Rosa26 reporter (Rosa26R) allele (Soriano, 1999) into mice with p120ctn floxed alleles. Then, by crossing with Wnt1Cre lines, the fate of eye cells with p120ctn ablation could be monitored in the progeny by histochemical staining for β-galactosidase. We took p120ctn$^{fl/+}$;Wnt1Cre;Rosa26R mice as control because they displayed no eye defects. We stained eyes at different stages but did not find any evidence for severe defects in either NCC genesis or NCC migration in p120$^{fl/fl}$;Wnt1Cre mice (Fig. 3B). Therefore, ocular defects in p120$^{fl/fl}$;Wnt1Cre mice might be due to a differentiation failure after correct migration and homing.

To investigate the mechanism underlying the ocular defect in p120$^{fl/fl}$;Wnt1Cre mice, we analyzed the expression levels of different cadherins and their associated catenins. Immunofluorescence staining revealed that the level of N-cadherin in mutant eyes was lower in the corneal iridocorneal and angle endothelium than in control eyes (Fig. 3C). Similarly, expression of β-catenin was also downregulated in the iridocorneal angle and corneal endothelium in mutant mice (Fig. 3C). Therefore, abnormal cell sorting following N-cadherin dysregulation could be the basis of the ocular defects.

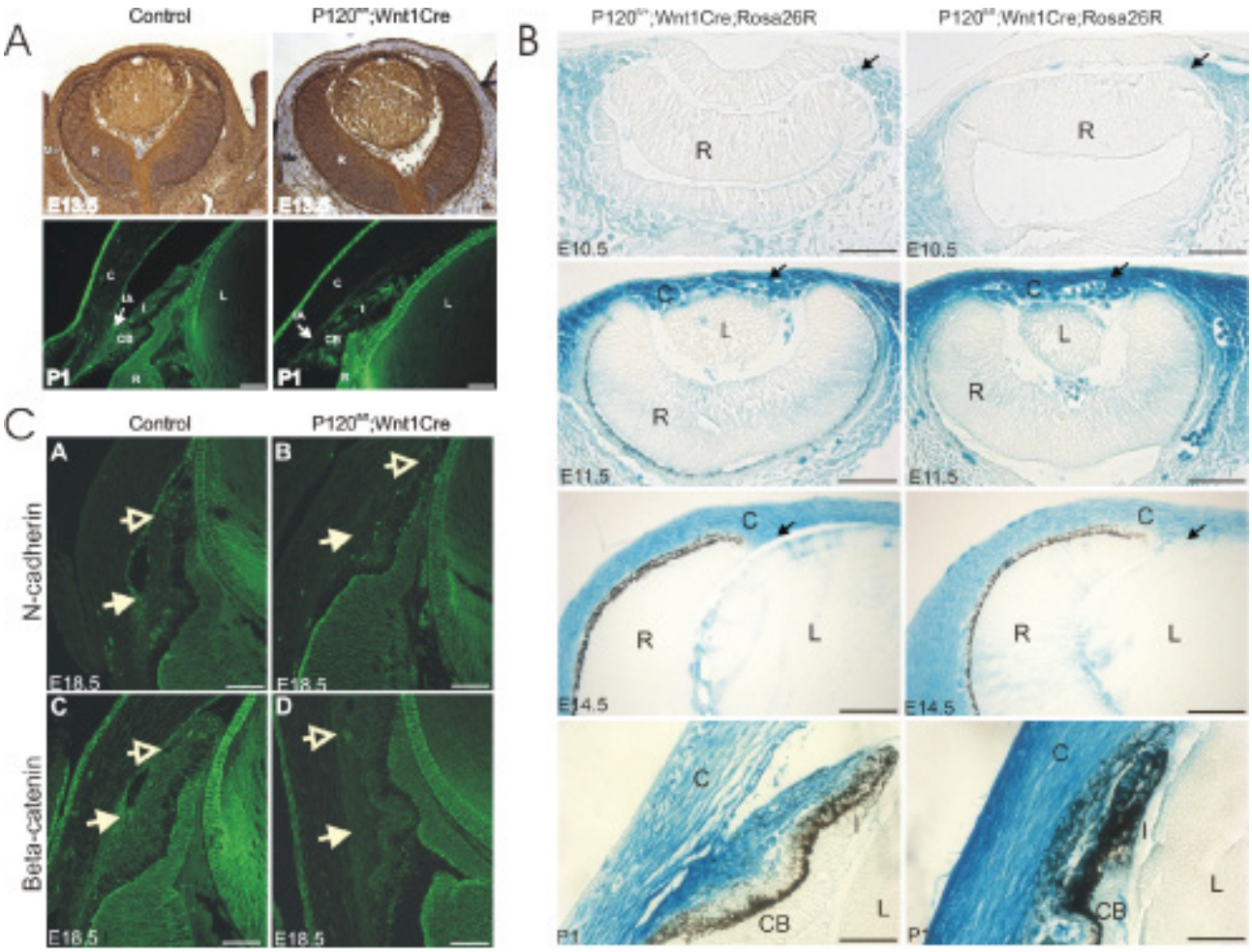

Figure 3. Analysis of eyes in mice with specific ablation of p120ctn in neural crest (NC) progenitor cells. (A) Analysis by immunostaining of the expression pattern of p120ctn in the developing eye. This indicated successful depletion of p120ctn in neural crest (NC)-derived ocular cells in p120$^{fl/fl}$;Wnt1Cre mice. (B) *In vivo* fate mapping of NC-derived ocular cells shows no defect in genesis or migration of NCC in p120$^{fl/fl}$;Wnt1Cre mice. (C) N-cadherin and β-catenin are downregulated in eyes of p120$^{fl/fl}$;Wnt1Cre mice compared to heterozygous p120$^{fl/+}$;Wnt1Cre control mice.

We then started the exploration of the molecular mechanism underlying the eye defects at the cell level. To this end, we analyzed the effect of shRNA-mediated p120ctn knockdown in the human trabecular meshwork cell (TMC) line GTM3 (a kind gift of the Alcon laboratories). These experiments are ongoing but showed already that the efficient knockdown of p120ctn in these TMC cells (Fig. 4A) results in downregulation of both cadherins and β-catenin (Fig. 4B), fully in line with our *in vivo* observations in the conditional KO mice.

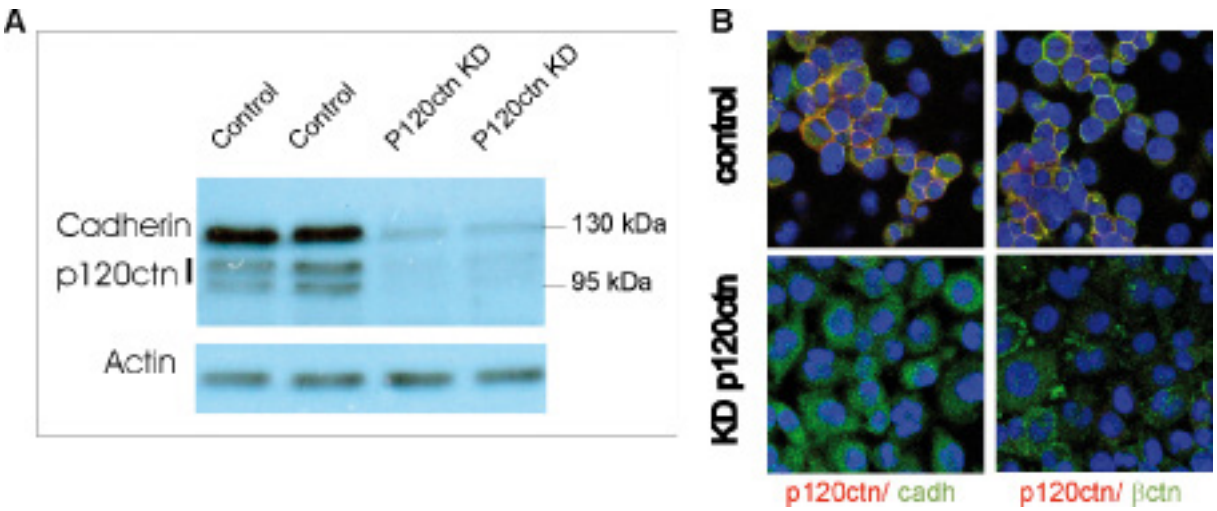

Figure 4. Effects of p120 down-regulation on adherens junctions in trabecular meshwork cells GTM3. The cells were transfected with a plasmid encoding p120-shRNA. (A) Cell lysates of established cell lines were subjected to Western blotting with antibodies directed to, respectively, p120ctn, pan-cadherin, and actin. The knockdown of p120ctn also caused downregulation of the cadherin expressed. (B) Co-immunofluorescence staining with antibodies to, respectively, p120ctn and pan-cadherin (left panels); or with antibodies to, respectively, p120ctn and β-catenin (right panels). p120ctn (red stain), cadherin and β-catenin (green stain) are enriched along the cell-cell junctions in p120ctn-expressing cells (top panels), whereas in p120-shRNA-expressing cells p120ctn is downregulated and residual cell-cell junctions show no staining for either cadherin or β-catenin (bottom panels).

In addition to malformations of the ocular anterior segment structures, exencephaly was observed. We started the analysis of this phenotype by using whole mount in situ hybridizations for transcripts of *Wnt1* (illustrated in Fig. 5), *Fgf8*, *Otx2* and *En1* (not shown). None of these markers used showed alterations in the mutant embryos as compared to the wild type embryos. Expression of other markers will now be explored at both the mRNA and the protein level.

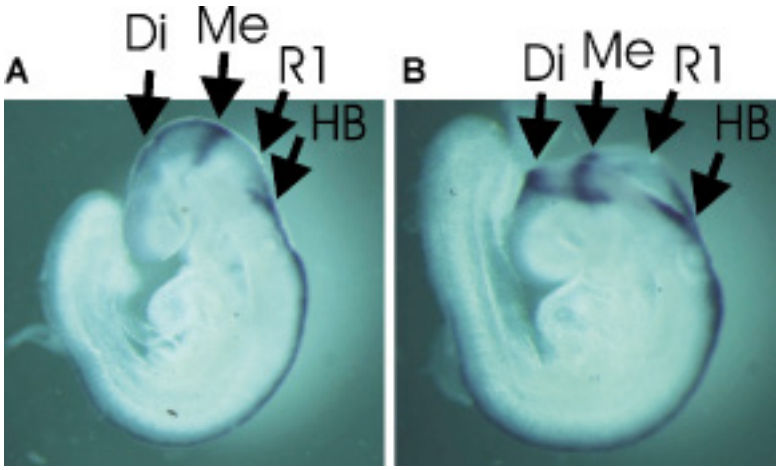

Figure 5: Whole-mount *in situ* hybridization for early brain marker Wnt1 in mouse embryos. (A) Wild-type and (B) p120$^{fl/fl}$;Wnt1Cre E9.5 mouse embryos. *Wnt1* expression shows: a transverse band at the posterior end of the midbrain and in a stripe along the dorsal midline of the mesencephalon (Me), diencephalon (Di) and hindbrain (HB) with a gap of expression in the R1 region of the metencephalon.

3. Role of Delta-Protocadherins in Neural Tissues

With more than 80 different protocadherin (Pcdh) genes in man and mouse and an increasing number of splice variants, these genes comprise a major group within the cadherin gene superfamily (Hulpiau and van Roy, 2009). They differ from classic cadherin genes in numerous aspects and are expressed predominantly in neural tissues (Redies et al., 2005). Protocadherins can be divided into clustered α-, β- and γ-protocadherin genes, and nonclustered δ-protocadherin (δ-Pcdhs) genes. In the present project we aim to characterize selected human δ-protocadherins (δ-PCDHs) and their mouse orthologs and to examine their roles in brain development and neurological disorders such as mental illness, dementia and brain tumors. Part of our research strategy is to conditionally knock out the *Pcdh10* gene in the mouse, and another part is to ectopically express different isoforms of human PCDH11, also in the mouse.

It was recently demonstrated that mice with total *Pcdh10* knockout in the germline develop severe defects in growth of striatal axons and thalamocortical projections (Uemura et al., 2007). These mice die within several weeks after birth, which prevents functional and pathological studies at an older age. To avoid this early death, we started the generation of conditional *Pcdh10* knockout mouse models that allow deletion of Pcdh10 in a tissue- and time-specific manner. On the one hand, we are establishing a model in which all isoforms of Pcdh10 can be knocked out by the Cre-LoxP technology (Fig. 4A). We are quite happy now that, after a long disappointing period of technical failures, correctly targeted ES cells have been identified by us recently. These ES cells will now be used for blastocyst injections and for further generation of conditional *Pcdh10* knockout mice.

On the other hand, we are generating a mouse model in which only the long isoforms of Pcdh10 are conditionally knocked out, resulting in lack of the conserved cytoplasmic domains CM1 and CM2 in the encoded Pcdh10 proteins (Fig. 4B). This model will be used to explore the role of these conserved domains in various intracellular signaling pathways. These mice with various floxed parts of the *Pcdh10* gene will then be crossed with different Cre mice in order to elucidate the role of Pcdh10 in important processes, such as controlled cell proliferation, cell migration, cell differentiation, and programmed cell death. To date, we are happy that mice with correctly targeted *Pcdh10* alleles were born and are currently crossed with first Flp-e transgenic mice to remove the neoR cassette followed by different Cre lines to obtain mice with tissue-specific knockout of long isoforms of Pcdh10.

Besides studying δ-protocadherins during mouse development, we are trying to interpret their functions during development of the frog *Xenopus tropicalis* development. For this purpose, we first cloned the cDNA probes for each gene and analyzed the expression patterns by *in situ* hybridization on different stages of *X. tropicalis* embryos. The results revealed that several δ-protocadherin genes were expressed in the neural tissues of *X. tropicalis*. Both Pcdh10 and Pcdh19 are expressed in the eyes and brain. Pcdh20 is expressed in the olfactory vesicle and otic vesicle. We will investigate the functional implications of the above protocadherins by applying the morpholino (MO)-knockdown technique as well as overexpression experiments. Preliminary data revealed eye defects in the Pcdh10 MO-knockdown embryos, including smaller eyes and disorganized retina and lens. We are currently working on the mechanism underlying this phenotype.

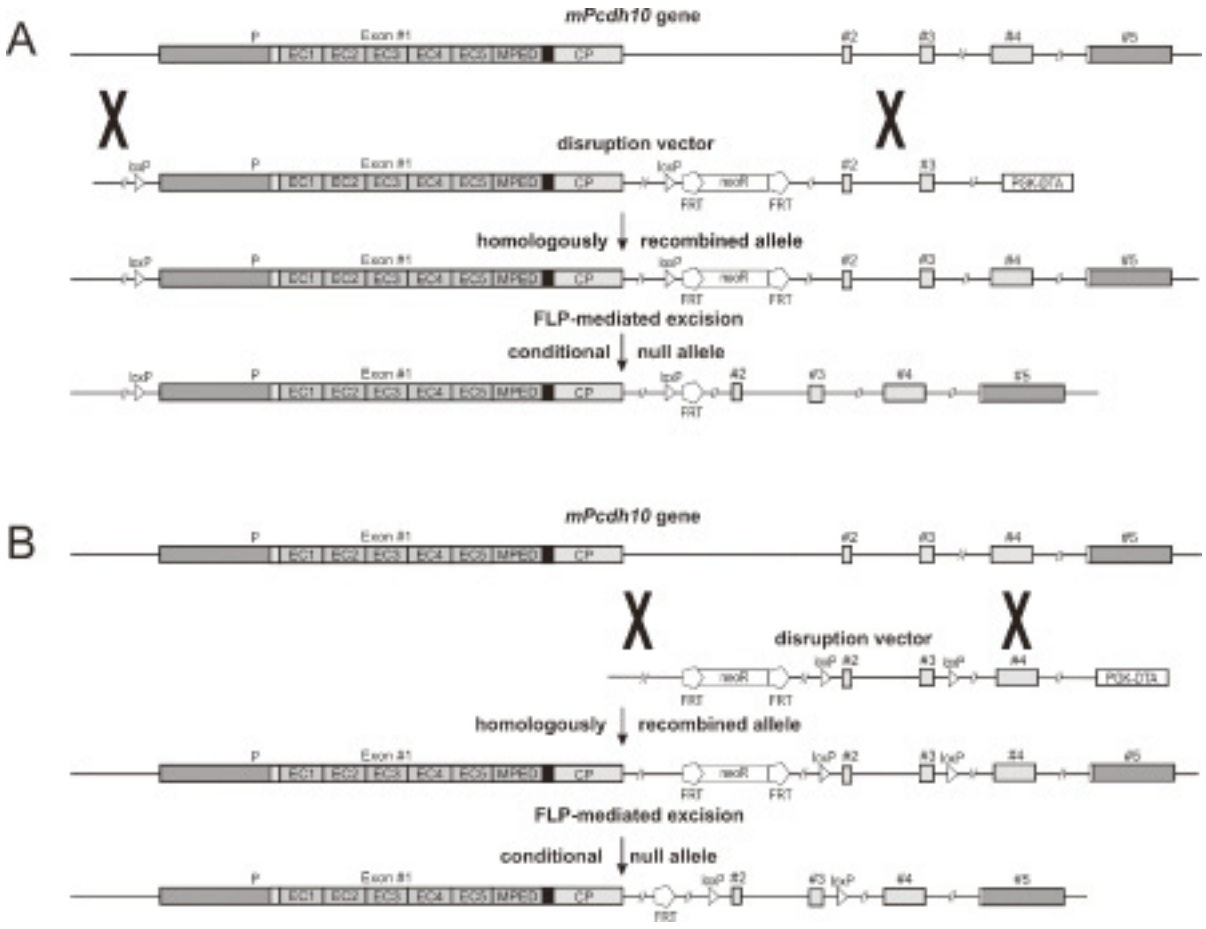

Figure 4. Construction of *Pcdh10* knockout alleles affecting either all isoforms of protocadherin-10 (A) or only the long isoform (B). Embryonic stem (ES) cells with a homologously recombined *Pcdh10* allele can be selected by using a positive selection marker (neoR gene) in combination with loss of the negative selection marker (in a modified procedure: gancyclovir-sensitive TK, HSV thymidine kinase instead of DTA, diphtheria toxin subunit A). A floxed, conditional null *Pcdh10* allele is obtained upon excision of the neo^R gene by the action of the FLP recombinase at FRT sites. Action of Cre recombinase on the floxed alleles deletes most of the PCDH10 protein in (A) but only the elongated cytoplasmic tail of the longer isoform in (B). The latter tail comprises the CM1 and CM2 conserved motifs.

In humans, the closely related δ-protocadherins **PCDH11X** and **PCDH11Y** are encoded by chromosomes X and Y, respectively. PCDH11X has mammalian and vertebrate orthologs but PCDH11Y exists only in man (Wilson et al., 2006). Genetic variation in PCDH11X was most recently reported to be associated with susceptibility to late-onset Alzheimer's disease (Carrasquillo et al., 2009). A cytoplasmic variant of PCDH11Y has been associated with the Wnt signaling pathway and tumor formation (Terry et al., 2006; Yang et al., 2005). To generate informative animal models, we attempt to ectopically and conditionally express human PCDH11X and PCDH11Y in the mouse. For this purpose, we constructed tetracycline inducible constructs complementing the *hprt* locus (to achieve single vector integration). cDNA inducibility by doxycyclin was validated before transfection into hprt-deficient ES cells. Induced proteins were localized via an attached E-tag. In the meantime, the constructs have been transfected and correct vector integrations were confirmed by Southern blot analysis. Chimeric mice were born and found to transmit the transgene to their germline. At present, these mice have been bred to homozygosity and are crossed also with various Cre mouse lines in order to induce tissue-specific ectopic expression of PCDH11 isoforms. Eventually, this will be followed by breeding to various mouse tumor models, in particular prostate cancer models.

Because our knowledge of the signaling functions of δ-Pcdhs is quite limited, we recently initiated screenings by Y2H (Yeast 2-Hybrid) and MAPPIT (MAmmalian Protein-Protein Interaction Trap)

(Eyckerman et al., 2001) to identify novel intracellular molecules specifically interacting with these Pcdhs. For PCDH11X, three strong and two moderate interactors were identified and the molecular mechanism of interaction and its functional implications are under investigation. One of the strong interactors is dynein light chain 1 (DYNLT1), which is a part of the dynein motor complex but has also been reported to have dynein motor-independent functions (reviewed in Vallee et al., 2004). DYNLT1 has been shown to influence actin dynamics during neurite outgrowth (Chuang et al., 2005) and to have a negative effect on neurogenesis (Gauthier-Fisher et al., 2009). Using mutated or shortened constructs of the PCDH11X cytoplasmic tail and of DYNLT1 in an analytical MAPPIT assay, the interaction domains of the two partners were narrowed down. The results obtained with these constructs were unequivocal and the interaction seemed to be isoform-specific, as it could not be confirmed for PCDH11Y. Furthermore we found that DYNLT1 also interacts with PCDH10. For both PCDH10 and PCDH11X this occurred in a DYNLT1 phosphorylation dependent way. We are currently attempting to identify the functional implication of the PCDH-DYNLT1 complexes by endogenous pulldown experiments, analysis of selected tissue samples, and siRNA-mediated knockdown. We use these approaches routinely in our department. Functional consequences will be assessed by assays of cell aggregation, analysis of neurite extension, evaluation of dendritic spine mobility, etc.

Apart from our functional analyses, we are also interested in the mechanisms controlling the expression of the δ-PCDHs. In one approach, we searched for microRNAs regulating the mRNA stability of the δ-PCDHs. This was complemented by an additional study, where the 3'UTRs of different δ-PCDHs were fused to a luciferase reporter gene, followed by testing of various microRNAs for their respective ability to inhibit translation. So far, our experiments identified two microRNAs negatively controlling either *PCDH1* or *PCDH7* expression levels. Interestingly, the expression of the microRNA targeting the PCDH7 transcript shows an inverted correlation with the increased PCDH7 mRNA levels in advanced tumor types of neural origin. Future research will focus on the functional analysis of the observed interplay between microRNAs and δ-PCDHs, both in normal neuronal cells and in tumors derived from the central nervous system.

4. NBPF1: a novel neuroblastoma suppressor gene?

We identified the ***NBPF1* gene** (Neuroblastoma BreakPoint Family member 1) while cloning the **breakpoint** of a constitutional **translocation** t(1;17)(p36.2;q11.2) discovered in a **neuroblastoma** (NB) patient (Laureys et al., 1995; Vandepoele et al., 2008; Vandepoele et al., 2005). NBs originate from primitive, pluripotent, sympathetic nerve cells derived from the neural crest. These cells can differentiate into the different normal tissues of the sympathetic nervous system, such as the spinal sympathetic ganglia, Schwann cells, and adrenal chromaffin cells. As a result, NBs develop where these neural cells are normally located, most frequently in the adrenal medulla or in the chest cavity. NBs frequently have aberrations of the chromosomal regions 1p36 and 17q11, which are involved in the translocation that we cloned. Hence, we hypothesized that the t(1;17)(p36.2;q11.2) translocation, which we characterized, predisposed the patient to tumor development due to disruption of the *NBPF1* gene.

By quantitative RT-PCR we showed a decreased level of the *NBPF* transcripts in certain types of neuroblastoma cell lines (Vandepoele et al., 2008). Additionally, expression profiling of the *NBPF1* gene showed that its expression is significantly weaker in cell lines with heterozygous loss of *NBPF1* than in cell lines with a normal 1p chromosome (Vandepoele et al., 2008). A similar tumor-associated downregulation was observed for only 15-20% of the genes located in this region, which suggests

that the downregulation of at least some of these genes is functionally involved in neuroblastoma pathogenesis (Janoueix-Lerosey et al., 2004). This points to involvement of additional mechanisms besides loss of heterozygosity in the downregulation of some of these genes.

To investigate the factors controlling the expression of *NBPF1*, we isolated its promoter region. Interestingly, this promoter had been copied from an unrelated gene, *EVI5*, after the divergence between simians and prosimians, but before simian radiation (Vandepoele et al., 2009). Like *NBPF1*, *EVI5* was also first identified by virtue of its disruption by a constitutional translocation in a neuroblastoma patient (Roberts et al., 1998), suggesting that the *NBPF1/EVI5* genes are causally related to neuroblastoma. This link was further strengthened by a recent publication describing how copy number variations in an *NBPF* family member were associated with an increased chance of developing neuroblastoma (Diskin et al., 2009). Furthermore, we have shown that expression of NBPF1 in a human colorectal cell line severely suppressed colony formation in soft agar, demonstrating that NBPF1 might act as a tumor suppressor gene (Vandepoele et al., 2008), at least in colon cancer. So far, technical difficulties have prevented us from testing this hypothesis in neuroblastoma cell lines. As colorectal cancer is also characterized by frequent deletions or translocations of 1p36 (Schwab et al., 1996), we believe that our present data represent a first step in the elucidation of the potential tumor suppressive properties of *NBPF1*.

By searching for **NBPF1-interacting proteins**, we obtained additional evidence that NBPF1 has a role in formation of tumors in the nervous system. For instance, we found that the amino-terminal domain of NBPF1 interacts with the Chibby protein, an antagonist of the Wnt/Wingless pathway, which plays a key role in neural crest development and oncogenesis (Takemaru et al., 2003). However, further functional investigation of this interaction showed no influence of the binding between Chibby and NBPF1 on Wnt signaling, as measured by TOPFLASH activity. Moreover, no competition between β-catenin and NBPF1 was observed in their interactions with Chibby. This suggests the existence of additional, possibly NBPF-modulated functions for Chibby besides its repressor function in the Wnt pathway (Vandepoele et al., 2010). These putative functions of Chibby, which are independent of nuclear β-catenin but might involve cytoplasmic NBPF1, await further study.

Apart from investigating the interaction between NBPF1 and Chibby, we are also searching for other NBPF1-binding partners. Using affinity purification of endogenous NBPF1 and mass spectrometric analysis of the proteins in the complex, we identified a particular mitochondrial protein as another putative interaction partner for NBPF1. Although NBPF1 is a cytoplasmic protein and an interaction with a mitochondrial protein therefore appears to be an artifact, we demonstrated that NBPF1 interacts only with the precursor form, which is present in the cytoplasm, and not with the mature processed form, which is present within mithochondria. Therefore, we hypothesize that NBPF1 ensures proper folding of nascent polypeptide chains during protein translation or is required for proper shuttling from the cytoplasm to the mitochondria.

As the *NBPF1* gene might play an important role in both normal and cancerous neuronal cells, we are currently investigating the role of NBPF1 during the **cell cycle**. Transient overexpression of NBPF1 cDNA in HEK293T cells followed by flow cytometric analysis revealed that NBPF1-overexpressing cells were blocked in the G1-stage of the cell cycle, and this could be explained by increased expression of the CDK-inhibitor p21. We could show that this increased expression of p21 is dependent on p53, since no such increase was seen in NBPF1-expressing DLD1 colon cancer cells, mutated for the *TP53* gene.

Immunohistochemistry of human skin with a new, commercially available, antibody against NBPF (Santa Cruz, sc-82241) showed a strong and specific staining of the supra-basal layer of the epidermis. We used mouse skin as a negative control since the murine genome does not encode NBPF orthologs (Vandepoele et al., 2005) and could not observe any staining in the supra-basal layer of the mouse skin. Therefore, we conclude that the observed staining in the human skin is specific for NBPF. It is also worth mentioning that there is almost no signal observed in the proliferative basal layer of the skin, which is in line with the previously observed cell cycle block upon NBPF1 overexpression. Additionally, we stained human cervix, another tissue with a squamous epithelium. Although the staining was less homogeneous compared to the human skin stainings (possibly due to aging sections), we also found the highest signal in the supra-basal layer and not in the proliferative layer. On the other hand, a cervix tumor stained almost completely negative for NBPF, again nicely in correspondence with the putative tumor suppressor role of NBPF. In the future, we would like to expand the panel of normal tissues and their corresponding tumors so that a more significant conclusion can be drawn.

To further strengthen our present cell cycle data, we are presently exploring the use of the **COFRADIC technology**, which allows detection of differential expression at the protein level (in collaboration with Prof. Kris Gevaert, Ghent University). We prefer COFRADIC analysis over microarrays, since NBPF is found by us to be a cytoplasmic protein and therefore we assume that NBPF will influence rather RNA translation or protein stability than transcription, although at this moment we cannot exclude posttranscriptional influences on mRNA stability. In addition, we have recently started a collaboration with Prof. James M. Sikela at the University of Colorado on NBPF. His lab will perform a transcriptome-wide differential gene expression profiling of our colon cancer cell line derivatives DLD1Tr21/Mock and DLD1Tr21/NBPF1, the latter expressing NBPF1 in an inducible fashion. In that way, the COFRADIC experiments in Ghent and the mRNA-microarray experiments in the USA may nicely complement each other.

References

(Publications of Van Roy's research group are indicated by *)

- * Abe, K., O. Chisaka, F. van Roy, and M. Takeichi. 2004. Stability of dendritic spines and synaptic contacts is controlled by alphaN-catenin. *Nat. Neurosci.* 7:357-363.
- Anastasiadis, P.Z., and A.B. Reynolds. 2000. The p120 catenin family: complex roles in adhesion, signaling and cancer. *J. Cell Sci.* 113:1319-1334.
- Bertram, L., K. Mullin, M. Parkinson, M. Hsiao, T.J. Moscarillo, S.L. Wagner, K.D. Becker, G. Velicelebi, D. Blacker, and R.E. Tanzi. 2007. Is alpha-T catenin (VR22) an Alzheimer's disease risk gene? - art. no. e63. *J. Med. Genet.* 44:E63.
- Borchers, A., R. David, and D. Wedlich. 2001. Xenopus cadherin-11 restrains cranial neural crest migration and influences neural crest specification. *Development*. 128:3049-3060.
- Bronner-Fraser, M., J.J. Wolf, and B.A. Murray. 1992. Effects of antibodies against N-cadherin and N-CAM on the cranial neural crest and neural tube. *Dev. Biol.* 153:291-301.
- * Busby, V., S. Goossens, P. Nowotny, G. Hamilton, S. Smemo, D. Harold, D. Turic, L. Jehu, A. Myers, M. Womick, D. Woo, D. Compton, L.M. Doil, K.M. Tacey, K.F. Lau, S. Al-Saraj, R. Killick, S. Pickering-Brown, P. Moore, P. Hollingworth, N. Archer, C. Foy, S. Walter, C. Lendon, K. Iwatsubo, J.C. Morris, J. Norton, D. Mann, B. Janssens, H. J., M. O'Donovan, J. L., W. J., P. Holmans, M.J. Owen, A. Grupe, J. Powell, J. van Hengel, A. Goate, F. Van Roy, and S. Lovestone. 2004. Alpha-T-catenin is expressed in human brain and interacts with the Wnt signalling pathway but is not responsible for linkage to chromosome 10 in Alzheimer's disease. *Neuromol. Med.* 5:133-146.
- Carrasquillo, M.M., F. Zou, V.S. Pankratz, S.L. Wilcox, L. Ma, L.P. Walker, S.G. Younkin, C.S. Younkin, L.H. Younkin, G.D. Bisceglio, N. Ertekin-Taner, J.E. Crook, D.W. Dickson, R.C. Petersen, N.R. Graff-Radford, and S.G. Younkin. 2009. Genetic variation in PCDH11X is associated with susceptibility to late-onset Alzheimer's disease. *Nat. Genet.* 41:192-198.
- Chuang, J.Z., T.Y. Yeh, F. Bollati, C. Conde, F. Canavosio, A. Caceres, and C.H. Sung. 2005. The dynein light chain Tctex-1 has a dynein-independent role in actin remodeling during neurite outgrowth. *Dev. Cell*. 9:75-86.
- Danielian, P.S., D. Muccino, D.H. Rowitch, S.K. Michael, and A.P. McMahon. 1998. Modification of gene activity in mouse embryos in utero by a tamoxifen-inducible form of Cre recombinase. *Curr. Biol.* 8:1323-1326.
- Davis, M.A., R.C. Ireton, and A.B. Reynolds. 2003. A core function for p120-catenin in cadherin turnover. *J. Cell Biol.* 163:525-534.
- Davis, M.A., and A.B. Reynolds. 2006. Blocked acinar development, E-cadherin reduction, and intraepithelial neoplasia upon ablation of p120-catenin in the mouse salivary gland. *Dev. Cell*. 10:21-31.
- Ertekin-Taner, N., J. Ronald, H. Asahara, L. Younkin, M. Hella, S. Jain, E. Gnida, S. Younkin, D. Fadale, Y. Ohyagi, A. Singleton, L. Scanlin, M. de Andrade, R. Petersen, N. Graff-Radford, M. Hutton, and S. Younkin. 2003. Fine mapping of the alpha-T catenin gene to a quantitative trait locus on chromosome 10 in late-onset Alzheimer's disease pedigrees. *Hum. Mol. Genet.* 12:3133-3143.
- Eyckerman, S., A. Verhee, J. Van der Heyden, I. Lemmens, X. Van Ostade, J. Vandekerckhove, and J. Tavernier. 2001. Design and application of a cytokine-receptor-based interaction trap. *Nat. Cell Biol.* 3:1114-1119.
- Gauthier-Fisher, A., D.C. Lin, M. Greeve, D.R. Kaplan, R. Rottapel, and F.D. Miller. 2009. Lfc and Tctex-1 regulate the genesis of neurons from cortical precursor cells. *Nat. Neurosci.* 12: 735-744.
- Govek, E.E., S.E. Newey, and L. Van Aelst. 2005. The role of the Rho GTPases in neuronal development. *Genes Dev.* 19:1-49.
- Hay, E.D. 1995. An overview of epithelio-mesenchymal transformation. *Acta Anat (Basel)*. 154:8-20.
- Heck, D.H., Y. Zhao, S. Roy, M.S. LeDoux, and L.T. Reiter. 2008. Analysis of cerebellar function in Ube3a-deficient mice reveals novel genotype-specific behaviors. *Hum Mol Genet*. 17:2181-9.
- Herrenknecht, K., M. Ozawa, C. Eckerskorn, F. Lottspeich, M. Lenter, and R. Kemler. 1991. The uvomorulin-anchorage protein alpha-catenin is a vinculin homologue. *Proc. Natl. Acad. Sci. U.S.A.* 88:9156-9160.
- Hirano, S., N. Kimoto, Y. Shimoyama, S. Hirohashi, and M. Takeichi. 1992. Identification of a neural alpha-catenin as a key regulator of cadherin function and multicellular organization. *Cell*. 70:293-301.
- * Hulpiau, P., and F. van Roy. 2009. Molecular evolution of the cadherin superfamily. *Int. J. Biochem. Cell Biol.* 41:343-369.
- Ittner, L.M., H. Wurdak, K. Schwerdtfeger, T. Kunz, F. Ille, P. Leveen, T.A. Hjalt, U. Suter, S. Karlsson, F. Hafezi, W. Born, and L. Sommer. 2005. Compound developmental eye disorders following inactivation of TGFbeta signaling in neural-crest stem cells. *J Biol*. 4:11.
- Janoueix-Lerosey, I., E. Novikov, M. Monteiro, N. Gruel, G. Schleiermacher, B. Loriod, C. Nguyen, and O. Delattre. 2004. Gene expression profiling of 1p35-36 genes in neuroblastoma. *Oncogene*. 23:5912-5922.
- * Janssens, B., S. Goossens, K. Staes, B. Gilbert, J. van Hengel, C. Colpaert, E. Bruyneel, M. Mareel, and F. van Roy. 2001. aT-Catenin: A novel tissue-specific b-catenin-binding protein mediating strong cell-cell adhesion. *J. Cell Sci.* 114:3177-3188.

- * Keirsebilck, A., S. Bonné, K. Staes, J. van Hengel, F. Nollet, A. Reynolds, and F. van Roy. 1998. Molecular cloning of the human p120^{ctn} catenin gene (CTNND1): Expression of multiple alternatively spliced isoforms. *Genomics*. 50:129-146.
- Laureys, G., F. Speleman, R. Versteeg, P. van der Drift, A. Chan, J. Leroy, U. Francke, G. Opdenakker, and N. Van Roy. 1995. Constitutional translocation t(1;17)(p36.31-p36.13;q11.2-q12.1) in a neuroblastoma patient. Establishment of somatic cell hybrids and identification of PND/A12M2 on chromosome 1 and NF1/SCYA7 on chromosome 17 as breakpoint flanking single copy markers. *Oncogene*. 10:1087-93.
- Luo, Y., F.A. High, J.A. Epstein, and G.L. Radice. 2006. N-cadherin is required for neural crest remodeling of the cardiac outflow tract. *Dev. Biol.* 299:517-528.
- Martin, E.R., P.G. Bronson, Y.J. Li, N. Wall, R.H. Chung, D.E. Schmechel, G. Small, P.T. Xu, J. Bartlett, N. Schnetz-Boutaud, J.L. Haines, J.R. Gilbert, and M.A. Pericak-Vance. 2005. Interaction between the alpha-T catenin gene (VR22) and APOE in Alzheimer's disease. *J. Med. Genet.* 42:787-792.
- Miyashita, A., H. Arai, T. Asada, M. Imagawa, E. Matsubara, M. Shoji, S. Higuchi, K. Urakami, A. Kakita, H. Takahashi, S. Toyabe, K. Akazawa, I. Kanazawa, Y. Ihara, and R. Kuwano. 2007. Genetic association of CTNNA3 with late-onset Alzheimer's disease in females. *Hum. Mol. Genet.* 16:2854-2869.
- Nagafuchi, A., M. Takeichi, and S. Tsukita. 1991. The 102 kd cadherin-associated protein: Similarity to vinculin and posttranscriptional regulation of expression. *Cell*. 65:849-857.
- * Redies, C., K. Vanhalst, and F. van Roy. 2005. Delta-protocadherins: Unique structures and functions. *Cell. Mol. Life Sci.* 62:2840-2852.
- Reynolds, A.B., J.M. Daniel, Y.Y. Mo, J. Wu, and Z. Zhang. 1996. The novel catenin p120cas binds classical cadherins and induces an unusual morphological phenotype in NIH3T3 fibroblasts. *Exp. Cell Res.* 225:328-37.
- Soriano, P. 1999. Generalized lacZ expression with the ROSA26 Cre reporter strain. *Nat. Genet.* 21:70-1.
- Takemaru, K.I., S. Yamaguchi, Y.S. Lee, Y. Zhang, R.W. Carthew, and R.T. Moon. 2003. Chibby, a nuclear beta-catenin-associated antagonist of the Wnt/Wingless pathway. *Nature*. 422:905-9.
- Taneyhill, L.A. 2008. To adhere or not to adhere: The role of Cadherins in neural crest development. *Cell Adhes. Migr.* 2:223-30.
- Terry, S., L. Queires, S. Gil-Diez-de-Medina, M.W. Chen, A. de la Taille, Y. Allory, P.L. Tran, C.C. Abbou, R. Buttyan, and F. Vacherot. 2006. Protocadherin-PC promotes androgen-independent prostate cancer cell growth. *Prostate*. 66:1100-13.
- Thiery, J.P. 2003. Epithelial-mesenchymal transitions in development and pathologies. *Curr. Opin. Cell Biol.* 15:740-746.
- Tronche, F., C. Kellendonk, O. Kretz, P. Gass, K. Anlag, P.C. Orban, R. Bock, R. Klein, and G. Schutz. 1999. Disruption of the glucocorticoid receptor gene in the nervous system results in reduced anxiety. *Nat. Genet*. 23:99-103.
- Uemura, M., S. Nakao, S.T. Suzuki, M. Takeichi, and S. Hirano. 2007. OL-protocadherin is essential for growth of striatal axons and thalamocortical projections. *Nat. Neurosci.* 10:1151-1159.
- Vallee, R.B., J.C. Williams, D. Varma, and L.E. Barnhart. 2004. Dynein: An ancient motor protein involved in multiple modes of transport. *J. Neurobiol.* 58:189-200.
- * Vandepoele, K., V. Andries, and F. Van Roy. 2009. The NBPF promoter has been recruited from the unrelated EVI5 gene before simian radiation. *Mol. Biol. Evol.* 26:1321-1332.
- * Vandepoele, K., V. Andries, N. Van Roy, K. Staes, J. Vandesompele, G. Laureys, E. De Smet, G. Berx, F. Speleman, and F. van Roy. 2008. A constitutional translocation t(1;17)(p36.2;q11.2) in a neuroblastoma patient disrupts the human *NBPF1* and *ACCN1* genes (p. 1-12). *PLoS ONE*. 3:e2207
- * Vandepoele, K., K. Staes, V. Andries, and F. van Roy. 2010. Chibby interacts with NBPF1 and clusterin, two candidate tumor suppressors linked to neuroblastoma. *Exp. Cell Res.* 316:1225-1233.
- * Vandepoele, K., N. Van Roy, K. Staes, F. Speleman, and F. van Roy. 2005. A novel gene family NBPF: Intricate structure generated by gene duplications during primate evolution. *Mol. Biol. Evol.* 22:2265-2274.
- * Vanpoucke, G., S. Goossens, B. De Craene, G. Gilbert, F. van Roy, and G. Berx. 2004. GATA-4 and MEF2C transcription factors control the tissue-specific expression of the alpha-T-catenin gene *CTNNA3*. *Nucleic Acid Res.* 32:4155-4165.
- * Vermeulen, S.J., F. Nollet, E. Teugels, K.M. Vennekens, F. Malfait, J. Phillippé, F. Speleman, M.E. Bracke, F.M. van Roy, and M.M. Mareel. 1999. The aE-catenin gene (CTNNA1) acts as an invasion-suppressor gene in human colon cancer cells. *Oncogene*. 18:905-916.
- Wilson, N.D., L.J. Ross, T.J. Crow, and E.V. Volpi. 2006. PCDH11 is X/Y homologous in Homo sapiens but not in Gorilla gorilla and Pan troglodytes. *Cytogenet. Genome Res.* 114:137-139.
- Xiao, K.Y., D.F. Allison, K.M. Buckley, M.D. Kottke, P.A. Vincent, V. Faundez, and A.P. Kowalczyk. 2003. Cellular levels of p120 catenin function as a set point for cadherin expression levels in microvascular endothelial cells. *J. Cell Biol.* 163:535-545.
- Yang, X., M.W. Chen, S. Terry, F. Vacherot, D.K. Chopin, D.L. Bemis, J. Kitajewski, M.C. Benson, Y. Guo, and R. Buttyan. 2005. A human- and male-specific protocadherin that acts through the wnt signaling pathway to induce neuroendocrine transdifferentiation of prostate cancer cells. *Cancer Res.* 65:5263-71.

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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.

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Interactions between areas investigated using awake monkey (f)MRI.

The previous cycle of the GSKE was very successful for my group. In a series of combined ***perturb-and-measure*** techniques, we could investigate ***causal functional interactions*** between remote brain areas and the interactions between activity in a given brain region and behavior. We were amongst the first to implement the combination of intracranial microstimulation with functional magnetic resonance imaging (fMRI) in the awake monkey which resulted in a Science and a Journal of Neuroscience paper (Ekstrom et al., 2008, 2009) and two other papers that are currently in preparation. Moreover, thanks to the support of the GSKE, we were able to combine fMRI in monkeys performing three different tasks requiring spatial attention with a reversible lesion of an important node within the fronto-parietal attention network (see below for a detailed description). This study revealed rapid compensatory mechanisms throughout the brain evoked by a sudden and small cortical lesion. The manuscript is submitted for publication (Gerits et al.,).

In addition to this series of 'perturb and measure' experiments, we also started a series of experiments in which we pushed the limits of the spatial resolution of the awake behaving monkey fMRI experiments. For example we were able to obtain reliable maps at an isotropic resolution of 0.75 mm in awake monkeys which revealed the detailed topographic organization of area MT and its neighbours (Kolster et al. J. Neuroscience 2009). Furthermore, we started a series of combined fMRI-pharmacological experiments, for example in monkeys that perform self-administration cocaine experiments (Mandeville et al. Neuropsychopharmacology). Last but not least, the GSKE funding allowed us to start a series of studies in which we perform fMRI-guided electrophysiological recordings focusing on category selectivity in inferotemproal cortex and disparity defined 3D-shape selectivity (in collaboration with Dr. Janssen and Vogels).

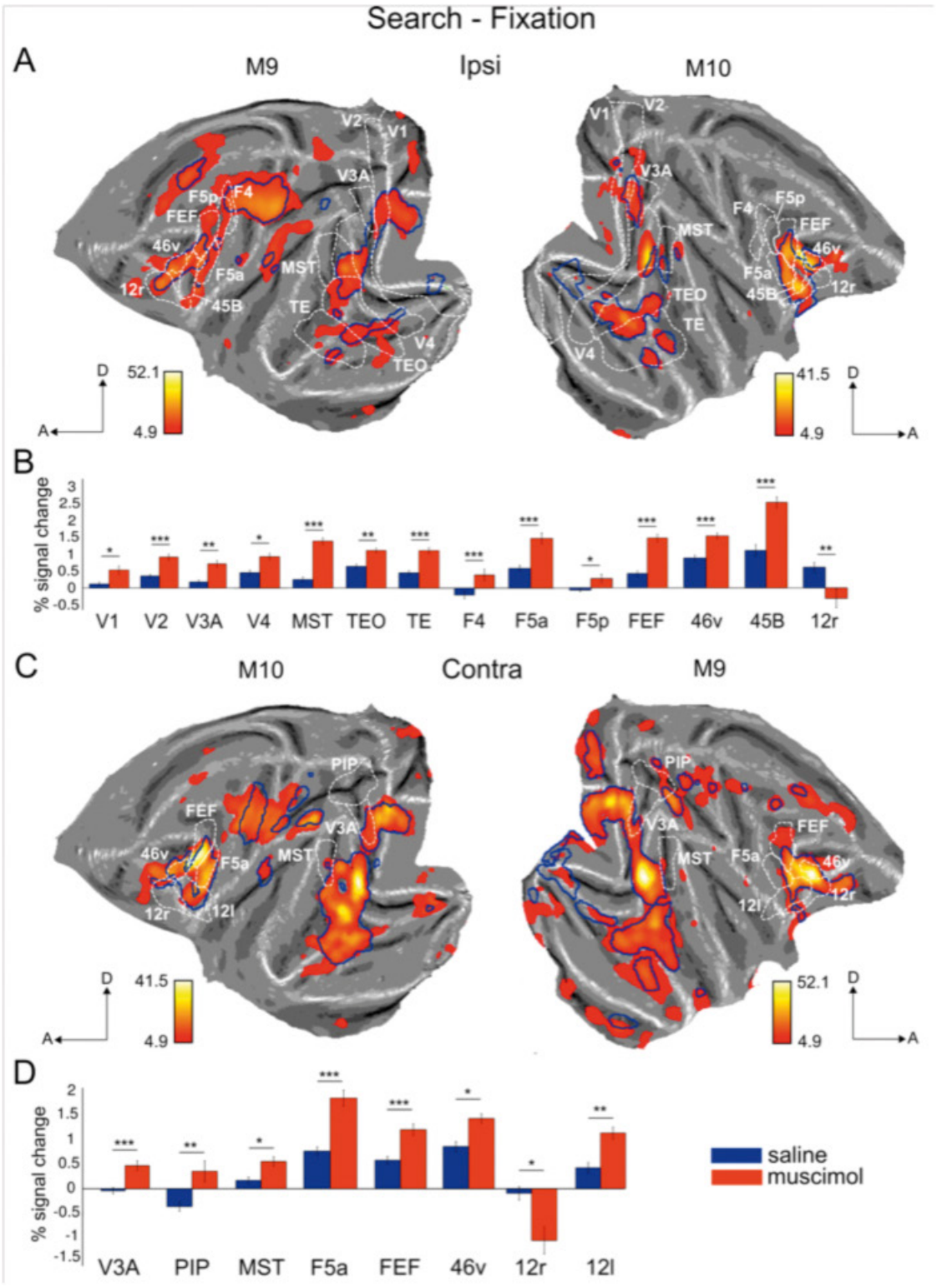
The aforementioned microstimulation-fMRI experiments and high spatial resolution fMRI experiments have been described in the reports of 2008 and 2009. Here I will focus on the experiments in which we reversibly inactivated an important node of the parieto- frontal attention network (area LIP within the intraparietal sulcus). Using a neuronavigation system and based on prior visual search-specific fMRI maps acquired in the same individuals, we inactivated LIP using the $GABA_A$ agonist muscimol. Monkeys were performing either a conjunction visual search task, a pop-out visual search task, a fixation task, or a detection task. In agreement with earlier studies, inactivation of LIP caused a behavioral deficit during the search and detection tasks. Moreover, inactivation of LIP caused increased task-driven fMRI activity and boosted the number of search-activated voxels in several down- and upstream areas relative to LIP. In a number of these areas the increase in fMRI activity is positively correlated with behavioral performance levels indicating **compensatory functional changes** in the search network.

Adaptive compensatory mechanisms in functional task networks caused by a reversible parietal lesion in monkeys. Annelies Gerits, John Arsenault, Hauke Kolster, Guy Orban, Claire Wardak, Wim Vanduffel

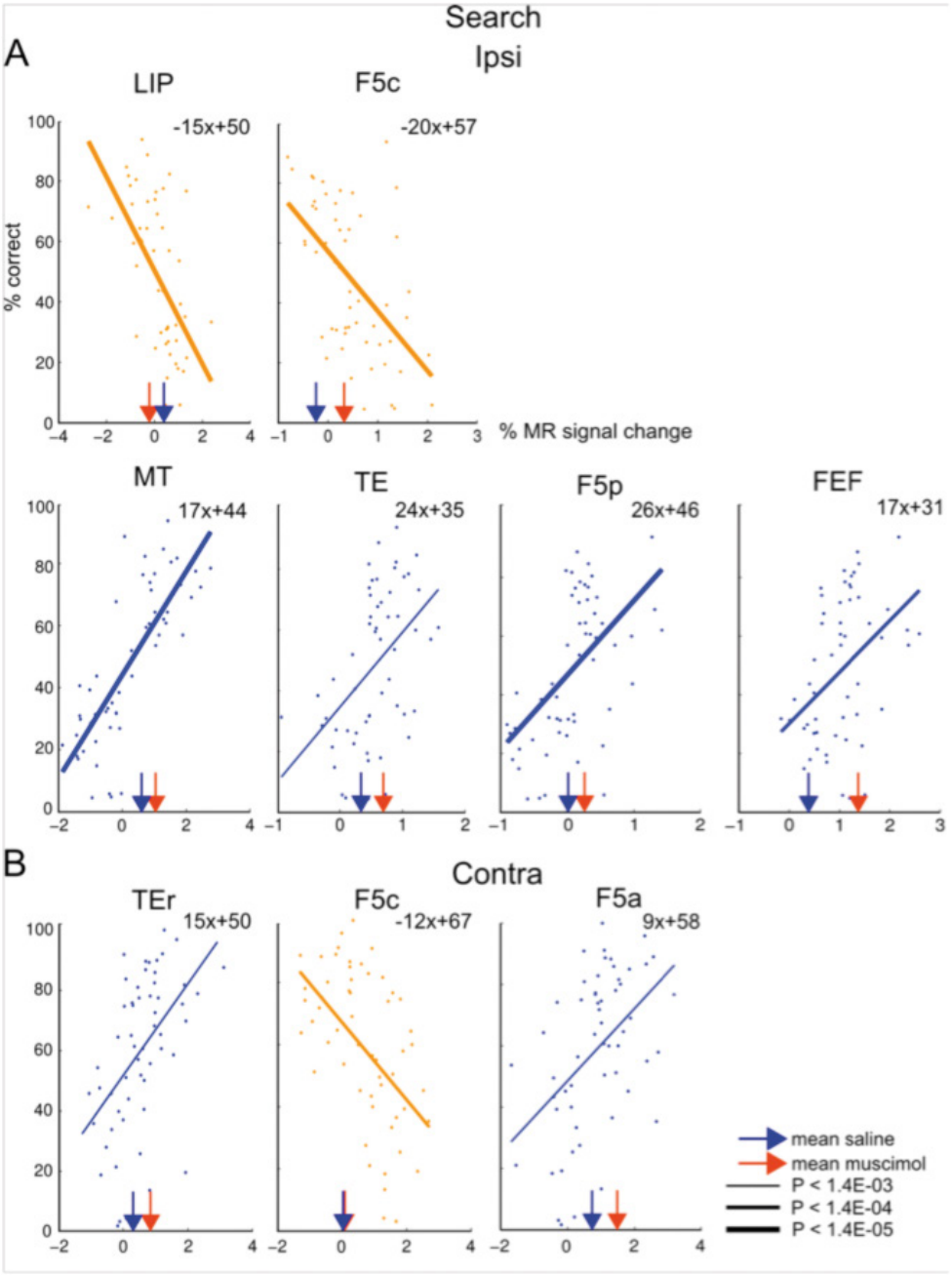
The brain has a remarkable capacity to recover after lesions, yet little is known about the underlying neural adaptations at the systems level. We combined monkey fMRI with reversible inactivation methods to correlate behavioral with brain-wide functional changes caused by a focal lesion in the lateral intraparietal area (LIP). We confirmed that such lesions cause a behavioral deficit in macaques

performing three different covert spatial attention tasks. Unexpectedly, however, we observed profound lesion-induced *increases* in task-related fMRI activity in many ipsi- and contralesional areas -both up- and downstream relative to LIP. In several of these cortical areas, lesion-induced changes in fMRI activity were task-dependent and correlated positively with changes in behavioral performance -which indicates that rapid adaptive compensatory mechanisms are triggered in areas remote from the lesion. We hypothesize that task-dependent dis-inhibition triggers these rapid adaptive changes within specific functional networks.

Focal brain damage is usually followed by extensive structural and functional changes in non-damaged brain regions and it is generally assumed that recruitment of regions outside the lesion underlies functional recovery. Here, we aimed to investigate brain-wide functional adaptations after a focal chemically-induced reversible lesion in the parietal cortex of the monkey. To examine the task-dependency of the functional changes, we trained two monkeys on three similar cognitive tasks each with a strong spatial attention component: a conjunction search, pop-out search and a peripheral detection task. We attempted to affect cognitive rather than the eye-movement related LIP functions. Therefore, we specifically chose manual responses instead of saccades as operant behavior in the covert attention tasks while requiring the monkeys to fixate during the experiments. The three tasks activate extended and largely overlapping functional networks including frontal, parietal and occipital visual cortical areas as well as subcortical regions.
Currently, it is a matter of debate whether behavioral recovery after a lesion should be attributed to reduced or increased activity in areas at a distance from the lesion. Reversible deactivation experiments in animals combined with measurements of either metabolic or neuronal activity have revealed reduced activity in sites connected with the deactivated cortex. Moreover, long-range cortico-cortical connections are mainly excitatory in nature. Lesioning such connections should lead, at least initially, to reduced excitation -hence decreased neuronal activity in remote connected sites. Therefore, we hypothesized that reversible inactivation of LIP would also lead to reduced fMRI activity at the site of injection, which would further propagate as a reduction in task-related activity throughout the relevant functional network -exactly as found in motor-related networks after stroke in human patients (Ward et al., 2003). Contrary to this prediction, however, we found immediately after LIP inactivation profoundly increased fMRI activity in many up- and downstream cortical areas within functional networks of all three tasks.



In several of these areas, the lesion-induced increase in fMRI activity was task-dependent and correlated positively with changes in behavioral performance levels (see figure below). This indicates that at least part of the activity changes within these networks underlie rapid adaptive compensatory mechanisms.



Conclusion

A small reversible LIP lesion resulted in a behavioral deficit for a conjunction search, pop-out search and detection task and caused brain-wide functional reorganizations. These findings underscore the importance of task-dependent network interactions as opposed to functional specialization of individual areas. Increased deficit-correlated and task-specific fMRI activity was observed in both down- and upstream areas relative to LIP. We suggest that these very rapid increases in activity *compensate* for the loss of activity in an important node of the search network. We propose that this compensation is at least partially caused by excitatory long-range connections originating in LIP which affect networks of interneurons in remote sites. Setting speculation aside, this study paves the way for fMRI-guided single cell studies providing insights in the exact nature of the increased compensatory activity in remote areas.

Papers 2008 – January 2010

- Mandeville JB, Choi J-K, Jarraya B, Rosen BR, Jenkins BG, Vanduffel W. fMRI of cocaine self-administration in macaques reveals functional inhibition of basal ganglia. **Neuropsychopharmacology** *In Press*
- Nelissen K, Borra E, Gerbella M, Rozzi S, Luppino G, Vanduffel W, Rizzolatti G, and Orban GA. Action observation circuits in the macaque monkey cortex. **Journal of Neuroscience** *In press*
- Kwong KK, Reese TG, Nelissen K, Wu O, Chan ST, Benner T, Mandeville JB, Foley M, Vanduffel W, Chesler DA. Early time points perfusion imaging. **Neuroimage**. 2011 54(2):1070-82. 1.
- Wardak C, Vanduffel W, Orban GA (2010) Searching for a salient target involves frontal regions. **Cerebral Cortex** 20(10):2464-77.
- Nelissen K, Joly O, Durand JB, Todd J, Vanduffel W, Orban GA (2010) The extraction of depth structure from shading and texture in the macaque brain. **PlosOne** 4:1-11.
- Radhakrishnan H, Vanduffel W, Deng HP, Ekstrom L, Boas DA, Franceschini MA (2009) Fast optical signal not detected in awake behaving monkeys. **Neuroimage** 45:410-419.
- Kolster H, Mandeville JB, Arsenault JT, Ekstrom LB, Wald LL, Vanduffel W (2009) Visual Field Map Clusters in Macaque Extrastriate Visual Cortex. **Journal of Neuroscience** 29:7031-7039.
- Joly O, Vanduffel W, Orban GA (2009) The monkey ventral premotor cortex processes 3D shape from disparity. **Neuroimage** 47:262-272.
- Driver J, Blankenburg F, Bestmann S, Vanduffel W, Ruff CC (2009) Concurrent brain-stimulation and neuroimaging for studies of cognition. **Trends Cogn Sci** 13:319-327.
- Ekstrom LB, Roelfsema PR, Arsenault JT, Kolster H, Vanduffel W (2009) Modulation of the contrast response function by electrical microstimulation of the Macaque frontal eye field. **Journal of Neuroscience** 29:10683-10694.
- Peeters R, Simone L, Nelissen K, Fabbri-Destro M, Vanduffel W, Rizzolatti G, Orban GA (2009) The representation of tool use in humans and monkeys: common and uniquely human features. **Journal of Neuroscience** 29:11523-11539.
- Op de Beeck, H.P., Deutsch, J.A., Vanduffel, W., Kanwisher, N., DiCarlo, J.J. (2008). A Stable Topography of Selectivity for Unfamiliar :Shape Classes in Monkey Inferior Temporal Cortex. ***Cerebral Cortex***. 18 (7) 1676-1694
- Ekstrom L.B., Roelfsema P.R., Arsenault J.T., Bonmassar G., and Vanduffel W. (2008) Bottom-up dependent gating of frontal signals in early visual cortex. **Science**, 321 (5887): 414-417.
- Khachaturian M.H, Ekstrom L.B., Arsenault J.T., Tuch D.S., Vanduffel W. (2008) Focal reversible deactivation of cerebral metabolism affects water diffusion at distant sites. **MRM**, 60:1178–1189.

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Coding of action categories in primate cortex.

The recognition of biological movements is extremely important for reproductive success and survival. Single cell work in non human primates as well as lesion studies and functional imaging in humans have suggested that the rostral Superior Temporal Sulcus (STS) in the temporal lobe, and regions of the parietal and frontal cortex are important for action recognition. **We examined the analysis of dynamic action stimuli by temporal cortical neurons, including the rostral STS, of macaque monkeys.**

Previously, we studied the coding by macaque temporal cortical neurons of a parameterized set of dynamic visual images of simple actions like knocking, throwing and lifting and the stimuli were rendered as stick figures. We explored mainly the dorsal and ventral bank of the rostral STS (visual areas STP and TE). The results of the single cell recordings indicated that rostral STS neurons respond selectively to temporal segments of the action movies, but not to the whole action as such. We were able to distinguish different kinds of neuronal selectivities. Firstly, neurons, mainly in the ventral bank of the rostral STS, responded as well to the action movies as to static snapshots. These neurons clearly responded to form information. Secondly, other neurons, mainly in the dorsal bank of the rostral STS, responded much less to static snapshots than to the action movies, thus responding to motion information (Vangeneugden, Pollick and Vogels, *Cerebral Cortex*, 2009).

In the above described experiments we used simple arm movements in which most of the motion information was present in one point (the wrist). In the present project, we have been using more complex motion patterns, i.e. those of a walking and running human. These more complex locomotion actions have been widely used in psychophysical and functional imaging studies of biological motion perception in humans. Also, ongoing human and monkey fMRI studies in our research division use walking humans as stimuli (Jastorff and Orban, *Journal of Neuroscience*, 2009). The stimuli that we use are based on motion-capture data of real human subjects that were walking or running at different, controlled speeds on a treadmill. These data were obtained at ETH Zurich (collaboration with L. Van Gool). Unlike in previous neurophysiological studies of locomotion recognition, the actor does not translate across the screen in the movies, i.e. we use “treadmill” walking and running, which avoids a strong directional translation component. Importantly, our standard displays do not show a full body of a human but instead displays of cylinders connecting the joints (and a head) are used as stimuli. Despite the fact that this is an impoverished stimulus, humans perceive easily and effortless human locomotion and its direction in these displays.

First, we performed an extensive behavioral study of the perception of such impoverished, biological motion displays in monkeys. Although a vast literature exists on *human* biological motion perception in impoverished displays, e.g. point-light walkers, much less is known about the perception of impoverished biological motion displays in *macaques*. However, the latter is essential to link perception of humans and single cell response selectivities obtained in macaques. We trained 3 macaques in the discrimination of facing-direction (left versus right) and forward versus backward walking using the above discussed motion-capture-based locomotion displays in which the body features were represented by cylinder-like primitives. Discriminating forward versus backward locomotion requires motion information while the facing-direction/view task can be solved using motion and/or form. All monkeys required lengthy training to learn the forward-backward task, while the view task was learned more quickly. Once acquired, the discriminations were specific to walking and stimulus format but generalized across actors. Although the view task could be solved using form cues, there was a small impact of motion. Performance in the forward-backward task was highly susceptible to degradations

of spatio-temporal stimulus coherence and motion information. These results indicate that rhesus monkeys require extensive training in order to use the intrinsic motion cues related to forward versus backward locomotion (i.e. when no extrinsic, translatory cues are present) and imply that extrapolation of observations concerning human perception of impoverished biological motion displays onto monkey perception needs to be made cautiously. *These results are presented in a paper in the top visual psychophysics journal Journal of Vision (Vangeneugden et al., J. Vision, 2010)*

After the behavioral training, we conducted a single cell recording study in the two of the three trained animals, examining the contribution of motion and form information to the selectivity for locomotion actions. We employed the same stimuli as used in the behavioral study, supplemented by movies of walking in 6 different directions. Unlike in previous studies from Perrett and colleagues our stimuli did not contain a translatory motion component and thus selectivity for facing direction of the walker or for forward versus backward walking cannot be due to a spatial, translation mechanism. In the case of forward-backward locomotion, it must be due to a sensitivity for snapshot sequences or motion information, while selectivity for facing direction can be based on form (posture selectivity) and/ or motion information. We recorded in both dorsal and ventral banks of the rostral STS. The majority of the neurons were selective for facing direction, while a minority distinguished forward from backward walking. We employed a state-of-the-art classification machine learning algorithm (Support Vector Machines) to assess how well the population of recorded neurons could classify the different walking directions and forward from backward walking. Support vector machines using the temporal cortical population responses as input classified facing direction well, but forward and backward walking less so but still significantly better than chance. The latter fits the behavioral data in the same animals described above. Classification performance for forward versus backward walking improved markedly when the within-action response modulation was considered, reflecting differences in momentary body poses within the locomotion sequences. Responses to static pose presentations predicted the responses during the course of the action. Analyses of the responses to walking sequences wherein the start frame was varied across trials showed that some neurons also carried a snapshot sequence signal. Such sequence information was present in neurons that responded to static snapshot presentations and in neurons that required motion. In summary, our data suggest that most STS/ inferior temporal neurons predominantly signal momentary pose. In addition, some of these temporal cortical neurons, including those responding to static pose, are sensitive to pose sequence, which can contribute to the signaling of learned action sequences. *A paper describing this work appeared in the Journal of Neuroscience (Vangeneugden et al., J. Neurosci., 2011)*

In addition to this study of the analysis of visual dynamic action stimuli, **we studied the effect of stimulus history on the responses of inferior temporal (IT) neurons to static stimuli.** Part of the selectivity for dynamic action sequences and the discovered sequence selectivity (see above; Vangeneugden et al., *J. Neurosci.*, 2011) might be due to the effect of preceding stimuli in a sequence to the response to the next stimulus of that sequence. One well known effect of stimulus history in visual cortex is adaptation, being the reduction in the response upon repetition of a stimulus. In this series of studies, we are examining the stimulus selectivity of this adaptation effect in macaque temporal cortex. **In a first adaptation study, we measured adaptation in macaque IT for parameterized shapes by comparing tuning for test stimuli following a brief adaptation with predictions derived from different models of adaptation. We measured simultaneously, using the same electrode, single-cell spiking activity and local field potentials (LFPs).** Adaptation was similar during two tasks: passive fixation and an attention-demanding luminance detection task. We found consistent adaptation of spiking activity and LFP power in high- (gamma) but not low-frequency bands when repeating shapes. Contrary to sharpening models

of adaptation, repetition did not affect shape selectivity. The degree of similarity between adapter and test shape was a stronger determinant of adaptation than was the response to the adapter. Adaption still occurred when adapter and test stimulus did not spatially overlap, but adaptation was stronger for same, compared to different, adapters and test stimulus positions. These adaptation effects were similar for spiking and for gamma activity. In conclusion, adaptation in IT – at least when using short interstimulus intervals - is not explainable by sharpened tuning or mere firing-rate-dependent fatigue of the neuron, two mechanisms that have been proposed to underlie adaptation effects (Grill-Spector, Henson and Martin, *Trends in Cognitive Sciences*, 2006). Indeed, adaptation of IT spiking activity and LFPs in IT is strongly dependent on feature similarities in the adapter and test stimuli, in agreement with input, but not firing-rate fatigue models. *These results are published in Cerebral Cortex (De Baene and Vogels, Cerebral Cortex, 2010).*

In a second adaptation study, we investigated a recently proposed hypothesis that explains adaptation as resulting from the fulfillment of a perceptual expectation (or reduction in prediction error; Summerfield et al., *Nat. Neurosci*., 2008). This hypothesis is a top-down, feedback explanation of adaptation which disagrees with bottom-up mechanisms (such as input fatigue) that we propose (see above). The role of perceptual expectation related to adaptation is relevant for action recognition since one can predict in principle for highly familiar actions, such as locomotion, the occurrence of a particular posture/snapshot from the sequence of previous postures. We examined whether stimulus repetition probability – a manipulation of the expectation of stimulus repetition - affects adaptation of spiking activity and local field potentials (LFPs) in macaque inferior temporal cortex, using a protocol similar to that of Summerfield et al. Monkeys were exposed to 2 randomly interleaved trials, each consisting of either 2 identical (rep trial) or 2 different stimuli (alt trial). Trials were presented in repetition (rep) blocks consisting of 75% of rep trials and 25% of alt trials, or in alternation (alt) blocks having opposite repetition probabilities. For both spiking and LFP activities, the stimulus-selective adaptation did not differ significantly between rep and alt blocks. Also, the number of preceding rep or alt trials and the trial position within a block did not affect adaptation. This absence of any effect of stimulus repetition probability on adaptation suggests that adaptation in IT is not caused by contextual factors related to perceptual expectation and our findings agree with bottom-up or local network fatigue models of adaptation. *A paper describing this work appeared in Cerebral Cortex (Kaliukhovich and Vogels, Cerebral Cortex, 2011).*

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- Kaliukhovich DA, Vogels R. (2011) Stimulus Repetition Probability Does Not Affect Repetition Suppression in Macaque Inferior Temporal Cortex. *Cerebral Cortex*, in press.
- Vangeneugden, J., De Mazière, P.A., Van Hulle, M.M., Jaeggli, T., Van Gool, L., Vogels, R. (2011) Distinct Mechanisms for Coding of Visual Actions in Macaque Temporal Cortex. *Journal of Neuroscience ,31*(2), 385-401
- Mysore, S.G., Vogels, R., Raiguel. S.E., Todd, J.T., Orban, G.A. (2010) The selectivity of neurons in the macaque fundus of the superior temporal area for three-dimensional structure from motion. *Journal of Neuroscience, 30*(46), 15491-508
- Manyakov, N., Vogels, R., Van Hulle, M. (2010). Decoding Stimulus-Reward Pairing From Local Field Potentials Recorded From Monkey Visual Cortex. *IEEE Transactions on Neural Networks, 21* (12), 1892-1902.
- Frankó, E., Seitz, A., Vogels, R. (2010). Dissociable neural effects of long term stimulus-reward pairing in macaque visual cortex. *Journal of Cognitive Neuroscience, 22* (7), 1425-1439.
- Verhoef, B., Vogels, R., Janssen, P. (2010). Contribution of inferior temporal and posterior parietal activity to three-dimensional shape perception. *Current Biology, 20* (10), 909-913.
- Vangeneugden, J., Vancleef, K., Jaeggli, T., Van Gool, L., Vogels, R. (2010). Discrimination of locomotion direction in impoverished displays of walkers by macaque monkeys. *Journal of Vision, 10* (4), 22.1-22.19.
- Vogels, R. (2010). Mechanisms of visual perceptual learning in macaque visual cortex. *Topics in Cognitive Science, 2* (2), 239-250.
- De Baene, W., Vogels, R. (2010). Effects of adaptation on the stimulus selectivity of macaque inferior temporal spiking activity and local field potentials. *Cerebral Cortex 20*(9), 2145-65.
- Vangeneugden, J., Pollick, F., Vogels, R. (2009). Functional Differentiation of Macaque Visual Temporal Cortical Neurons Using a Parametric Action Space. *Cerebral Cortex, 19* (3), 593-611.
- Verhoef, B., Kayaert, G., Frankó, E., Vangeneugden, J., Vogels, R. (2008). Stimulus similarity-contingent neural adaptation can be time and cortical area dependent. *Journal of Neuroscience, 28* (42), 10631-10640.
- De Baene, W., Ons, B., Wagemans, J., Vogels, R. (2008). Effects of category learning on the stimulus selectivity of macaque inferior temporal neurons. *Learning & Memory, 15* (9), 717-727.
- Mysore Gopalrao, S., Vogels, R., Raiguel, S., Orban, G. (2008). Shape selectivity for camouflage-breaking dynamic stimuli in dorsal V4 neurons. *Cerebral Cortex, 18* (6), 1429-1443.
- Matarazzo, L., Frankó, E., Maquet, P., Vogels, R. (2008). Offline processing of memories induced by perceptual visual learning during subsequent wakefulness and sleep: A behavioral study. *Journal of Vision, 8* (4), 1-9.
- Op de Beeck, H., Wagemans, J., Vogels, R. (2008). The representation of perceived shape similarity and its role for category learning in monkeys: a modeling study. *Vision Research, 48* (4), 598-610.
- Köteles, K., De Mazière, P., Van Hulle, M., Orban, G., Vogels, R. (2008). Coding of images of materials by macaque inferior temporal cortical neurons. *European Journal of Neuroscience, 27* (2), 466-482.

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