
08/10/2016

BELGIAN BRAIN COUNCIL

# The BELGIAN BRAIN CONGRESS : a mix of

# neurobiologists

# clinicians

# patients

# industry partners

frontiers Abstract Book

# 6TH BELGIAN BRAIN CONGRESS: THE GENE-ENVIRONMENT TANGO IN THE HEALTHY AND DISEASED BRAIN

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October 08 2016, 8:00 AM – 6:30 PM, MICX Mons International Congress Xperience,
Av. Melina Mercouri 9, MONS, Belgium

The interplay between gene expression and environment is a major actor of most of neurological and psychiatric disorders as well as normal brain functioning. This congress offers therefore the opportunity to address a lot of different questions ranging from basal ganglia function to narcolepsy. In line with our mission to bridge the different points of view, scientific sessions will approach basic science, neurology and psychiatry and will give the opportunity to patients' associations to take an active part to the discussion.

A better understanding of the relation between our heredity and our environment, socio-cultural, economic or natural, is essential to improve the prevention, the detection and the treatment of brain disorders. Belgian and international experts in Autism, Epilepsy, Narcolepsy, Multiple Sclerosis, Endocrine disruptors and Learning and Memory will present their recent findings on this complex interaction. Young researchers will be given the opportunity to present their most recent findings in poster sessions and the 10 better abstracts will be chosen for an oral e-poster session. Moreover, their registration fee will be waived by the BBC.

We hope that this program will convince you to participate. The town of Mons, European Capital of Culture in 2015, is proud to invite all participants to discover the town and its new congress centre, the MICX in collaboration with the University of Mons, the CHU Ambroise Paré and the CHP Chêne aux Haies

## Belgian Brain Congress 2016

On October 8th 2016, the town of Mons will welcome the 6th edition of the Belgian Brain Congress. entitled “The gene-environment tango in the healthy and diseased brain”. Unique in its form and objectives, the BBC congress opens the dialogue between all relevant stakeholders and expertise for a better understanding and a more efficient management of brain disorders.

Clinicians, researchers, representatives of patients and pharmaceutical industry are invited to participate to this important meeting taking place every two years in Belgium.

The interplay between gene expression and environment is a major actor of most of neurological and psychiatric disorders as well as normal brain functioning. This congress therefore offers the opportunity to address a lot of different questions ranging from basal ganglia function to narcolepsy.

In line with the BBC mission to bridge the different points of view, scientific sessions will approach basic science, neurology and psychiatry and will give the opportunity to patients’ associations to take an active part to the discussion.

A better understanding of the relation between our heredity and our environment, socio-cultural, economic or natural is essential to improve the prevention the detection and the treatment of brain disorders. During the congress, Belgian and international experts, as well as young researchers in Autism, Epilepsy, Narcolepsy, Multiple Sclerosis Endocrine disruptors and Learning and Memory will present their recent findings on this complex interaction.

Dear Participant,

You will find herein the abstracts of presentations at the 6th Belgian Congress, held October 8, 2016 at the MICX congress center in Mons – Belgium on the general topic "The gene-environment tango in the healthy and diseased brain".

You might be surprised to find out that this is not an abstract book like any others. It stands out indeed by the fact that each abstract in English is accompanied by a summary in lay language translated into the major two Belgian languages, Dutch and French. The reason for this is that the Belgian Brain Congresses are interdisciplinary gathering basic and clinical neuroscientist, but also patients and their representatives as well as industry partners. Patients are thus central to the meeting and every effort is made to get them informed and involved.

Three poster prizes of 250€ each will be awarded to the best posters selected by a multidisciplinary jury that comprises representatives from the patients' organizations and from the Belgian Association of Pharmacists.

All abstracts are made available online by the publisher "Frontiers" (www.frontiersin.org). In addition, during the congress webcasts will be made with interviews from selected poster presenters, invited lecturers and patients' representatives. These will be available shortly after the congress on the website of the Belgian Brain Council (http://www.braincouncil.be/).

This abstract book was in part financed by the financial support the Federation Wallonie-Bruxelles, the Fonds voor Wetenschappejijk Onderzoek (FWO), the Fonds National de la Recherche Scientifique (FNRS), the University of Mons, as well as a number of non-academic industrial sponsors. We are grateful to all of them for their continuing support.

We hope that you will find this abstract book a useful and interesting support for interactions during the congress. On behalf of the organizing committee of the Belgian Brain Congress, we are grateful to the presenters and speakers as well as to you for your participation.

Laurence Ris Jean Schoenen

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**PROGRAMME**

| | |
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| 08.00 – 08.45 | Registration and poster hanging |
| 08.45 – 09.00 | Welcome address Dirk Van Roost / Laurence Ris |
| **09.00 – 10.30** | **Session 1** |

Chair: Henrique Sequeira & Charles van der Straten Waillet

| | |
|---|---|
| 09.00 – 09.20 | Alban de Kerchove d'Exaerde (ULB): Basal ganglia and addiction |
| 09.20 – 09.40 | Bénédicte Dubois (KUL): Genetics of Multiple Sclerosis |
| 09.40 – 10.10 | Pierre Thomas (Lille, Fr): Treatment resistance in psychiatry |
| 10.10 – 10.30 | Panel discussion with patients' associations |
| 10.30 – 11.15 | Poster viewing & coffee |

Poster Jury: Rufin Vogels, Jean Schoenen, Chris Baeken, Marie-Noëlle Simonart, Rebecca Muller, Alain Chaspierre

| | |
|---|---|
| **11.15 – 12.45** | **Session 2** |

Chair: Robert Poirrier & Mohamed Abbass

| | |
|---|---|
| 11.15 – 11.35 | Kees Van Heeringen (UGent): Gene-environment interaction models of suicide |
| 11.35 – 11.55 | Jean-Pierre Bourguignon (ULg): Brain endocrine disruption: enough science for regulation? |
| 11.55 – 12.25 | Claudio L Bassetti (Bern, CH): The Etiology of Narcolepsy |
| 12.25 – 12.45 | Panel discussion with patients' associations |
| 12.45 – 14.30 | Satellite Symposia & lunch |

**14.30 – 16.00 Session 3**

Chair: Gianni Franco and Joelle Kapompole

Médico-social workshop on interdisciplinary management of patient's environment with Alain Chaspierre, Jean Paulius, Philippe Cazals, Claudio Colantoni, Lia Leroy

Poster viewing

Chair: Rufin Vogels & Jean Schoenen

14.30 – 15.15 e-poster session

**16.00 – 17.30 Session 4**

Chair: Roland Pochet & Lia Leroy

16.00 – 16.20 Massimo Pandolfo (ULB): Genetics of the Epilepsies

16.20 – 16.40 Claudia Bagni (VIB, KUL): FMRP protein and Fragile X syndrome

16.40 – 17.10 Pieter Hoekstra (Groningen, NL): Autism spectrum disorder: overlap with attention deficit/hyperactivity disorder

17.10 – 17.30 Panel discussion with patients' associations

17.30 – 17.45 Poster prizes by Rufin Vogels & Jean Schoenen Patients Prize by Rebecca Muller and Lia Leroy

18.00 – 18 :05 Conclusions by Laurence Ris

18.05 Cocktail and Cultural event

Neuropsychiatric Symposia organized by the UMHAP unit of Experimental psychopathology

– J-A. Elosegi (HAP): Cognitive disorders in proteinopathies
– L. Lefebvre (UMONS): Non-drug therapies for Alzheimer's
– T. Pham (UMONS): Emotional processes in psychopathy and therapeutic implication

The **BELGIAN BRAIN COUNCIL (BBC)**

announces

the **2016 « CEFALY TECHNOLOGY PRIZE »**

of 15,000€ for a research project
in the clinical and/or basic neurosciences

Eligibility criteria

- Research group comprising at least 1 applicant who is member of a *BBC member* society/association
- A project investigating a *specific research question* concerning the brain, its disorders and their management ; the project can be part of a larger program funded by other sources
- A *budget* on utilisation for operational costs and/or equipment and/or personnel

Jury: the executive committee of the BBC + 2 external experts

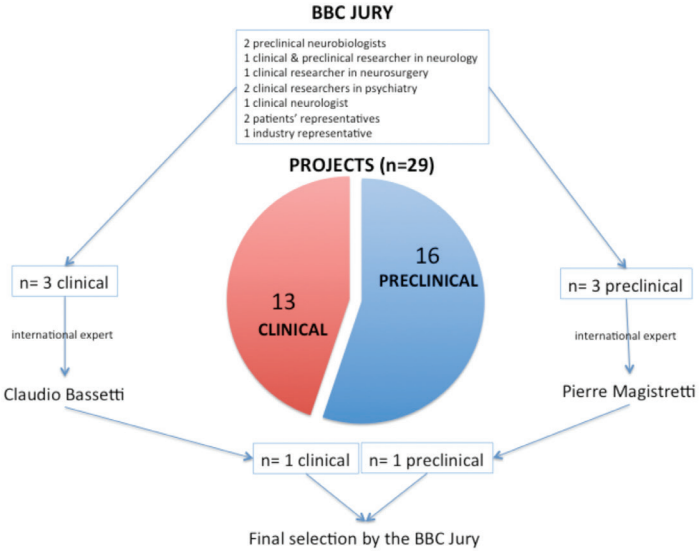
Evaluation : report and presentation of results at the Belgian Brain Congress at the latest 2 years after reception of the Prize

Application forms available at www.braincouncil.be to be sent to jschoenen@ulg.ac.be with a short CV of the principal investigator

Deadline: April 30th 2016

Info : rpochet@ulb.ac.be

Applications and Selection process




The Cefaly Technology Prize of the Belgian Brain Council will be officially handed out during the 6th Belgian Brain Congress – Mons MICX – October 8, 2016.

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**LIST OF ORGANIZERS:**

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Bart STULENS (Medtronic)

**SPEAKERS:**

Pierre Thomas, university Lille 2, Lille, France

Kees Van Heeringen, Dept of Psychiatry and Medical Psychology, Ghent University, Ghent, Belgium

Jean-Pierre Bourguignon, Université de Liège-CHU, Liège, Belgium

Alban De Kerchove D'ExaErde, Université Libre de Bruxelles, Brussels, Belgium

Benedicte Dubois, KULeuven, Leuen, Belgium

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Gianni Franco, CHU Dinant Godinne, Dinant, Belgium

Pieter Hoekstra, University Medical Center, Groningen, Netherlands

Claudio Bassetti, University Hospital, Bern, Switzerland

# Genetics of the Epilepsies

Massimo Pandolfo[1]

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Genetic epilepsies are a complex group of disorders, with heterogeneous etiologies and clinicopathological features. The use of high-throughput approaches to sequence DNA and to detect CNVs is revealing a landscape of mutations in genetic epilepsies, affecting a variety of genes involved in neuronal excitability (e.g. ion channels), synaptic transmission, neuronal metabolism (e.g. GLUT1) or network development (e.g. the "toropathies"). A number of distinct genetic epilepsy syndromes may be defined on the basis of their electro-clinical characteristics, associated neurological, psychiatric or general physical features, imaging, neuropathology, and mode of inheritance. Phenotypes may include, in addition to epilepsy, variable degrees of intellectual disability, elements of autism spectrum disorders, other psychiatric disorders, and motor impairment. In some cases, these co-morbidities derive from uncontrolled seizure activity (epileptic encephalopathies), but in other cases they are direct, multifaceted consequences of global brain dysfunction. When phenotypes are accurately defined, the underlying genetics usually becomes less heterogeneous, more consistently pointing to specific pathogenic pathways. Conversely, however, the full phenotypic spectrum of mutations of a given gene may turn out to be surprisingly large and apparently heterogeneous, challenging any simplistic explanation of pathogenesis. Mutations may be de novo, or, when inherited, show reduced penetrance and variable expressivity. Understanding the genetics of these conditions will improve diagnosis, reveal pathogenic mechanisms, and eventually lead to better treatment.

Résumé en français: L'épilepsie existe sous plusieurs formes, ce qui rend son diagnostic complexe. L'analyse approfondie et détaillée du phénotype (ensemble des caractéristiques visibles d'une personne) c'est-à-dire de la partie visible du génotype, notre patrimoine génétique, permet de mieux appréhender son héritage génétique. C'est ainsi que l'analyse phénotypique peut révéler, en plus de l'épilepsie, des degrés variables de déficiences intellectuelles, d'autisme, d'autres troubles psychiatriques et moteurs. Mais c'est par la recherche au niveau de la génétique des patients "épileptiques" que le diagnostic optimal poura être fait, cette recherche doit aussi permettre de découvrir les mécanismes cellulaires altérés et peut-être de nouveaux traitements.

Samenvatting in het Nederlands: Er bestaan verschillende soorten epilepsie en types epileptische aanvallen, wat de diagnose complex maakt. Grondige en gedetailleerde analyse van het fenotype (alle zichtbare kenmerken van een persoon), dit is het zichtbare gedeelte van het genotype (ons genetisch materiaal), laat toe om onze genetische

erfenis beter te begrijpen. Het is daarom dat fenotypische analyse, naast epilepsie, ook verschillende graden van intellectuele stoornissen, autisme en andere psychiatrische- en bewegingsstoornissen kan onthullen. Door bij patiënten met epilepsie onderzoek op niveau van de genetica te verrichten, kan een optimale diagnose gesteld worden. Dergelijk onderzoek moet ook toelaten om gewijzigde cellulaire mechanismen en misschien ook nieuwe behandelingen te ontdekken.

**Keywords: Epilepsy, phenotype, Genotype, comorbidities, Pathogenic Mechanism**

# Brain endocrine disruption: enough science for regulation?

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Fetal and early postnatal life periods are critical for brain development. Thyroid hormones and neurotransmitters play a crucial role in that process and their alteration could explain some neurodevelopmental disorders. The number of synthetic chemicals present in the environment has increased dramatically during the past decades, as did fetal exposure. Some of those chemicals can alter functioning of the endocrine system (Endocrine Disruptors, EDs) and interfere with brain development. PCBs provide a classical example with a mode of action involving thyroid hormones. A ubiquitous chemical like bisphenol A is another example with a mechanism that could imply disordered GABA neurotransmission. Studies have shown that the cost of health disorders attributable to EDs in the EU is 160 billions Euros per year. Pesticides are the most important EDs and neurodevelopmental disorders including cognitive impairment, autism and possibly attention deficit hyperactivity disorder account for the most important cost. This raises the issue of limiting exposure either by ban on chemicals based on scientific evidence and, in some instances, the precautionary principle. The task is beyond measure: Among 143,000 synthetic chemicals listed in the EU, it is estimated that only few % have been tested for ED properties; 1,036 have been identified as possible EDs based on peer-reviewed literature; 445 pesticides or biocides were screened for ED identification using the EC criteria, resulting in 13 to 31 confirmed as EDs. Raising awareness of the public particularly in critical and vulnerable periods like pregnancy is thus important inasmuch EDs can cause transgenerational effects likely involving epigenetic mechanisms.

Résumé en français: Le développement du cerveau implique des hormones comme celles produites par la thyroïde et des neurotransmetteurs comme le GABA. Les perturbateurs endocriniens (PEs) comme les PCBs et le bisphénol A interfèrent avec ces processus, d'où un risque de réduction des capacités intellectuelles ou de maladies comme l'autisme. Les PEs entraînent un coût annuel de 160 milliards d'euros pour l'UE, dont la plus grande partie vient des troubles du neurodéveloppement. Les critères proposés par la Commission européenne pour identifier les PEs sont tels que très peu de substances sont susceptibles d'être réglementées. Dès lors, l'éducation des consommateurs, en particulier les femmes enceintes, est essentielle.

Samenvatting in het Nederlands: Bij de ontwikkeling van de hersenen zijn hormonen zoals deze die door de schildklier aangemaakt worden en neurotransmitters zoals GABA betrokken. Endocriene hormoonontregelaars (EH) zoals PCB's en bisphenol A beïnvloeden dit proces en creëren een risico op vermindering van de verstandelijke bekwaamheid of op ontwikkeling van ziektes zoals autisme. Deze endocriene hormoonontregelaars liggen aan de basis van een jaarlijkse kost van 160 miljard euro binnen de EU, het grootste deel ervan veroorzaakt door neuro-ontwikkelingsstoornissen. De criteria die de EU voorstelt om de EH te identificeren zijn van dien aard dat slechts zeer weinig ervan onder deze reglementering zullen vallen. Daarom is de opvoeding van de gebruikers, in bijzonder van zwangere vrouwen, zeer belangrijk.

**Keywords: Endocrine Disruptors, Thyroid Hormones, Brain Development, Bisphenol-A, Pesticides**

# Using genetics to better understand multiple sclerosis

Benedicte Dubois[1]

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Neurological diseases have a substantial and growing impact in our society. Multiple Sclerosis is one of the most common neurological disorders. Life-time risk of developing the disease is 1/500 in north-western Europe. Approximately 1.3 million individuals worldwide and 11,000 individuals in Belgium suffer from the disease. Onset of the disease typically occurs in early adulthood, between 20 and 40 years of age, at the start of building out a family and a professional career. The disease leads to significant physical and cognitive disability and hence has an important impact on the personal, social and professional life of patients and their relatives. The currently available treatments are only partially effective. The pathogenesis of the disease has not been unravelled yet, but the past years have seen exciting progress in the field. A large number of genetic risk factors have been identified, and patients differ in which combination of genetic risk factors they carry. We now are facing the challenge of translating this list of genes into an improved understanding of disease mechanisms and hopefully to better treatments.

Résumé en français : La sclérose en plaque est une maladie inflammatoire du cerveau et de la moelle épinière pour laquelle il n'existe actuellement que des traitements partiellement actifs. On ne comprend pas encore très bien les causes de la maladie mais des facteurs héréditaires semblent influencer la prédisposition. Nous décrirons l'état actuel de la science en matière d'hérédité et de sclérose en plaque et nous expliquerons comment de telles connaissances peuvent conduire à un progrès au niveau de l'approche de cette maladie.

Samenvatting in het Nederlands Multiple sclerose (MS) is een ontstekingsziekte van hersenen en ruggenmerg waarvoor tot nu toe slechts gedeeltelijk werkzame behandelingen beschikbaar zijn. De oorzaak van de ziekte is nog onvoldoende begrepen, maar erfelijke factoren kunnen de vatbaarheid ervoor beïnvloeden. We gaan in op de huidige stand van de wetenschap rond erfelijkheid en MS, en bespreken hoe dergelijke inzichten kunnen leiden tot vooruitgang in de aanpak van deze ziekte.

**Keywords: Multiple Sclerosis, cognitive disability, physical disability, Genes, Risk factors**

# Treatment resistance in psychiatry

Pierre Thomas[1]

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Mental illnesses have long been the subject of passionate debates about their origin and nature . Advances in neuroscience with sophisticated exploration methods of human brain function have allowed to better understand how the psychiatric diseases merge and perpetuate. To date, available pharmacological treatments show significative but limited efficiency, requiring precise clinical and para-clinical assessments and treatment algorithms. Research conducted on brain functions, developpment and plasticity favors a re-conceptualization of mental illness with therapeutic innovations mainly in the field of neuromodulation.

Résumé en français: L'origine et la nature des troubles mentaux ont longtemps fait l'objet de débats passionnés. Des méthodes d'exploration sophistiquées de la fonction cérébrale chez l'homme ont permis de réaliser des avancées sur le terrain des neurosciences et de mieux comprendre la façon dont les maladies psychiatriques se combinent et se perpétuent. À ce jour, les traitements pharmacologiques disponibles présentent une efficacité significative mais limitée, exigeant des évaluations cliniques et paracliniques ainsi que des algorithmes de traitement extrêmement précis. Les recherches menées sur les fonctions, le développement et la plasticité du cerveau favorisent une reconceptualisation du trouble mental par le biais d'innovations thérapeutiques, principalement dans le domaine de la neuromodulation.

Samenvatting in het Nederlands Het ontstaan en de aard van geestesziekten vormde lange tijd stof voor discussies. Doorbraken in de neurowetenschap dankzij geavanceerde onderzoeksmethodes van de werking van het menselijke brein zorgden voor een beter inzicht in het ontstaan en het voortbestaan van psychiatrische aandoeningen. Vandaag laten de beschikbare farmacologische behandelingen een duidelijke, maar beperkte efficiëntie zien, waarbij uiterst nauwkeurige klinische en paraklinische evaluaties en behandelingsalgoritmen nodig zijn. Onderzoek naar de functies, de ontwikkeling en de plasticiteit van de hersens monden uit in een andere visie op geestesziekten via therapeutische vernieuwingen, hoofdzakelijk op het vlak van neuromodulatie.

**Keywords: Schizophrenia, brain imaging, Neuromodulation, Hallucinations, Mental Disorders**

# Understanding the role of basal ganglia neuronal populations and genes in addiction and motor control using transgenic models

Alban De Kerchove D'Exaerde[1]

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Dopamine is a key neurotransmitter involved in motor and motivational functions. One of the main targets of the dopaminergic neurones is the striatum. The dorsal striatum, divided into the dorsolateral striatum (DLS) (innervated by the sensorimotor cortex) and the dorsomedial striatum (DMS) (innervated by prefrontal and other associative cortices), is critically involved in a variety of motor functions, including regulation of motor activity, motor skill learning and motor response to psychostimulant and neuroleptic drugs, whereas the ventral striatum, is essential for motivation and drug reinforcement. Both parts of striatum contain two efferent dopaminoceptive medium spiny neurons (MSN) populations, that compose the so called direct (dMSN) and indirect (iMSN) pathways. To decipher the role of the different MSN populations, we performed subregion- and cell-population selective ablation of striatal neurones generating new animal models and evaluated their behavioural consequences in motor learning, drug addiction and response to dopaminergic drugs. To further decipher the physiology of the indirect pathway we evaluated the role of a new specific gene of the iMSN, the ecto-5'-nucleotidase, in striatal specific behaviours and the cellular and behavioural consequences of the absence of glutamate NMDA receptor in this neuronal population. Finally, we characterized a new gene that has a key role in drug addiction by acting via glutamatergic afferents to the ventral striatum.

Résumé en français: Les noyaux de la base sont un ensemble de noyaux cérébraux sous-corticaux qui jouent un rôle essentiel dans l'apprentissage moteur et les circuits de la récompense. Ils sont affectés dans des maladies comme la maladie de Parkinson, la dépendance aux drogues et la schizophrénie. Grâce à des approches utilisant des souris transgéniques permettant de cibler précisément des populations neuronales ou des gènes, on comprend de mieux en mieux la physiologie précise de ces noyaux tant à une échelle moléculaire que comportementale. Cette meilleure compréhension pourrait potentiellement permettre de développer des approches thérapeutiques plus ciblées. »

Samenvatting in het Nederlands: De basale kernen vormen een geheel van diep onder de hersenschors gelegen hersenkernen die een essentiële rol spelen in het leren van bewegingen en in het beloningscircuit. De basale kernen zijn betrokken bij aandoeningen zoals de ziekte van Parkinson, drugverslaving en schizofrenie. Door gebruik

te maken van transgene muizen, waarin welbepaalde zenuwcelpopulaties of genen gewijzigd zijn, begrijpen wij gaandeweg de precieze fysiologie van deze kernen beter, zowel op moleculaire schaal als op gebied van het gedrag. Dit betere begrip zal mogelijks toelaten om een nieuwe en doelgerichte therapeutische aanpak te ontwikkelen.

**Keywords: Basal Ganglia, Dopamine, Drug Addition, motor learning, Transgenic mice**

## Acknowledgements

This work was supported by FRS–Fonds de la Recherche Scientifique (FNRS) and Action de Recherche Concertée. A.d.K.d.E. is a Senior Research Associate of the FRS–FNRS and is an investigator of Walloon Excellence in Lifesciences & BIOtechnology (WELBIO).

# Autism spectrum disorder: overlap with attention deficit/hyperactivity disorder

Pieter J. Hoekstra[1*]

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Attention-deficit/hyperactivity disorder (ADHD) is associated with functional impairments in different areas of daily life. One such area is social functioning. Children with ADHD often have conflicts with adults and peers, and suffer from unpopularity, rejection by peers, and a lack of friendships, in part as a consequence of their ADHD symptoms. Comorbid oppositional defiant or conduct disorder aggravates these impairments. In some cases the inadequate social behavior of children with ADHD may be phenomenologically and etiologically related to autism spectrum disorder (ASD). However, the causes and consequences of ASD symptoms in ADHD are understudied. Also, the relative contributions of ADHD, on the one hand, and comorbid disorders, on the other, to the course of social impairments are unknown. Social dysfunctioning in children with ADHD appears to increase their risk of later psychopathology other than ADHD. Thus far effective treatment for social dysfunctioning is lacking. Future research should address the exact nature and long-term consequences of social dysfunctioning in children with ADHD, and focus on development of effective treatment strategies.

Résumé en français: Titre: TDAH et Autisme Les troubles déficitaires de l'attention (TDAH) couvrent différents dysfonctionnements de la vie de tous les jours. Parmi ceux-ci figure le fonctionnement social (capacité à interagir facilement et avec succès avec d'autres). Les enfants souffrant de TDAH ont souvent un fonctionnement social altéré. Il est aujourd'hui montré qu'entre 20 à 50 % de ces cas sont associés à des symptômes autistiques mais des traitements efficaces sont encore absents. Les recherches futures devraient se focaliser sur la nature exacte et les conséquences à long termes sur les enfants TDAH ayant un fonctionnement social altéré et proposer de meilleures stratégies de traitements.

Samenvatting in het Nederlands: ADHD en sociaal onhandig gedrag Hoewel kinderpsychiaters vanouds proberen discrete stoornissen te onderscheiden zoals ADHD en autisme is het eerder regel dan uitzondering dat kinderen kenmerken vertonen van meer dan een stoornis. Veel kinderen met ADHD laten problemen zien met sociale interactie en communicatie en of vertonen rigide gedrag. Sinds kort is het nu mogelijk om bij een persoon tegelijkertijd de diagnose ADHD en autisme te stellen. Er zijn steeds meer gegevens die erop wijzen dat qua erfelijkheid, oorzakelijke factoren en

betrokken hersengebieden ADHD en autisme veel overlap vertonen. In de lezing zal een overzicht van de nieuwste inzichten worden gegeven.

**Keywords: adolescents, environment;, ADHD, ASD, Children**

# The multi-tasking protein FMRP wires mammalian brain

Claudia Bagni[1,2,3], Emanuela Pasciuto[3], Nuria Dominguez[3]

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Deficiencies in the fragile X mental retardation protein (FMRP) lead to the most frequent form of inherited intellectual disability and Autism Spectrum Disorders (ASDs), the fragile X syndrome (FXS), with symptoms manifesting during infancy and early childhood. Because FMRP is expressed at very early stages of embryonic development, we hypothesize that FXS is the result of complex regulatory mechanisms occurring prenatally and at early postnatal stages, when synaptogenesis occurs. FMRP regulates the positioning of neurons in the cortical plate during embryonic development, affecting their multipolar-to-bipolar transition (MBT). We identified a few FMRP-regulated targets crucial for MBT in embryonic brain, among them N-cadherin.

Spontaneous network activity and high-resolution brain imaging at earlier postnatal stages revealed embryonic defects in the establishment of excitatory and inhibitory neuronal networks. At early post-natal stages the-amyloid precursor protein (APP), involved in Alzheimer's disease, plays a role in synapse formation, and is upregulated in FXS and other intellectual disabilities. In FXS, APP signals through the metabotropic receptor that, activating the MAP kinase pathway, leads to synaptic and behavioral deficits. Proper control of APP processing is crucial for healthy spine formation and function(s). We propose that the affected brain wiring in FXS is in part the result of dysregulated mRNA metabolism that starts during the first weeks of life and persists with remnants into adulthood.

**Résumé en Français:** Autisme et Alzheimer Le syndrome du X fragile est une maladie genetique responsable de deficiences mentales et d'autisme. Les symptomes se manifestent pendant l'enfance. La proteine (FMRP), appelee Proteine du X fragile, est exprimee a des stades precoces du developpement embryonnaire et est diminuee chez les enfants ayant le syndrome. Notre hypothese est que ce syndrome apparait des la naissance, lorsque la synaptogenese se developpe. De maniere analogue, il a ete montre qu'une autre proteine l'APP (responsable de la formation des plaques amyloides) joue aussi un role dans la synaptogenese, interagit avec la proteine du X fragile qui, lorqu'elle est diminuee, altere la synaptogenese.

**Samenvatting in het Nederlands:** Autisme en Alzheimer. Het fragiele X syndroom is een genetische aandoening die mentale achterstand en autisme veroorzaakt. De symptomen treden op tijdens de kinderjaren. De proteine (FMRP), ook proteine van het fragile X syndroom genoemd, komt voor in vroegtijdige stadia van de ontwikkeling van het embryo, en vermindert dan bij kinderen met het syndroom. Onze hypothese is dat dit syndroom tot uiting komt bij de geboorte, bij de ontwikkeling van de synaptogenese.

Analoog daarmee werd aangetoond dat een andere proteine, APP (verantwoordelijk voor de vorming van amyloide neerslag) ook een rol speelt in de synaptogenese, in interactie met de proteine van het fragile X syndroom dat, wanneer dit vermindert, de synaptogenese aantast.

**Keywords: fragile X mental retardation protein, fragile X syndrome, Alzheimer's disease, autism spectrum disorders, synaptogenesis, intellectual disabilities, amyloid precursor protein (APP)**

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# Etio-pathogenesis of Narcolepsy

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Narcolepsy is a chronic hypothalamic disorder that affects 1 in 2000 people and presents with sleep-wake (excessive daytime sleepiness, disturbed sleep), motor (cataplexy, sleep paralysis, REM sleep behavior disorder), neuropsychiatric (hallucinations, depression), and metabolic (obesity) disturbances. Narcolepsy is most commonly caused by the selective loss of neurons of the posterior hypothalamus that produce the neuropeptide hypocretin (HCRT). Ablation of hypocretin or hypocretin receptors also leads to narcolepsy phenotypes in animals. Several observations suggest an immune-mediated process: 1) narcolepsy is associated with the HLA-DQB1*06:02 allele and with the T-cell receptor α locus, 2) infections (H1N1, streptococcus) and vaccinations (H1N1, Pandemrix®) were reported to increase the risk of narcolepsy; 3) elevated anti-Tribbles homolog 2 and anti-streptolysin O antibodies are found in (some) narcoleptics close to disease onset; 4) post--mortem histopathology study of a single patient's brain showed an hypothalamic inflammation dominated by cytotoxic CD8 T cells, 5) rare cases of narcolepsy-like syndromes can be seen in the course of autommune disorders ; 6) few (but not all) observations suggest a favorable effect of immunoglobuline treatment on the course of narcolepsy. Data from our research team/collaborations* further supporting the hypothesis of an underlying autoimmune process in narcolepsy will be presented including: a) the first ever observation of hypocretin-reactive CD4+ T cell clones in narcoleptic patients (but not in HLA-controls), b) the observation of five patients with both narcolepsy and multiple sclerosis (one of whom improved with natalizumab), c) a survey of Swiss patients who developed narcolepsy following FSME and H1N1 vaccinations. *Johannes Mathis, Ulf Kallweit, Markus Schmidt (Neurology Department, Bern) Ramin Khatami (Klinik Barmwelweid, Aarau) Mauro Manconi (Neurocenter of Southern Switzerland, Lugano) Daniela Latorre, Eric Armentani, Federica Sallusto (Center of Medical Immunology, Institute for Research in Biomedicine Institute, Bellinzona)

Lay Summary: Narcolepsy is a disorder which affects 1of 2000 people and presents with excessive daytime sleepiness, cataplexy (loss of muscle control triggered by emotions), disturbed sleep, hallucinations, depression and obesity. Recent observations, including data from our research team, suggest that narcolepsy may represent an autoimmune disorder, which arises from a combination of genetic predisposition and such environmental factors as infections and vaccinations eventually leading to a selective loss of hypocretin neurons in the brain. The final proof of this autoimmune hypothesis

would have profound consequences for the (early) diagnosis and (immunomodulatory) treatment of the disease.

Résumé en français: La narcolepsie est un trouble qui affecte 1 personne sur 2000 et présente une somnolence diurne excessive, de la cataplexie (perte de contrôle musculaire déclenchée par des émotions), des troubles du sommeil, des hallucinations, de la dépression et de l'obésité. Des observations récentes alliées à nos résultats suggèrent que la narcolepsie peut être classée parmi les maladies auto-immunes, résultant d'une combinaison de prédispositions génétiques et de facteurs environnementaux (infections, vaccinations, etc.) pour aboutir à une perte sélective des neurones à orexine, un neuropeptide qui régule l'éveil et l'appétit. La validation de cette hypothèse de l'auto-immunité aura des conséquences importantes lors du diagnostic précoce et du traitement de la maladie.

Samenvatting in het Nederlands: Narcolepsie is een aandoening die 1 persoon op 2000 raakt en die gepaard gaat met excessieve slaperigheid overdag, met kataplexie (verlies van controle over de spieren na emoties), met slaapstoornissen, met hallucinaties, met depressie en obesitas. Recente observaties gekoppeld aan onze resultaten suggereren dat narcolepsie kan geplaatst worden binnen de auto immuun ziekten, voortkomend uit een genetische voorbestemdheid en omgevingsfactoren (infecties, vaccinaties, enz.) en uiteindelijk leidend tot een selectief verlies van orexine neuronen, een neuropeptide dat het wakker worden en de eetlust reguleert. De validatie van deze hypothese van auto immuniteit zal gevolgen hebben voor een vroegtijdige diagnose en de behandeling van de ziekte.

**Keywords: Narcolepsy, Autoimmune Diseases, hypocretins/orexins, cataplexy., Hallucination, Depression, Obesity**

# Gene-environment models of suicide

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Suicide can be regarded as the endpoint of complex gene-environment interactions. Suicide risk factors may be either distal (early adverse life events or genetic factors determining responses to stressors) or proximal (precipitant stress). Interactions of such stress and several genes have indeed been identified in suicidal individuals, involving e.g. the promoter region of the serotonin transporter gene (5HTTLPR), which may affect suicide risk via changes in functional brain connectivity. Epigenetics provide an intriguing explanation for the relationship between early adverse events, recent stress, genes and suicidal behaviour. For example, a significant effect of childhood abuse on the genetic regulation of hippocampal glucocrtocoid receptors has been demonstrated in individuals who took their own lives. Early-life adversity is also associated with epigenetic modification of genes involved in neuronal plasticity, neuronal growth, and neuroprotection. Thus, via (epi-)genetic mechanisms functional and structural characteristics of "the suicidal brain" may predispose individuals to suicidal behaviour when confronted with environmental stressors.

Résumé en français: Le suicide peut être vu comme la résultante d'une interaction complexe entre des facteurs génétiques qui déterminent notre réponse au stress et des facteurs environnementaux qui peuvent avoir des effets à long terme (difficultés dans l'enfance) ou à court terme (évènement précipitant l'action). Il a été démontré que des évènements stressants pouvaient induire une modification de la régulation de certains gènes responsables de notre capacité de réponse au stress. Cette modification porte le nom d'épigénétisme car il ne s'agit pas d'une modification du gène en lui-même (mutation) mais d'une modification de son utilisation par les cellules.

Samenvatting in het Nederlands : Zelfdoding kan men zien als het resultaat van een complexe interactie tussen genetische factoren die bepalend zijn voor de manier waarop wij stress verwerken en omgevingsfactoren met gevolgen op lange termijn (problemen tijdens de jeugdjaren) dan wel op korte termijn (gebeurtenis die de daad uitlokt). Het werd aangetoond dat stressvolle gebeurtenissen kunnen leiden tot een wijziging van de afstelling van bepaalde genen die onze bekwaamheid om met stress omgaan bepalen. Deze wijziging wordt epigenetisme genoemd, gezien het niet echt een wijziging van de genen (mutatie) betreft maar een veranderd gebruik ervan door de cellen.

**Keywords: serotonin transporter gene, Epigenetics Mechanisms of Plasticity, childhood abuse, BDNF, Glucocorticoids, Prefrontal Cortex, Stress, Psychological**

# Study of the SV2A protein role in Epilepsy

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The SV2A protein is a glycoprotein present in the membranes of most synaptic vesicles. The SV2 protein family includes SV2A but also two other isoforms, SV2B and SV2C [1,2]. Although it is highly conserved during evolution, its physiological role remains largely unknown. However, it has recently been demonstrated that levetiracetam (Keppra®) a very effective drug in epilepsy binds to this SV2A protein [3]. Moreover, in epileptic foci resected in humans with intractable temporal lobe epilepsy, SV2A expression is down-regulated while SV2C is up-regulated [4]. Currently we do not know much about the normal function of SV2A and its possible involvement in diseases such as epilepsy. This project aims to better understand how the SV2A protein may be involved in the occurrence of epilepsy. For this purpose, we engineered a mouse line that allows the conditional removal of SV2A in hippocampal region (CA3 and dentate gyrus (DG)) from the postnatal stages 15 (P15) (Sv2A-cKO). We observed a significant reduction of SV2A proteins and transcripts concentrations in hippocampus of Sv2A-cKO animals in comparison with the wild-type (WT). Together, these results confirmed the efficiency of the invalidation of SV2A in our mouse model. In parallel, we did not measure any significant change in SV2B or SV2C expression, two other member of SV2 family, implying the absence of compensation phenomenon. Finally, our preliminary results show that SV2A-cKO adult animals did not exhibit spontaneous seizure or a decreased threshold of seizures in a PTZ model. Ongoing experiments are designed to identify more precisely Sv2A-cKO phenotype.

Résumé en français: Les mécanismes cellulaires qui sont la cause de l'épilepsie sont encore très mal connus. Notre groupe a récemment mis en évidence (cf référence 4) des modifications de la concentration d'une protéine nommée SV2 (qui existe sous 2 isoformes SV2a et SV2b) chez des patients épileptiques. SV2 est localisée au niveau des terminaisons neuronales mais de fonctions inconnues. Afin d'élucider le ou les rôles de cette protéine nous avons mis au point un modèle de souris qui nous permet de modifier la concentration de SV2 et ainsi d'étudier, au niveau cellulaire, l'effet de cette modification.

Samenvatting in het Nederlands: Mechanismen op celniveau die epilepsie veroorzaken, zijn nog erg slecht gekend. Onze werkgroep heeft onlangs aangetoond dat bij patiënten met epilepsie de concentratie van een eiwit genaamd SV2 (voorkomend als twee iso-vormen SV2a en SV2b) veranderd is. SV2 bevindt zich op de zenuwuiteinden, maar de functie ervan is nog ongekend. Om de rol van dit eiwit op te helderen, hebben we een muismodel ontwikkeld dat toelaat de concentratie SV2 te wijzigen en dus de uitwerking van deze wijziging op celniveau te onderzoeken.

**Keywords: Epilepsy, Temporal Lobe, Hippocampus, SV2A., Synaptic Vesicles, mouse models**

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# Behavioral alterations occurring in the 6 Hz corneal kindling model of limbic epilepsy

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Epilepsy is a neurological disorder which yearly affects 2.4 million people of all ages and social backgrounds (1). Despite many steps that have been taken towards the development of more effective therapies, antiepileptic drugs still fail to control seizures in approximately 30% of patients with epilepsy (2). Together with chronic unpredictable seizures, a variety of comorbidities, such as memory and cognitive dysfunctions and psychiatric symptoms, affects 1 in 2 patients with epilepsy, further deteriorating their quality of life (3). Unarguably, the validation of robust animal models which mimic drug refractoriness and comorbidities is a hot topic in therapeutic epilepsy research. Resistance to antiepileptic drugs was recently observed in the 6 Hz corneal kindling model (4) and this non-invasive, inexpensive and reliable model could thus provide a valid alternative to well-established models of epilepsy. However, due to its novelty, an in-depth characterization of the molecular alterations and behavioral changes occurring in the 6 Hz corneal kindling model has not yet been provided. Therefore, after inducing the "fully kindled state" (defined as 10 consecutive generalized seizures) in male NMRI mice via sub-convulsive corneal stimulations, we performed an elaborate battery of behavioral tests in order to evaluate spontaneous locomotor activity, possible cognitive impairments, and psychiatric-like symptoms. Afterwards, we evaluated neuronal activation comparing c-Fos positive cells in the dentate gyrus of mock-stimulated and kindled mice. We show that 6 Hz corneal kindling does not induce anxiety-like behavior in NMRI mice, as no significant differences between mock-stimulated and kindled mice are observed neither in the time spent in the center of the open field arena nor in the open arms of the elevated plus maze. However a consistent hyper-locomotion in the kindled mice is demonstrated by significantly increased distance travelled in the open field test and number of entries in the Y maze spontaneous alternations test. This hyperactive feature induces a strong bias in the mouse tail suspension and novelty suppressed feeding tests, making it difficult to evaluate depression-like behavior. Furthermore 6 Hz corneal kindling strongly impairs short-term memory, as it induces a decreased preference and time spent in the novel arm in the delayed Y-maze test. No differences are observed in the Y-maze spontaneous alternations test used to evaluate working memory. Our kindling paradigm also induces strong neuronal activation in

the dentate gyrus, a region often recruited in pharmacoresistant forms of epilepsy. To the best of our knowledge, our behavioral characterization depicts for the first time psychotic-like symptoms occurring in the 6 Hz corneal kindling model. Although it requires more research to elucidate whether or not those symptoms can be reversed using anti-psychotic drugs, these data suggest that the 6 Hz corneal kindling model may be used not only to investigate mechanisms of epileptogenesis but also to mimic some comorbidities of epilepsy.

Résumé en Français Un patient épileptique sur deux souffre également de troubles psychotiques. Afin de pouvoir étudier le mécanisme de ces troubles additionnels, nous avons utilisé un modèle animal rendu épileptique par une série de stimulations électriques de 6 Hz de la cornée. Notre étude a porté sur la réalisation de tests comportementaux qui détectent ces troubles additionnels. Nos résultats ont montré que ces souris épileptiques présentaient deux comportements psychotiques additionnels : une augmentation de l'activité locomotrice et des troubles de la mémoire à court termes. Des analyses morphologiques ont également montré que des neurones du gyrus dentelé, une région du cerveau connue pour être impliquée chez les patients présentant une épilepsie pharmaco-résistante, étaient activés. Ce modèle animal épileptique et psychotique devrait permettre d'analyser les aspects bénéfiques de drogues connue comme anti-psychotiques à la fois sur l'épilepsie et le comportement psychotique.

Samenvatting in het Nederlands: Eén op twee epilepsiepatiënten vertoont ook een geheugen-, verstandelijke of psychiatrische stoornis. Er bestaat een goedkoop, niet-invasief en betrouwbaar muismodel van limbische epilepsie, dat tot stand gebracht wordt door elektrische prikkeling (6Hz) van het hoornvlies. Nochtans ontbreekt een diepgaande kennis over de moleculaire en gedragsveranderingen ervan. We onderwierpen daarom aldus epileptisch geprikkelde muizen én schijn-geprikkelde muizen aan een batterij van gedragstests. We konden aantonen dat het muismodel geen angststoornis, maar wel een overmatig loopgedrag en een stoornis van het kortetermijngeheugen vertoont, bovendien een versterkte zenuwactiviteit in de gyrus dentatus, een hersengebied vaak betrokken bij farmacoresistente epilepsie. Verder onderzoek is vereist om te achterhalen of deze symptomen omkeerbaar zijn door antipsychotische middelen.

**Keywords: Epilepsy, kindling model, psychiatric symptoms, Pharmacoresistant epilepsy, Behavior**

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# Connexin43 hemichannel inhibition exerts anticonvulsant effects against experimentally induced seizures

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Epilepsy is one of the most common chronic neurological disorders and is characterized by spontaneous, repetitive seizures. Despite the medical advances over the years, 30 % of patients still suffer from intolerable side effects or drug resistance. Pharmacoresistancy can mainly be explained by the similar mechanisms of action of the marketed drugs, which all act on neuronal targets. Hence, the emphasis in antiepileptic drug discovery is being placed in the discovery of compounds that have distinct profiles of activity than the currently available anti-epileptic drugs. Therefore in this study, we investigated (non-neuronal) astrocytic connexin43 (Cx43) hemichannels as a possible target to treat epilepsy. Cx43 hemichannels are built up by six Cx43 membrane proteins and are located between the astrocytic cytosol and extracellular environment. Various pathological stimuli can activate Cx43 hemichannel opening, leading to the release of small signaling molecules (gliotransmitters, e.g. glutamate, D-serine, adenosine triphosphate), which in their turn can influence neurotransmission and epileptic seizures. To assess the role of Cx43 hemichannels in seizures, we used a newly developed, selective Cx43 mimetic peptide that only inhibits Cx43 hemichannels, without interfering with Cx43 gap junction coupling. We found that this peptide exerts anticonvulsant effects in different acute models of seizures (i.e. the 6 Hz mouse model of refractory seizures and the focal pilocarpine rat/mouse model). Additionally, an anticonvulsant effect was found in a chronic electrical model of epilepsy (6 Hz corneal kindling mouse model of refractory seizures). In the future, it is of very high importance to unravel the potential role of Cx43 hemichannels in epileptogenesis. This study points out the essential role of astrocytes and Cx43 hemichannels in epilepsy and might lead to novel therapy strategies to treat pharmacoresistant patients. If the Cx43 mimetic peptide affects epileptogenesis, it can give rise to the discovery of disease-modifying and/or anti-epileptogenic drugs.

Résumé en français : Titre - Étude d'une nouvelle cible thérapeutique pour le traitement de l'épilepsie : la connexine43. L'épilepsie est une maladie neurologique très fréquente caractérisée par des crises convulsives spontanées et répétées. Malgré les progrès médicaux récents, 30% des patients souffrent encore d'effets secondaires ou de

résistances aux médicaments prescrits contre l'épilepsie. Il est donc important de trouver de nouvelles cibles thérapeutiques. Dans ce travail, nous nous sommes intéressés à une protéine qui forme un canal dans la paroi de certaines cellules de notre cerveau : les astrocytes. La présence de ce canal permet la libération de molécules qui peuvent agir sur les neurones et induire ou exacerber les crises d'épilepsie. Nous avons testé une molécule capable de bloquer ce canal et avons démontré que cette molécule réduisait les crises d'épilepsie dans un modèle murin d'épilepsie résistante.

Samenvatting in het Nederlands: Titel: Studie van een nieuwe therapeutische aanpak voor de behandeling van epilepsie: connexin43. Epilepsie is een veelvoorkomende neurologische ziekte die gepaard gaat met spontane en herhaalde stuiptrekkingen. Ondanks recente medische vooruitgang blijven 30% van de patiënten lijden onder bijwerkingen of reageren ze niet op de voorgeschreven medicatie tegen epilepsie. Nieuwe behandelingsvormen zijn dus noodzakelijk. In dit werk ging onze aandacht naar een proteïne die een kanaal vormt in de wand van bepaalde hersencellen: de astrocyten. Dit kanaal laat toe dat moleculen vrijkomen die invloed kunnen hebben op de neuronen en epilepsieaanvallen uitlokken of verergeren. We hebben een molecule getest die dit kanaal kan blokkeren en konden aantonen dat deze molecule de kans op een epilepsieaanval deed afnemen bij muizen met resistente epilepsie.

**Keywords: connexin43 hemichannels, Epilepsy, Animal Models, Seizures, Peptides**

# Neural sources from resting state eeg oscillations in angelman syndrome

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Angelman syndrome (AS) is a neurodevelopmental genetic disease due to deficient UBE3A expression. We recorded and analyzed high density electroencephalogram (128 electrodes) by signal averaging and EEG inverse solution model estimation in 6 children with AS (aged 2-5) while in a default-mode (resting state). We found a consistent theta oscillation (5Hz) originating in somatosensory associative cortex (BA7) that could be explained by the contribution of the hippocampal network able to produce theta oscillation on the cortical mantle. We hope this new approach can be used to better understand the origin of the emergence of recurrent theta oscillations in AS patients.

Résumé en français : Titre : Enregistrement de l'activité cérébrale des patients souffrant du syndrome d'Angelman. Le syndrome d'Angelman est une affection génétique qui perturbe le développement du cerveau menant à un retard mental sévère et des troubles de la motricité et de l'équilibre. Nous nous intéressons à l'activité électrique du cerveau chez les enfants atteints de ce syndrome. Cette activité est enregistrée grâce à l'électroencéphalographie qui permet de détecter les modifications de connexions entre les différentes zones du cerveau au repos et lors de tâches cognitives. Une meilleure compréhension du fonctionnement du cerveau de ces enfants pourrait permettre de mieux les stimuler afin d'améliorer leur condition.

Samenvatting in het Nederlands: Titel: Het registreren van hersenactiviteit bij patiënten die lijden aan het syndroom van Angelman. Het syndroom van Angelman is een genetische aandoening die de ontwikkeling van de hersenen stoort, wat leidt tot mentale achterstand en een storing van de motoriek en het evenwicht. Wij interesseerden ons voor de elektrische activiteit van de hersenen bij kinderen die aan deze ziekte lijden. Deze activiteit wordt geregistreerd met een elektro-encefalografie die toelaat vast te stellen of er veranderingen optreden in de verbindingen tussen de diverse hersenzones, bij rust en tijdens het uitoefenen van cognitieve taken. Een beter begrip van het functioneren van de hersenen bij deze kinderen zou toelaten hen te stimuleren, en hun toestand te verbeteren.

**Keywords: Angelman Syndrome, EEG, oscillations, synchrony, neural networks, Epilepsy, source localization, Cortex**

# The effect of trigeminal nerve stimulation (TNS) on the noradrenergic signaling in the brains of healthy volunteers

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Introduction Trigeminal nerve stimulation (TNS) is currently under investigation as a non-invasive treatment for refractory epilepsy. In TNS the trigeminal nerve is stimulated through an electrode placed on the forehead. The number of open label and randomized controlled trials with TNS in epilepsy are limited, which show encouraging results of responder rate and seizure frequency reduction. Based on the anatomical projections of the vagus nerve and trigeminal nerve to the brainstem nucleus of the solitary tract and locus coeruleus, the mechanism of action of TNS is hypothesized to be similar to that of invasive vagus nerve stimulation (VNS) (1). Previous experiments at the LCEN3-lab demonstrated in a translational setting using hippocampal microdialysis and lesioning experiments in epileptic rats, that VNS-induced NA release plays a crucial role in the seizure suppressing mechanism of action of VNS (2). Non-invasive measurements of VNS-induced NA release by means of P300 event-related potential recordings in epilepsy patients was able to distinguish responders and non-responders to VNS treatment (3). Methods To investigate the effect of TNS on the noradrenergic signaling in the brain, we performed a pilot study in which P300 event-related potentials were recorded in 21 healthy volunteers (10 M, 11 F) between 18 and 30 years old. The P300 event-related potential can be extracted from the EEG and is elicited by performing an auditory oddball paradigm, in which a random sequence of frequent low standard tones and rare high oddball tones are presented. The subject was instructed to press a predefined button after presentation of an oddball tone while ignoring the standard tones. Each subject performed the auditory oddball task in three conditions: TNS (120 Hz, 250 μs, 30s ON/30s OFF), sham stimulation (2 Hz, 250 μs, 1s ON/90s OFF) and no stimulation. The stimulation intensity was defined individually on beforehand to the maximum tolerable output current of TNS (range: 2.8 mA – 7.4 mA). The conditions were randomized and separated by a 30-minute break to avoid a carry-over effect. Results There was no statistically significant effect of the stimulation condition on the mean P300 amplitude or latency. However, the analysis showed a higher P300 amplitude in the condition TNS compared to no stimulation. This increase was higher than the pre-defined cut-off score of > 1 μV, which was considered as clinically relevant based on the results of a similar study with P300 measurements in epilepsy patients

treated with VNS (3). Based on the statistical analysis it cannot be concluded that the conditions sham and no stimulation were equivalent. There was a statistically significant difference in mean P300 amplitude for the variable "sex", in which females had a significantly higher P300 amplitude compared to males. Variables "age", "tobacco use", "alcohol use" and "coffee consumption" did not influence the mean P300 amplitude. The P300 latency was not affected by any tested variable. Conclusions In conclusion, although not statistically significant, TNS induced a clinically relevant increase in P300 amplitude compared to no stimulation, reflective of noradrenalin release in the brain. More volunteers will be recruited to investigate whether this clinically relevant effect might also become statistically significant.

Résumé en Français: Le nerf trijumeau est un nerf crânien transmetteur de la douleur mais aussi de mouvement. Des travaux récents ont montré que sa stimulation avait un effet bénéfique sur les épileptiques. Ce travail vise à déterminer les conditions optimales de stimulation à appliquer et à affiner ce traitement parmi des sous-groupes de patients épileptiques.

Samenvatting in het Nederlands: De nervus vagus is een hersenzenuw die verantwoordelijk is voor ervaren van pijn in het gezicht, maar ook instaat voor beweging van het gezicht. Recent onderzoek heeft aangetoond dat stimulatie van deze zenuw een positief effect heeft op aanvallen bij mensen met epilepsie. Dit werk heeft als doel de voorwaarden om optimaal te kunnen stimuleren te bepalen en subgroepen van patiënten met epilepsie die het meest baat zouden hebben bij deze behandeling, te identificeren.

**Keywords: Epilepsy, trigeminal nerve stimulation, P300 event-related potential, noradrenalin, mechanism of action**

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# Shining light on the role of Parvalbumin interneurons in cortical spreading depression

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As a pathological phenomenon, Cortical Spreading Depression (CSD) is the most likely cause of migraine aura. CSD has also been implicated in the physiopathology of traumatic brain injury and ischemic/hemorrhagic stroke. It is even likely that after traumatic brain injury, depolarization waves such as CSD exacerbate brain damage. Using electrophysiological recordings and DC-coupled amplification this process can be visualised as slow and large negative potential drops with amplitudes in the range of -5 to -25 mV. While the macroscopic changes related to CSD are now clear, microscopic mechanisms and cell-cell interactions need further study. Optogenetics is an efficient and well-established method in which viral vectors are used to induce expression of light-sensitive ion channels like Channelrhodopsin-2 (ChR2) into the membranes of specific cell types, such as neurons, allowing the researcher to manipulate the ion fluxes of these cells independently using light only. Our starting hypothesis was that suppressing inhibitory neurons will modulate velocity and frequency of CSD. Since 50 % of the GABA-ergic cortical interneurons express parvalbumin (PV), we focused on investigating PV(+) interneurons. In the experimental group we injected a Cre-dependent vector (AAV2/7-CMV-FLEX-ChR2-mCherry) in adult P120 PV-ires-cre mice (C57Bl/6J background, n=5) in order to get cell-specific ChR2 expression in PV(+) interneurons. We used a total of 6 C57Bl/6J mice (4 injected with a nonspecific neuronal vector AAV2/7-CMV-ChR2-eGFP and 2 blanks) as control group. All animals were injected in the left hemisphere of the visual cortex (-3.2mm AP, 2.5mm LM (BR); depth 400 um; 15x50 nl; Drumond Nanoject II) and recovered at least for 4 weeks. CSD was induced in an acute preparation as follows. Animals were anesthetized with a combination of urethane and chlorprothixene. Two craniotomies for each hemisphere (4 in total) were prepared having one positioned above the visual cortex and one above the prefrontal cortex. CSD waves were constantly induced by placing cotton balls on the visual cortices which were impregnated with 1M KCl solution. CSD waves were recorded in the prefrontal cortex using Ag/AgCl electrodes embedded in glass capillaries and an optical fiber was positioned above the visual cortex of the transduced hemisphere for laser stimulation (10 Hz, 50mW/mm$^2$, 60 mins). Our results so far demonstrate that there is no effect on the duration of CSD waves between the two mouse strains or upon an optogenetic activation of either the complete set of

neurons or the PV(+) interneurons subtype. In contrast, we found an effect caused by the genetic background when comparing C57Bl/6J mice and PV-Cre mice where in the latter genotype CSD waves propagate with a higher frequency. In addition, optogenetic activation of PV(+) interneurons increases the frequency even further. These results suggest both the involvement of the genetic background and the role of the excitation-inhibition balance on CSD initiation and propagation. Implementing the optogenetics toolbox in CSD research provides a unique opportunity to look into cell type-specific mechanisms of CSD onset/propagation and can facilitate dissecting different physiopathological feedback loops.

Résumé en français: La propagation de la dépression corticale (CSD) est une onde de dépolarisation de neurones qui est à la base de la migraine mais est aussi impliquée lors d'un traumatisme crânien. Afin d'analyser de manière précise quels étaient les sous-populations de neurones responsables de cette onde de dépolarisation, nous avons utilisé sur des rats la technique dite d'optogénétique qui permet de visualiser l'activité de neurones ciblés. Nos résultats suggèrent qu'à la fois le patrimoine génétique et l'équilibre excitation-inhibition jouent un rôle lors de l'initiation et la propagation de la CSD.

Samenvatting in het Nederlands : de verspreiding van de corticale depressie (CSD) is een depolarisatie golf van neuronen die aan de basis ligt van migraine, maar ook voorkomt bij een hersentrauma. Om een nauwkeurige analyse te maken van de subgroepen van neuronen de deze golf veroorzaken hebben we bij ratten gebruik gemaakt van de zogenaamde optogenetische techniek, die toelaat de activiteit van geviseerde neuronen te visualiseren. Onze bevindingen suggereren dat zowel het genetisch patrimonium als het evenwicht prikkeling/remming een rol spelen bij het ontslaan en de verspreiding van CSD.

**Keywords: Cortical Spreading Depression, optogenetics, parvalbumin interneurons, Migraine with Aura, Cortex, Electrophysiology**

# Does regional sunlight irradiance influence habituation of visual evoked potentials in migraine?

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Background. Deficient habituation of visual evoked potentials (VEP) is a redundant neurophysiological finding in interictal episodic migraine sufferers. However, not all studies were able to retrieve this feature. The discrepancies can probably not be explained on the sole basis of methodology. Migraine is a multifaceted disease that arises from the interaction of a genetic predisposition with an enabling environment. Aim and methods. We hypothesized that environmental factors such as regional sunlight irradiance could modulate the activity of visual pathways in migraine patients and account for some discrepancies between VEP studies. We performed a retrospective analysis of studies that evaluated VEP habituation in episodic migraine and correlated the available data with in-situ mean solar radiation. Results. Twenty-six studies were included. Mean sunlight irradiance was significantly higher in locations of studies reporting deficient habituation (133.3+/-41.05 vs. 99.43+/-42.81 W/m*2,p=0.022). Interestingly, the VEP habituation slope positively correlated with sunlight irradiance in both migraineurs (n=351,p<0.001) and healthy controls (n=348, p<0.001,figure 1). Moreover, there was a significant difference between VEP slopes reported in controls from northern compared to southern countries (-0.34vs.-0.28 μV/block, p<0.01). Conclusions. This study suggests that variations in sunlight irradiance may induce adaptive modifications in visual processing that are reflected in VEP habituation studies and could in part account for the different results found in geographically distant centers. Increased sunlight irradiance seemingly reduces the habituation capability exacerbating the habituation deficit in migraine patients. However, other causal factors, such as genetic differences, likely play a role.

Résumé en français: Bien que l'existence d'un déficit d'habituation aux potentiels évoques visuels dans la migraine ait été démontrée, toutes les études ne retrouvent pas cette altération. Afin d'expliquer cette variabilité et d'étudier le rôle de l'irradiation solaire sur la modulation de l'activité des voies visuelles, nous avons réalisé une analyse rétrospective de la littérature des études de potentiels évoques visuels. Cette analyse a montré que l'irradiation solaire était plus importante dans les endroits du monde où les résultats rapportaient un déficit d'habituation, suggérant un rôle des facteurs

environnementaux tels que l'irradiation solaire dans les modifications du traitement de l'information sensorielle.

Samenvatting in het Nederlands: Hoewel het bestaan van een deficit in elektrofysiologische reacties van de hersenen op visuele gebeurtenissen in de omgeving bij migraine aangetoond werd, vinden niet alle studies deze kwaliteitsvermindering terug. Om deze verschillen te verklaren en om de rol van de zonlichtuitstraling op de visuele aanpassing te bestuderen, maakten we een retrospectieve literatuurstudie met betrekking tot studies inzake het opwekken van visueel vermogen. Deze analyse toonde aan dat in bepaalde streken op aarde waar de resultaten melding maken van een deficit in de gewenning, de zonnestraling groter was. Er wordt gesuggereerd dat omgevingsfactoren, zoals zonnestraling een rol zouden kunnen spelen in de wijzigingen waarop zintuiglijke informatie verwerkt wordt.

**Keywords: Migraine, visual evoked potential (VEP), Sunlight irradiance, Habituation deficit, Electrophysiology**

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# Familial history of migraine influences habituation of visual evoked potentials

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Background. Lack of habituation of visual evoked potentials (VEP) is a common finding in migraine patients between attacks. Previous studies have suggested an electrophysiological familial aggregation pattern associated with migraine. The aim of this study was to evaluate the influence of a positive familial history of migraine on VEP amplitude and habituation. Methods. We recorded 6 blocks of 100 VEP during continuous pattern-reversal stimulation in 30 patients with migraine between attacks (MO) and in 30 healthy volunteers of whom 15 had a 1st degree relative suffering from migraine (HVm) and 15 had not (HV). Results. Both MO and HVm had a significant deficit of VEP habituation and similarly reduced N1-P1 1st block amplitudes, compared to HV (habituation slope: MO= 0.033,HVm= 0.021, HV= -0.025, HV vs. MO p=0.002, HV vs. HVm p=0.036; mean N1-P1 amplitude in the first block: MO= 9.08μV, HVm=9.29μV, HV=12.19μV. HV vs. MO p=0.041, HV vs. HVm p=0.076) (Fig 1 & 2). The first block N1-P1 amplitude was negatively correlated with the habituation slope for both MO (ρ= -.44, p=0.015) and HVm (ρ= -.56, p=0.031) while no significant correlation was found in HV (ρ= .17, p=0.53). There were no differences in VEP latencies between the groups. Conclusions: Our study suggests that lack of habituation of visual evoked potentials is probably a genetically determined endophenotypic trait which is associated with both migraine and migraine susceptibility. We hypothesize that genetic diversity of populations could account for some of the discrepancies between electrophysiological studies performed in migraine and for interindividual variations among the subgroups.

Résumé en français: Sur la base des études précédentes en électrophysiologie, nous avons testé si le déficit d'habituation aux potentiels évoqués visuels avait une composante héréditaire. Pour ce faire, nous avons comparé les réponses aux potentiels visuels de migraineux et de sujets sains avec ou sans antécédents familiaux de migraine. Nous avons observé chez les sujets sains avec antécédents familiaux de migraine une réponse similaire aux patients. Ce résultat suggère que le déficit d'habituation pourrait être génétiquement déterminé mais que d'autres facteurs seraient nécessaires pour développer la maladie.

Samenvatting in het Nederlands: Op basis van vroegere studies over elektrofysiologie hebben we onderzocht of het deficit in elektrofysiologische reacties van de hersenen op visuele gebeurtenissen in de omgeving erfelijk bepaald is. Daartoe hebben we een vergelijking gemaakt tussen de antwoorden van personen die lijden aan migraine en gezonde personen, al dan niet met familiale antecedenten van migraine. Bij gezonde personen met familiale antecedenten van migraine vonden we antwoorden gelijkwaardig aan deze van de patiënten. Dit laat ons veronderstellen dat het deficit in de gewenning erfelijk bepaald zou kunnen zijn, maar dat andere factoren noodzakelijk zijn om de ziekte te ontwikkelen.

**Keywords: visual evoked potential (VEP), Migraine Disorders, Genetics, family history., Habituation deficit**

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# A machine-learning classifier for episodic migraine based on visual evoked gamma band activity

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Introduction. Objective and reliable biomarkers of migraine may be of interest for diagnosis and research purposes. Neuroimaging-based machine-learning classifiers are promising but hampered by availability and cost issues. Conversely, evoked potential are of easy access and affordable. They have provided increasing evidence that sensory information processing is impaired in migraine. We have used gamma band oscillations (GBOs) of visual evoked potentials (VEPs) to compute a machine-learning neural network classifier in episodic migraine. Materials and methods. We analyzed GBOs from VEPs (6x100 responses). Recordings were performed in two matched samples: a training sample composed of 43 migraine patients (EM) and 20 healthy volunteers (HV) and a validating sample of 18 EM and 10 HV. A logistic regression model of the training sample was performed to evaluate the relevance of the predictor variables. Ten neural networks were automatically generated based on late component frequency, n3-p4 and p4-n4 slopes, 1st block n1-p2 amplitude and age. Results. The logistic regression model of the training sample reached a significant classification rate of 79% (EM: 88%; HV: 60%, p=0.002). The best neural network was able to classify the groups with an accuracy of 73% in the training phase and 89% in the subsequent validation (success rate HV: 90%; EM: 88%). The mean global accuracy within the training and validating samples were 69% (63-78%) and 84% (82-89%). Conclusion. This machine-learning neural network classifier based on visual GBOs provides an accurate and cost-efficient tool for objective migraine diagnosis. Further training and validation studies with new cohorts are warranted.

Résumé en français: Classification des migraineux épisodiques sur base des oscillations gamma lors de potentiels évoques visuels. Malgré des différences électrophysiologiques décelées de manières répétées chez les patients migraineux, il n'existe pas encore de biomarqueur assez sensible qui permette de les distinguer des sujets sains. Dans cette étude, nous avons utilisé les oscillations gamma des réponses aux potentiels évoques visuels pour entrainer et ensuite tester un classifieur informatique. Ce classifieur a permis de sensiblement distinguer les patients migraineux des sujets sains, ce qui suggère un potentiel futur outil diagnostic simple et efficace.

Samenvatting in het Nederlands: Classificering van migrainelijders op basis van de gamma trillingen tijdens elektrofysiologische reacties van de hersenen op visuele gebeurtenissen in de omgeving. Ondanks elektrofysiologische verschillen die regelmatige te merken zijn bij migrainepatiënten, bestaat er nog steeds geen bio marker gevoelig genoeg om het onderscheid te maken met gezonde personen. In deze studie maakte we gebruik van gamma trillingen bij deze reacties om een geïnformatiseerde classificering uit te werken en te testen. Deze classificering laat toe om migrainepatiënten en gezonde personen duidelijk te onderscheiden, en opent een potentieel om in de toekomst op een eenvoudige en efficiënte manier de diagnose te stellen.

**Keywords: machine-learning, visual evoked potentials, Migraine, gamma oscillations, diagnosis**

## Acknowledgements

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# Distinct cerebral metabolic patterns related to high pain sensitivity in episodic or chronic migraine patients and healthy volunteers

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Introduction Allodynia, i.e. pain evoked by a non-painful stimulus, is prevalent in chronic pain and in migraine where it augments with disease severity and chronicity [1]. Central sensitization is thought to be the culprit [2]. It is not known, however, which central areas are involved. The aim of the present study was to evaluate whether brain metabolism in subjects that are more sensitive to pain is different between migraine patients and healthy controls. Subjects and methods Quantitative heat sensory testing on the forehead and 18FDG-PET were performed in 55 subjects: 20 healthy volunteers (HV, 21-59 years, 5M), 21 patients with episodic migraine in the interictal phase (MO, age range: 20-63 years, 5M) and 14 patients with chronic migraine (CM, age range: 22-62 years, 1M). The 3 cohorts were subdivided according to the median heat pain threshold into subgroups with low and high pain thresholds. PET results were compared between these subgroups in each cohort. Data analyses were restricted to areas of the pain/salience matrix. Results There was no significant difference in heat pain thresholds between HV (median: 43.7 °C), MO median: 44.2°C) and CM (median: 43.3°C) ($p=0.64$). In an SPM-ANOVA, a contrast modelling the potential gradual effect of increased differences in pain sensitivity in relation to disease severity showed significant metabolic changes in bilateral thalamus and midbrain ($p < 0.001$). Additional analyses revealed that hypometabolic areas in subgroups with a low heat pain threshold differed between HV (anterior cingulate and somatosensory cortices), MO (lower pons and somatosensory cortex) and CM (midbrain and thalamus) (Figure 1). Conclusion Overall migraine patients do not have reduced heat pain thresholds. However, hypometabolic areas related to high thermal pain sensitivity are strictly cortical in HV, but comprise the pons in episodic migraine and are restricted to midbrain and thalamus in chronic migraine. The distinct central correlates of heat pain sensitivity in migraine patients might therefore represent a biomarker of migraine and its chronification. Legend to figure Figure 1. Hypometabolic areas in low pain threshold subgroups in HV (green), MO (orange) and CM (red). $p < 0.01$ for display purpose.

Résumé en français: Titre: Métabolisme cérébral distinct en relation avec la sensibilité à la douleur entre sujets sains, migraine épisodique et migraine chronique. Les migraineux ont une sensibilité anormale à la douleur. Les mécanismes cérébraux en sont

inconnus. Nous avons comparé le métabolisme cérébral chez des sujets sains et chez des migraineux épisodiques ou chroniques et corrélé les résultats avec le seuil douloureux. Les aires cérébrales hypométaboliques liées à un seuil douloureux bas diffèrent entre groupes: régions corticales chez les sujets sains, aires corticales et sous-corticales dans la migraine épisodique, régions sous-corticales dans la migraine chronique .Le contrôle central de la douleur semble modifié distinctement dans les formes de migraine, ce qui pourrait en constituer un biomarqueur et avoir des implications thérapeutiques.

Samenvatting in het Nederlands: Titel: Het aparte hersenmetabolisme gerelateerd tot de gevoeligheid voor pijn bij gezonde personen en personen die lijden aan episodische en chronische migraine. Personen die lijden aan migraine zijn abnormaal gevoelig voor pijn. De hersenmechanismen daarvan zijn onbekend. We vergeleken het hersenmetabolisme van gezonde personen en van personen die lijden aan episodische of chronische migraine en vergeleken de resultaten met de pijndrempel. De hypo metabolische hersengebieden gelinkt aan een lage pijndrempel verschillen tussen groepen: gebieden in de hersenschors bij gezonde personen, gebieden in de hersenschors en subcorticale gebieden bij episodische migraine, subcorticale hersenschorsgebieden bij chronische migraine. De centrale controle van pijn blijkt anders te zijn bij de vormen van migraine, wat een biomarker zou kunnen vormen met gevolgen voor de behandeling.

**Keywords: episodic migraine, chronic migraine, Cerebral metabolism, heat pain sensitivity, FDG-PET**

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# Mutations in the ABCC6 gene are associated with an increased risk for ischaemic stroke

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Background:Ischemic stroke (IS) results from a complex interplay between environmental and genetic risk factors. Because of the increased IS incidence in pseudoxanthoma elasticum (PXE) – an autosomal recessive connective tissue disease with skin, eye and cardiovascular (CV) symptoms due to ABCC6 mutations –, the higher CV risk in carriers of one ABCC6 mutation and the established role of the ABCC6 transporter in myocardial ischemia, ABCC6 was hypothesized to be a candidate risk factor for IS. Methods and Results: In a three-generation family, we established segregation of a known ABCC6 mutation (p.Arg1314Gln) in 18 individuals with IS and/or CV disease at young age. Two family members were identified as having PXE, due to co-inheritance of a second ABCC6 mutation. In an independent cohort of 424 IS patients, we identified 18 carriers of one ABCC6 mutation compared to 2 carriers in controls. None showed clinical features of PXE. Carriers were heterogeneous in age, familial history and stroke type. The calculated Odds Ratio was 5.4975 (p=0.023; 95% CI 1.2-23.8). No interaction with other CV risk factors was noted. To study the cellular consequences of ABCC6 deficiency in the brain, we focussed on BMP signaling as perturbation of BMP ligands and receptors has been previously associated with ABCC6 and its role in myocardial ischemia. Preliminary results of immunofluorescent staining in the brain of the Abcc6 knock-out mouse confirms BMP dysregulation with increased BMP4 expression. Such overexpression has already been associated with impaired stroke recovery. Conclusion: The perfect segregation of an ABCC6 mutation in affected members of a multi-generation family with cerebro- and cardiovascular disease suggests heterozygous ABCC6 mutations to be a significant risk factor for IS. This was confirmed by a high incidence of ABCC6 mutations in cryptogenic IS patients compared to controls. The mechanism underlying this increased susceptibility involves BMP signalling, similar to the effect of ABCC6 deficiency in myocardial ischemia. As demonstrated by the diagnosis of two PXE patients in our first family, identification of ABCC6 mutation carriers has important implications for genetic counselling and follow-up of these families.

Résumé en français: Cette étude montre que les mutations du gène ABCC6, qui sont à l'origine d'une pathologie du tissu conjonctif appelée pseudoxanthome élastique (PXE), sont également fréquentes chez des patients ayant un infarctus cérébral mais ne présentant pas de symptômes de PXE. Les mutations ABCC6 peuvent donc être considérées comme un facteur de risque indépendant pour les infarctus cérébraux agissant via la voie des BMP, protéines de croissance impliquées dans la prolifération cellulaire. Ces résultats indiquent qu'il pourrait être utile de procéder à une analyse ABCC6 chez les patients ayant un infarctus cérébral avec peu ou pas de facteurs de risque classiques. Les résultats de cette analyse ont un impact considérable sur les conseils génétiques et le suivi du patient et de sa famille.

Samenvatting in het Nederlands Dit onderzoek toont aan dat genetische fouten (mutaties) in het ABCC6 gen, verantwoordelijk voor pseudoxanthoma elasticum (PXE), frequent voorkomen bij patiënten met een herseninfarct die geen symptomen van PXE vertonen. ABCC6 mutaties kunnen beschouwd worden als een onafhankelijke risicofactor voor herseninfarcten, op basis van een verstoorde BMP signalisatie in de hersenen. Deze resultaten wijzen op het nut om bij patiënten met een herseninfarct, waarvoor geen of onvoldoende klassieke risicofactoren gevonden worden, een ABCC6 analyse uit te voeren. De resultaten van deze analyse hebben een belangrijke impact op de genetische adviesverstrekking en opvolging van de patiënt en zijn familie.

**Keywords: ABCC6, ischaemic stroke, BMP signaling, BMP4, susceptibility factors, Genetic counselling**

# Transplantation of fluorescent neural stem cells in healthy and stroke-affected mice

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Transgenic mouse line Thy1-YFP selectively expressing fluorescent proteins in neurons has been developed to study many aspects of neuronal structure and connectivity. Taking advantage of the long term expression and repeated expression of Yellow Fluorescent Protein (YFP) labeled cells being minimally toxic and providing robust fluorescent signal (Feng et al.) we used YFP labeled stem cells. Neural stem cells were purified from the THY1 YFP-16 transgenic mouse line to study the birth and fate of transplanted YFP labelled neuronal stem cells in the healthy and stroke-affected mouse brain. During embryonic development THY1 – YFP positive cells were first observed at E12.5 and they were followed during differentiation of the nervous tissue. Thy1-positive cells were mostly present in prosencephalon, rombencephalon the spinal cord and in peripheral nerves of the embryo. Number of THY1 – YFP positive cells was 22% of the total cells and remained constant along the differentiation. Analysis on both RT-PCR and immunocytochemical level revealed that Thy1 positive cells during embryonic and in vitro differentiation were first nestin/SOX2 positive, which was gradually replaced by expression of MAP2, β3-tubulin and NeuN. Neural stem cells isolated from THY1 – YFP mouse strain transplanted in the striatum of the healthy and stroke affected mouse brain differentiated into mature neurons and were detectable even after 14 weeks, the end point of our experiment. Stroke region attracted transplanted cells but did not affect signal of Thy1. This study revealed that neural stem cells during in vitro differentiation and after transplantation in the brain followed the same pattern observed during development of embryo.

Résumé en français: Il est aujourd'hui possible de transplanter des cellules souches et leurs dérivés (p.ex. des cellules souches neurales). Cette nouvelle approche thérapeutique ouvre la voie vers le remplacement de neurones défectueux ou morts observés dans les maladies neurodégénératives. Ce concept doit être validé par la parfaite connaissance du devenir de ces cellules transplantées. Notre travail décrit le devenir de cellules souches neurales de souris une fois transplantées dans le cerveau de souris normales ou ayant subi un accident vasculaire cérébral. Le suivi de ces cellules a pu être réalisé grâce au marquage préalable de ces cellules par un marqueur fluorescent.

Samenvatting in het Nederlands: Het is tegenwoordig mogelijk om stamcellen of de daarvan afgeleide cellen (bv. stamcellen van het zenuwstelsel) te transplanteren. Deze therapeutische benadering effent de weg naar het vervangen van gebrekkige of dode hersencellen bij degeneratieve hersenaandoeningen. Dit concept dient gevalideerd te worden door een gedegen kennis van het lot van zulke getransplanteerde cellen. Ons werk beschrijft het lot van neurale muizestamcellen na transplantatie in de hersenen van normale muizen of in de hersenen van muizen die een beroerte hebben opgelopen. Het opvolgen van deze cellen wordt mogelijk gemaakt door een voorafgaande markering met een fluorescerende merker.

**Keywords: Neural Stem Cells, Yellow fluorescent protein, Transgenic mice, Stem Cell Transplantation, Stroke Conference.**

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# Development of potent neuromedin u receptor agonists for regulating feeding behavior

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Diseases such as diabetes and obesity have become major health concerns worldwide. To address this issue, our group is attempting to contribute through a focus on neuromedin U (NMU), a highly conserved neuropeptide regulator of feeding, energy homeostasis and glycemic control. It exerts its biological effects via two G protein-coupled receptors, NMUR1 and NMUR2. NMUR1 is mostly found in the periphery whereas NMUR2 is most abundant in the central nervous system. Both central and pheripheral administration of the peptide reduce food intake and body weight in rodents. The anorexigenic effect of NMU renders NMUR agonists attractive as potential therapeutics in the treatment of diabetes and obesity [1]. NMU-8 (H-Tyr-Phe-Leu-Phe-Arg-Pro-Arg-Asn-NH2), a natural occurring fragment of NMU, is taken as lead molecule for the synthesis of novel analogues. A first batch of analogues is prepared on basis of the available structure-activity relationships described in the literature [2,3]. Mainly two types of modifications were initially performed, namely chirality switches and the introduction of different N-capping groups. In a second set of NMU-ligands, more advanced modifications are performed, such as the introduction of unnatural/constrained amino acids or N-alkylated glycines ('peptoid') analogues in the NMU-sequence. A third generation of compounds was synthesized and contains analogues in which the most promising modifications of the previous generations were combined. The in vitro characterization of these peptides has been performed by an inositol phosphate accumulation assay. Additionally, the plasma stability of these analogues has been investigated. The results of the in vitro characterization present the discovery of high potency agonists. Compared to NMU-8, more active agonists on both NMURs were discovered. Our experiments revealed, for instance, that acetylation of the N-terminus leads in general to an increase of activity. When replacing Tyr1 by 7-OH-Tic or Dmt, extremely potent agonists for both receptors were obtained as well. Moreover, an improved plasma stability of these compounds is observed. The replacement of Phe4 by 7-OH-Tic, Dmt, Oic, 1'Nal or 2'Nal leads to ligands with a comparable activity to NMU-8, but an increased plasma stability emerged. The most promising ligands were tested in an in vivo model to study their effect on food intake, and promising results were obtained. Summary: This research aims to further unveil the

role of the neuropeptide neuromedin U (NMU), and more specific its function in the regulation of food intake. Since diseases like obesity and diabetes have become major health concerns, the anorexigenic effect of NMU renders NMUR agonists attractive as novel therapeutics in the treatment of obesity and diabetes.

Résumé en français: Titre: Développement de nouvelles molécules capables d'activer le récepteur de la Neuromédine pour le contrôle de la prise alimentaire. Le but de notre recherche est de mieux comprendre le rôle d'une petite molécule protéique produite par le système nerveux, la Neuromédine, dans la régulation de la prise alimentaire. Il a en effet été démontré que cette petite molécule avait des effets suppresseurs de l'appétit en agissant sur un récepteur spécifique appelé NMUR. De nouvelles molécules thérapeutiques ciblant ce récepteur NMUR pourraient permettre de lutter contre les problèmes d'obésité et de diabète.

Samenvatting: Titel: Ontwikkeling van nieuwe moleculen die de receptor van neuromedine kunnen activeren en de voedselinname reguleren. Het doel van ons onderzoek is een beter inzicht te krijgen in de rol van neuromedine, een kleine eiwitmolecule die wordt geproduceerd door het zenuwstelsel, bij de regulering van de voedselinname. Er werd namelijk aangetoond dat deze molecule een eetlustremmend effect heeft door haar actieve werking op de NMUR-receptor. Nieuwe therapeutische molecules die gericht zijn op deze NMUR-receptor, kunnen helpen in de strijd tegen obesitas en diabetes.

**Keywords: neuromedin U, Obesity, peptide synthesis, inositol phoshate accumulation assay, NMUR agonists**

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# In vivo effects of anti-glutamate decarboxylase 65 antibodies on the hippocampus

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Anti-GAD 65 antibodies have been detected in patients suffering from various pathologies such as stiff person syndrome, cerebellar ataxia, limbic encephalitis and type 1 diabetes. It binds the smaller isoform of GAD that converts the excitatory glutamate into inhibitory GABA at the level of GABAergic neurons. It has been proved that in vivo injections of anti-GAD65 antibodies induce motor deficits (Hansen et al., 2013), increase of extracellular glutamate concentration (Manto et al., 2011), anxious behavior (Geis et al., 2011) and also alteration of spatial strategies and cognitive capacities (Hampe et al., 2013). These effects are epitope specific. It has been demonstrated that b78 recognizes an epitope located at the C-terminus (512-540) while the b96.11 target is situated in the center of the protein (308-365). These two specific anti-GAD65 human monoclonal antibodies derived from a patient with autoimmune polyendocrine syndrome type 1 and have been isolated by the laboratory of Christiane Hampe (University of Washington). In our laboratory, previous research performed on hippocampal organotypic cultures showed that b78 GAD65 antibodies induce an inhibition of long term potentiation and also a microglial proliferation. So in this study we tend to verify these observations in vivo and to explore more deeply the specific effect of b78 and b96.11 GAD65 antibodies on the hippocampus. To do so, both anti-GAD65 antibodies are slowly injected in the third ventricle of mice. Electrophysiological measurements are conducted to study the long term potentiation of synaptic response on acute hippocampal slices. The inflammatory state of the hippocampus is examined by immunohistochemistry. Furthermore the microdialysis technique will be developed to quantify extracellular glutamate and GABA. Cognitive impairment will be studied by behavioral tests.

Résumé en français: Des anticorps anti-glutamate décarboxylase 65 (anti-GAD65) ont été décelés chez des personnes souffrant de diverses pathologies telles que l'encéphalite limbique, le « stiff person syndrome », l'ataxie cérébelleuse ainsi que le diabète de type 1. Ces anticorps sont produits par les patients et ciblent une protéine présente dans le cerveau, la GAD65. Celle-ci assure la conversion du neurotransmetteur excitateur glutamate en neurotransmetteur inhibiteur GABA au niveau des neurones. À l'heure actuelle, plusieurs équipes s'appliquent à mieux comprendre le mécanisme d'action ainsi que les effets de ces anticorps sur le système nerveux. Le laboratoire de Neurosciences

de l'UMONS s'est focalisé sur leur incidence dans l'hippocampe, une structure cérébrale indispensable dans le processus de mémorisation.

Samenvatting in het Nederlands: Anti-glutamate decarboxylase 65 (anti-GAD65) antilichamen werden ontdekt bij personen die lijden aan diverse ziektes zoals limbische encefalitis, het "stiff person syndrome" , de cerebelleuse ataxie en diabetes 1. Deze antilichamen worden aangemaakt door patiënten en zijn gericht op een proteïne aanwezig in de hersenen, de GAD65. Deze staat in voor de omvorming van de neurotransmitter die glutamaat opwekt en de neurotransmitter die GABA belemmert op het niveau van de neuronen. Momenteel proberen meerdere onderzoeksteams het mechanisme en de invloed van deze antilichamen op het zenuwstelsel beter te begrijpen. Het Neurowentenschappelijk Laboratorium van de UMons focust op het voorkomen ervan in de hypocampus, een hersenstructuur die noodzakelijk is voor het geheugenproces.

**Keywords: GAD65-antibodies, Long-Term Potentiation, Limbic Encephalitis, Hippocampus, Memory**

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# Neonatal seizures: 2 cases of channelopathies

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Neonatal seizures constitute one of the most common neurologic issue in the neonatal period. It is a challenge for both clinicians and basic science researchers. When an underlying brain injury such as hypoxia-ischemia, stroke or hemorrhage, malformation or metabolic etiology has been excluded, a genetic origin is therefore suspected; especially in case of intractable seizures ( Early infantile epileptic encephalopathies EIEE). Knowledge of an increasing number of genes involved in epilepsy and access to next-generation sequencing technologies (New Generation Sequencing, NGS) has helped to clarify of a greater number of supposed genetic epilepsies, and to establish a better genotype-phenotype correlation and a better understanding of the pathophysiological mechanisms involved, therefore allowing the use of the most appropriate treatment. We report on 2 cases of neonatal seizures with genetic origin. The first patient, a female infant weighing 3.510 kg was born at 38 weeks, 1/7 day gestation by caesarean delivery indicated for foetal distress . On the 2nd day of life, she presented repeated myoclonic seizures with upward deviation of the eyes and cyanosis of the lips which lasted approximately one minute. Neurologic examination revealed severe hypotonia. Electrolytes, calcium, glucose, cerebrospinal fluid examination, brain MRI were all normal. Electroencephalography showed burst-suppression pattern. Seizures were refractory to many antiepileptic drug like Phenobarbital, pyridoxine, pyridoxal phosphate, folic acid. Genetic tests showed a SCN2A mutation. Detailed review of her family history did not revealed affected family members. Topiramate was started without any seizure recurrence at 18months of age (cf fig. in supplemental data). Pharmacological studies suggest that its mode of action is multifactorial and involves blockade of voltage-dependent sodium channels. The second patient , a female infant weighing 2.620 kg was born at 39 weeks gestation by normal delivery . It is the first child of non-consanguineous parents. The pregnancy was uneventful. On the 2nd day of life, she presented generalized tonico-clonic seizures. Neurologic examination revealed mild hypotonia. Electrolytes, calcium, glucose, cerebrospinal fluid examination, brain magnetic resonance imaging were all normal. EEG showed bilateral epileptic activity and discontinuous pattern. Genetic test revealed a KCNQ2 mutation ,which is first associated with benign familial neonatal seizures), also responsible for about 10% of EIEE with neonatal onset . She responded to topiramate (TPM). Pharmacologic properties of TPM include modulatory effects on Na+ channels. Voltage-gated sodium channels and KCNQ potassium channels co-localize on the neuronal membrane. The response

to sodium-channel blockers in patients with potassium-channel disorders could be explained by structure–function approaches showing that modulation of one channel may significantly affect the function of the channel complex. Both children presented later psychomotor developmental delay. The clinical history of two patients shows the contribution of NGS technology to guide therapeutic management and expands our knowledge of the clinical spectrum of these severe pathologies. This technique should be offered promptly in case of neonatal seizures to adjust immediately the anti-epileptic treatment.

Résumé en français : Nous présentons ici deux cas de convulsions néonatales d'origine génétique. L'histoire clinique de ces deux patientes montre l'apport des techniques de séquençage de nouvelle génération qui permettent d'élucider un plus grand nombre d'épilepsies à début néonatal pour orienter la prise en charge thérapeutique et élargir nos connaissances sur le spectre clinique de ces pathologies souvent sévères. Le premier cas est une enfant née par césarienne à 38 semaines présentant dès le 2e jour de vie des crises de contractions musculaires (crises myocloniques) résistantes à plusieurs antiépileptiques. L'analyse génétique a mis en évidence une mutation du gène SCN2A, connu pour donner un phénotype d'épilepsie en perturbant des canaux sodium voltage-dépendants. La fréquence des crises a considérablement diminué sous Topiramate. Le deuxième cas est une enfant née à 39 semaines et présentant dès le 2e jour de vie des crises tonico-cloniques généralisées. L'analyse génétique a révélé une mutation au niveau du gène KCNQ2. Les crises ont définitivement cédé après administration du topiramate. Les propriétés pharmacologiques du topiramate comprennent des effets modulateurs des canaux ioniques.

Samenvatting in het Nederlands: We brengen hier twee gevallen van genetisch bepaalde neonatale stuipen naar voor. De klinische voorgeschiedenis van beide patiënten toont aan dat de nieuwe generatie sequentiële technieken die toelaten opeenvolgende epilepsieaanvallen van bij de geboorte beter te verklaren en aldus de therapeutische aanpak te bepalen en onze kennis te vergroten over het klinisch beeld van deze meestal zware pathologie. Het eerste geval betreft een kind op 38 weken geboren met keizersnede dat vanaf de tweede levensdag stuipen kreeg (myoclinische crisissen) resistent aan meerde anti-epileptica. De genetische analyse toonde aan dat een mutatie van het gen SCN2A, dat bekend staat als een fenotype van epilepsie door het verstoren van voltage-afhankelijke natriumkanalen. Het aantal epilepsieaanvallen daalde aanzienlijk door toediening van Topiramate. Het tweede geval betreft en kind geboren na 39 weken dat vanaf de tweede dag veralgemeende tonico-clonische crisissen vertoonde. De genetische analyse toonde een mutatie van het gen KCNQ2 aan. De crisissen hielden definitief op na toediening van Topiramate. Tot de farmacologische eigenschappen van Topiramate behoort de beïnvloeding door de ionische kanalen.

**Keywords: Neonatal seizures, Genetics, KCNQ2 mutation, SCN2A mutation, Topiramate**

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# Oligodendrocytopathy and astrocytopathy precede myelin loss and blood-brain barrier disruption in a mouse model of osmotic demyelination syndrome

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Osmotic demyelination syndrome (ODS) is a non-inflammatory disorder of the CNS myelin that occurs following too rapid correction of chronic hyponatremia. The physiopathology remains unclear although hypothetical mechanisms include blood-borne myelinotoxic factors or deleterious osmotic fluctuations focally in white and gray matter-mixed rich regions. To morphologically and functionally investigate the development of ODS in vivo, we generated a novel murine model of ODS. Eriochrome and anti-MBP stainings revealed typical demyelinating lesions in the thalamus, mesencephalon, pons and subcortical regions at 48 hours post-correction in ODS mice brains. Lesions were associated with a significant decrease of APC+ and Cx47+ oligodendrocytes, starting as soon as 24 hours post-correction. Oligodendrocytopathy was temporally and spatially correlated with the loss of astrocyte markers (ALDH1L1, AQP4, S100β) and both with the areas affected by demyelination. Using IgG immunostaining and Evans Blue extravasation assay, we demonstrated that blood-brain barrier disruption started at 48 hours post-correction. Following osmotic insult, Iba1+ microglial cells infiltrated the brain tissue within 12 hours post-correction, while acquiring an activated morphology, from quiescent type A to types B, C and D at latter time points. IL-1β and LIF mRNA, known to influence myelin integrity, were both significantly upregulated in the thalamus of ODS mice. ODS mice showed inabilities to perform motor tasks (Rotarod and Grip strength) and impairments in brainstem auditory evoked potentials. In conclusion, this murine model of ODS reproduces the demyelinating lesions observed in human pathology and raise new questions about the early role played by astrocytes or microglial cells in demyelination.

Résumé en français : Nous avons mis au point un nouveau modèle murin mimant le syndrome de démyélinisation osmotique humain. Ce modèle nous a déjà permis de mettre en évidence une implication précoce des cellules gliales (astrocytes et oligodendrocytes) dans le développement de ce syndrome. De façon plus générale, nos études visent à mieux comprendre les processus impliqués dans la démyélinisation. Ces recherches pourraient permettre l'étude de nouvelles cibles thérapeutiques.

Samenvatting in het Nederlands: We hebben een nieuw muismodel ontwikkeld dat het osmotisch demyelinisatiesyndroom (pontiene myelinolyse) bij de mens nabootst. Dit model liet ons reeds toe de vroegtijdige rol in het licht te stellen van astrocyten en oligodendrocyten bij het totstandkomen van dit syndroom. Dit onderzoek kan leiden naar het vinden van nieuwe therapeutische aanknopingspunten.

**Keywords: ODS, Animal Models, Astrocytes, oligodendrocytes, blood-brain barrier disruption**

# Oligodendrocyte regulation of neuronal plasticity during learning and ageing - a proteomic study

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Contextual memory is an intricate process involving numerous cellular mechanisms. Among those, synaptic plasticity and network rearrangement are known to be crucial. Both are governed by many molecular processes including phosphorylation and modulation of protein expression. However, little is known about the molecules involved in it. In the same way, during ageing, the plasticity of neuronal networks decreases leading to cognitive deficits even in absence of neurodegeneration but the molecular pathways involved in this process are not well understood. Here, we exploited the advantages of a quantitative proteomic approach using UHPLC – nanoESI-Triple TOF mass spectrometry to identify a great number of molecules in the rat hippocampus after a contextual fear conditioning session in young rats and in ageing F344 fisher rats. These rats present progressive cognitive decline and impairment of synaptic plasticity from the age of 20-24 months and are suitable models to study the neurobiology of ageing. Our results allowed us to highlight protein expression patterns, not only related to neuroplasticity, but also to myelin and perineuronal nets (PNN) structure. Since myelin and PNN are known to stabilize synaptic network, the regulation of expression of the proteins constituting PNN or myelin sheets can modulate neurite outgrowth in the ageing brain and during memory encoding. Our main results showed an inverse relationship between the expression of proteins involved in neurite outgrowth, which is increased after learning and decreased during ageing, and the expression of myelin associated proteins, which is temporally decreased after learning and increased in the ageing hippocampus. During ageing, the decreased expression of proteins involved in neurite outgrowth is combined to an increase expression of the proteins constituting the PNN. This last result suggests that during ageing, in the absence of neurodegeneration and before the appearance of cognitive decline, neuronal plasticity is inhibited by oligodendrocyte spreading and PNN stiffening.

Résumé en français : Bien que faisant l'objet de nombreuses études, les mécanismes cellulaires de la mémorisation restent à ce jour peu connus. En effet, s'il est actuellement reconnu que la mémoire réside sur des modifications des connections entre les neurones formant un réseau, les protéines impliquées dans ce processus n'ont pas été

clairement identifiées. Dans cette étude, nous avons tiré parti d'une technique innovante qui permet de mesurer l'expression de milliers de protéines en même temps dans des extraits de cerveau. Cette technique nous a permis de mettre en évidence le rôle de protéines particulières dans l'apprentissage et dans le vieillissement. Une meilleure compréhension de ces processus devrait permettre d'agir dans le futur sur les troubles de la mémoire et de la cognition.

Samenvatting in het Nederlands : Hoewel het onderwerp van talrijke studies, het cellulaire werkingsmechanisme hoe het geheugen juist functioneert blijft een belangrijk vraagteken. Ondanks de consensus dat het geheugen gevormd wordt door neuronale netwerken, zijn de onderliggende moleculaire processen slechts ten dele gekend. Om beter inzicht in deze processen te krijgen meet deze studieprocedure verschillende eiwitten met een innovatieve spectroscopie in een knaagdier model. Deze techniek kan verder inzicht verschaffen in het functioneren van bepaalde eiwitten in de hersenen en hoe specifiek hun rol is bij het verouderen. Een beter begrip hiervan kan belangrijk zijn voor het ontwikkelen van therapeutische interventies bij cognitieve- en geheugenstoornissen.

**Keywords: Hippocampus, myelin, Rats, perineuronal nets, Neurite outgrowth**

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# Investigating the role of sweet taste receptors in age-related neurodegeneration

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The sweet taste perception modality is primarily mediated by the sweet taste receptor (T1R2/T1R3 heterodimer) system. T1R2/T1R3 receptor heterodimers possess a diversity of ligand-binding sites which can be activated by various ligands including sugars, proteins, amino acids as well as artificial sweeteners. Recently, the widely expressed T1R2/T1R3 system has been implicated in multiple non-taste perception functions, including neurodegeneration, insulin secretion, mitochondrial activity and learning and memory functions. We have investigated the functional role of the sweet taste receptors in the central nervous system using T1R3 knockout (T1R3KO) mice. T1R3KO mice demonstrate significant cognitive/behavioral deficits, altered expression levels of neurosynaptic proteins and decreased dendritic spine density in the hippocampus – supporting the importance of the T1R3 receptor in memory functions. To characterize the multidimensional signaling capacity of the T1R2/T1R3 receptor system, we generated a controlled 'concentration-dependent' T1R2/T1R3 expression variation in neuronal cells to generate a proteomic constitutive signaling 'response profile' to these receptors. Data from our quantitative proteomic analyses of T1R2/T1R3 functionality suggest that sweet taste receptors are potentially directly involved in trophic hormone signaling, insulin signaling, DNA damage repair, and synaptic functions – pathways known to be impaired in age-related neurodegenerative processes. Moreover, identification of the protein interactors of the T1R2/T1R3 receptor complex, through SILAC-based-quantitative interactomics, revealed proteins involved in the regulation of protein folding, collagen synthesis, calcium homeostasis and autophagy. Interestingly, a number of these proteins are also known to be involved in the pathogenesis of different age-related disorders. Taken together, these data strongly suggest that the T1R2/T1R3 receptor system is involved in maintaining neurosynaptic activity and regulation of molecular processes linked to age-related neurodegenerative disorders.

Short Comment Our sweet taste perception ability is mediated by the T1R2/T1R3 receptor system. Their wide expression pattern (including brain tissue), suggest that T1R2/T1R3 receptors have also additional non-taste perception functionalities. Indeed, loss of T1R3 expression in mice resulted in memory and sociability impairments indicating that the T1R3 receptor is crucial for memory formation. Furthermore, quantitative proteomics and interactomics analyses of the T1R2/T1R3 system in human cells demonstrated alterations in multiple pathways known to be impaired in age-related neurodegenerative disorders (ND). Considering the potential involvement of T1R2/T1R3 in the pathogenesis of ND opens new perspectives for treatments.

Résumé en français: Titre: Recherche sur le rôle des récepteurs du goût dans les maladies neurodégénératives liées à l'âge. Notre perception du goût sucré est médié par des récepteurs localisés à la surface des cellules. Ces récepteurs, appelés T1R2/T1R3 sont présents dans de nombreux organes et notamment dans le cerveau suggérant des rôles supplémentaires. Effectivement lorsque nous supprimons l'expression du récepteur T1R3 chez la souris, nous observons une perte de mémoire et de sociabilité. Ce résultat démontre le rôle de T1R3 dans le processus de la mémoire. Par ailleurs, il a été montré que le système de signalisation de ces récepteurs est altéré dans des maladies neurodégénératives. Ces résultats permettent d'envisager de nouveaux traitements pour ces maladies.

Samenvatting in het Nederlands: Titel: Onderzoek naar de rol van smaakreceptoren bij ouderdom gerelateerde neurodegeneratieve ziektes. Onze smaak perceptie van zoet is geregeld door receptoren aan de oppervlakte van cellen. Deze receptoren, T1R2/T1R3 genoemd, zijn aanwezig in tal van organen, ook in de hersenen, wat zou kunnen wijzen op bijkomende functies. Inderdaad, wanneer we de receptor T1R3 bij een muis uitschakelen, zien we een verlies aan geheugen en sociaal gedrag. Dit resultaat toont de rol aan van T1R3 in het geheugenproces. Overigens, het werd aangetoond dat het systeem van signalisatie van deze receptoren een modificatie ondergaat bij neurodegeneratieve ziektes. Deze resultaten laten toe uit te kijken naar nieuwe behandelingen voor deze patiënten.

**Keywords: Sweet taste receptors, Aging, Neurodegenerative disorders, Proteomics, Neurosynaptic activity**

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# The synergistic GIT2-RXFP3 system in the brain and its importance in age-related disorders

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Aging is the most important risk factor for developing neurodegenerative disorders (ND), such as Alzheimer's disease. Furthermore, pathophysiological aging shares many common features with neurodegenerative processes, including mitochondrial/somatic DNA damage caused by oxidative stress, disruption of glucose metabolism and progressive loss of cellular stress resistance. This accumulation of cellular damage ultimately results in systemic dysfunction and eventual death. Given the similarities between aging and ND etiology, an enhanced appreciation of molecular aging pathomechanisms would significantly improve our ability to diagnose and/or treat the early stages of neurodegeneration. To address this, we identified GIT2 (G protein-coupled receptor kinase interacting protein 2) as a 'hub' protein that helps orchestrate the aging process. Hub proteins bridge multiple signaling pathways to coordinate multisystem processes such as aging or somatic metabolism. GIT2 is a known G protein-coupled receptor (GPCR) interacting protein and is known to be involved in both oxidative stress response and DNA damage repair processes. While GIT2 potentially presents itself as an important therapeutic target, GIT2 is considered as a non-canonical drug target. It is therefore important to identify proteins that can regulate GIT2 functions in order to gain control over the pathophysiological aging process with the potential to treat age-related disorders, such as neurodegeneration. Analyses of GIT2 knockout (GIT2KO) mice have led to the identification of a potential modulator of GIT2 functions: the relaxin family peptide receptor 3 (RXFP3). RXFP3 is a class A GPCR protein that was found to be consistently downregulated in GIT2KO mice and demonstrated several functional synergies with GIT2, including regulation of glucose metabolism and oxidative stress responsiveness. We generated in vitro RXFP3 constitutive cellular signaling signatures by progressive expression elevation followed by mass spectrometry-based analysis of the resultant cellular proteomic changes. We subsequently found strong evidence for a functional synergy between RXFP3 and GIT2 with respect to their shared roles in DNA damage repair, oxidative stress responsiveness, cell cycle arrest control and glucose metabolism functionalities. Further evidence supporting the existence of a synergisitic GIT2-RXFP3 system was provided by the demonstration of a physical interaction between both proteins as well as an RXFP3-dependent regulation of GIT2 expression. In conclusion, we propose that through a rational regulation of the RXFP3-GIT2 system new therapeutic strategies may be developed for neurodegenerative disorders.

Short comment : Aging is the strongest risk factor for developing neurodegenerative disorders, like Alzheimer's disease. Many synergies exist between aging-related processes and the development of these disorders, suggesting that they interconnect. We have identified GIT2 as a potential aging regulator, modulating stress resistance, metabolism and DNA repair mechanisms, hallmarks of aging and neurodegeneration. Being able to control GIT2 functions may represent an important objective for both aging and neurodegenerative research. Hence, we identified RXFP3 as a potential GIT2 regulator, showing strong functional synergy and comparable expression patterns to GIT2. We propose that the GIT2-RXFP3 interaction may be important in regulating age-related neurodegeneration.

Résumé en français: Le vieillissement est le principal facteur de risque pour le développement de maladies neurodégénératives, telles que la maladie d'Alzheimer. Les nombreuses synergies entre les processus de vieillissement et la survenue de ces maladies suggèrent l'existence d'une interconnexion. Nous avons identifié la protéine GIT2 comme un régulateur de vieillissement potentiel modulant la résistance au stress, le métabolisme et les mécanismes de réparation de l'ADN, trois processus atteints au cours du vieillissement et de la neurodégénérescence. Le contrôle potentiel des fonctions de la GIT2 pourrait donc constituer un objectif majeur pour la recherche sur le vieillissement et la neurodégénérescence. Nous avons identifié le gène RXFP3 comme régulateur potentiel de la GIT2, présentant une forte synergie fonctionnelle et des modèles d'expression comparables à la GIT2. Nous suggérons que l'interconnexion GIT2-RXFP3 est importante pour la régulation de la neurodégénérescence liée au vieillissement.

Samenvatting in het Nederlands: Het verouderen is de belangrijkste factor in het ontwikkelen van neurodegeneratieve ziektes, zoals bv de ziekte van Alzheimer. Het dikwijls samenvallen van de verouderingsprocessen en het voorkomen van deze ziekten suggereren dat er een verband bestaat. Wij hebben de proteïne GIT2 geïdentificeerd als een mogelijke regulator die weerstand tegen stress, het metabolisme en de herstelmechanismen voor het DNA moduleert, drie processen die aangetast worden tijdens het verouderen en de neurodegeneratie. De potentiële controle van de functies van het GIT2 zou dus een belangrijk doel kunnen worden in het onderzoek naar veroudering en de neurodegeneratie. We identificeerden het gen RXFP3 als mogelijke regulator van het GIT2. Wij suggereren dat het verband tussen GIT2-RXFP3 belangrijk is voor de regulering van de neurodegeneratie gelinkt aan de veroudering.

**Keywords: RXFP3, GIT2, Aging, neurodegeneration, GPCR**

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# The electrophysiological connectome is maintained in healthy elders: a power envelope correlation MEG study

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Previous functional magnetic resonance imaging (fMRI) studies have demonstrated major age-related modulations in resting-state functional connectivity (rsFC) with ageing that correlated with cognitive decline (for a review, see Sala-Llonch et al., 2015). However, these fMRI results might be biased by the age-related changes in neurovascular coupling and the high prevalence of neurological and psychiatric comorbidities in elders. We therefore studied using magnetoencephalography and a connectome approach the changes in static and dynamic rsFC with age in a group of highly selected young and old healthy subjects. Resting-state data were recorded with a whole-scalp MEG (Vectorview, Elekta) in two sex- and education-matched groups of 25 right-handed young (age=23±3 years, mean±standard deviation) and elder (68±2 years) healthy subjects. Participants were screened for depression, anxiety and dementia, and were free of psychotropic drugs. The connectome was estimated as rsFC matrices involving 40 cortical nodes of the default mode, visual, somatomotor, language, dorsal and ventral attentional resting state networks. Source-level rsFC maps were computed in the α (8-13Hz) and β (14-25Hz) bands using leakage-corrected envelope correlation (see Wens et al., 2015 for a detailed description) and normalized power (dynamic Statistical Parametric Mapping). The aforementioned pipeline has been applied either on entire MEG signals for static rsFC or on overlapped segments (10s window size with a 2s overlap step) of signals for the dynamic rsFC. The latter aims at exploring variation in stability (ratio of mean to standard deviation, across windows) and in variability (ratio of standard deviation to mean, across windows) indices between the two populations. Group differences were statistically assessed using unpaired, two-tailed permutation tests (1 million of permutations, p-value <0.05, family-wise error control as in Wens et al., 2015). No age-related difference was found in static rsFC in the α band, despite significant power decrease with age in the dorsal and ventral attentional network; while in the β band, one cross-networks coupling increased with age between the default mode and the ventral attentional networks concomitantly with power increase in the default mode and language networks. No age-related difference in dynamic rsFC and in power was observed in the α band, for both stability and

variability indices. In the β band, the dynamic rsFC variability decreased with age in the language network. Furthermore, the dynamic rsFC stability showed significant age-related changes in three intra-networks couplings (increase in the default mode and decrease in the visual networks) and two cross-networks couplings (increase between the somatomotor and visual networks and decrease between the visual and the default mode networks). Consequently, this study reveals that the electrophysiological connectome is globally maintained with ageing in healthy elders, having considered both static and dynamic rsFC investigations. Age-related changes in static and dynamic rsFC occurred both within and between networks, and mainly in the default mode networks. These findings suggest that the age-related evolution of rsFC reported in previous fMRI studies is mainly explained by age-related changes in neurovascular coupling or neuropsychiatric confounds.

Résumé en français: L'imagerie par résonance magnétique fonctionnelle (IRMf) a démontré des modifications importantes dans l'organisation fonctionnelle du cerveau avec l'âge. Ces résultats pourraient être biaisés par la technique d'IRMf qui fournit des renseignements indirects sur le fonctionnement neuronal et par l'existence de troubles neuropsychiatriques fréquents chez les personnes âgées. Dans cette étude, nous étudions en magnétoencéphalographie les variations de cette organisation fonctionnelle liées à l'âge chez des sujets jeunes et âgés soigneusement sélectionnés. L'étude démontre que l'organisation fonctionnelle est globalement préservée avec l'âge ce qui suggère que les résultats IRMf sont fortement influencés par le couplage neuro-vasculaire ou les troubles neuropsychiatriques.

Samenvatting in het Nederlands: De functionele magnetische resonantie beeldvorming (fMRI) heeft aangetoond hoe er bij het ouder worden belangrijke wijzigingen optreden in de functionele organisatie van de hersenen. Deze resultaten zouden kunnen negatief beïnvloed zijn door de techniek van het fRMI, die indirecte informatie geeft over het neuraal functioneren en door het bestaan van neuropsychiatrische problemen bij oudere personen. In deze studie maakten we gebruik van magneto-encefalografie om de variaties in deze functionele organisatie in relatie tot de leeftijd te onderzoeken bij zorgvuldig geselecteerde jongeren en bejaarden. De studie toont aan dat de functionele organisatie globaal gezien behouden blijft bij het ouder worden, wat zou aantonen dat de fRMI resultaten sterk beïnvloed zijn door hun neuro-vasculaire koppeling of door neuropsychiatrische problemen.

**Keywords: Magnetoencephalography, resting state, Aging, seed-based functional connectivity, dynamic analysis**

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# Clathrin adaptor CALM/PICALM is involved in tau pathology in Alzheimer and other tauopathies

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Alzheimer's disease (AD) is the most common form of dementia. AD is characterized by two neuropathological hallmarks: neurofibrillary tangles (NFTs) and amyloid deposits [5]. NFTs are constituted of hyperphosphorylated tau proteins [3] and are observed in other tauopathies [4]. Recent genome-wide association studies (GWAS) have identified two single nucleotide polymorphisms in PICALM gene as genetic susceptibility loci for late-onset Alzheimer's disease (LOAD) [6-8]. PICALM is a key protein for clathrin-mediated endocytosis and autophagy and thus modulates tau pathology [9]. We hypothesized that PICALM may be dysregulated and may be mislocalized in neurodegenerative brains. This project aimed to analyse the level and localization of PICALM in the brains of various neurodegenerative diseases such as AD, Down syndrome (DS), Pick disease, progressive supranuclear palsy (PSP), corticobasal degeneration (CBD), fronto-temporal lobar degeneration (FTLD) with MAPT P301L mutation (FTLD-P301L), Lewy body dementia, diffuse type (LBD) and FTLD with TDP-43 positive inclusions (FTLD-TDP). We found that the PICALM immunoreactivities were found in tau positive inclusions in specific neurodegenerative diseases: AD, DS, Pick disease and in PSP, but not in CBD and FTLD-P301L cases [1,2]. Astrocytic tau-positive inclusions in tauopathies were devoid of PICALM immunoreactivity. Lewy bodies in LBD and TDP-43 positive inclusions in FTLD-TDP were PICALM negative. The level of soluble PICALM was decreased and inversely correlated with the level of soluble phosphotau in the post-mortem brain homogenates from tauopathies. PICALM decrease was significantly correlated with the levels of autophagy markers such as LC3-II and Beclin-1 in the brain lysates from various neurodegenerative disease brains [2]. These results indicate that there is a close relationship among abnormal PICALM processing, tau pathology and impairment of autophagy. Our results suggest that PICALM and phosphotau interaction occurs in AD, DS, Pick disease and PSP and

that PICALM dysregulation may be associated with autophagy dysfunction in various neurodegenerative diseases [1,2].

Résumé en français : Le traitement efficace de la maladie d'Alzheimer et d'autres maladies neurodégénératives passe par la connaissance de la modification des mécanismes cellulaires impliqués. Dans ce travail, nous nous sommes intéressés à l'autophagie (sert à éliminer certaines régions toxiques contenues dans la cellule, voire la conduire à la mort pour éviter de propager une infection ou si la cellule ne peut plus fonctionner correctement). Dans ce travail nous avons montré qu'à la fois la localisation et la concentration d'une protéine nommée PICALM impliquée dans l'autophagie était modifiée chez les patients souffrant de 3 maladies neurodégénératives: 1) Alzheimer, 2) Pick et 3) Steele-Richardson-Olszewski.

Samenvatting in het Nederlands De effectieve behandelingsstrategie van de ziekte van Alzheimer en andere neurodegeneratieve aandoeningen zijn gebaseerd op wat we weten over de onderliggende cellulaire werkingsmechanismen. Met dit onderzoek zijn we voornamelijk geïnteresseerd in autofagie (het proces om bepaalde toxische cellulaire stoffen te elimineren, celdood te bevorderen om infectie te vermijden of om of cellulaire malfuncties verder te voorkomen). Deze studie toont aan dat de lokalisatie en concentratie van het eiwit PICALM betrokken bij deze autofage processen en gemodificeerd is bij drie types van neurodegeneratieve aandoeningen: 1) De ziekte van Alzheimer, 2) Pick en 3) Steele-Richardson-Olszewski.

**Keywords: PICALM, tau, nFTS, Alzheimer's disease, Pick disease, Progressive Supranuclear Palsy, Autophagy, LC3, Beclin-1**

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# PHF-tau propagation in the presence of Amyloid ß pathology

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Alzheimer's disease (AD) is the most common form of dementia characterised by a progressive cognitive decline. Pathologically, it is characterized by two hallmarks: amyloid plaques mostly consisting of β-amyloid peptide (Aβ) derived from the successive cleavage of the amyloid precursor protein (APP), and neurofibrillary tangles (NFTs) [6]. NFTs are formed by the aggregation of paired helical filaments (PHF), composed of abnormally phosphorylated and aggregated microtubules associated tau proteins [3]. NFTs correlate strongly with the cognitive decline and can also be found in a group of neurodegenerative diseases called tauopathies [4]. The importance of tau in mediating the toxicity of Aβ peptides has been demonstrated in tau knock-out neurons being resistant to Aβ-associated toxicity [11]. Deletion of murine tau expression rescues excitotoxicity in transgenic APP mice [8,12]. We have previously reported that tau deletion reduced Aβ load [9] in 5xFAD mice overexpressing human APP (K670N/M671L, I716V, and V717I) and PS1 (M146L and L286V) [10]. In contrary, crossing 5xFAD with Tg30 overexpressing human double mutant tau exacerbated tau pathology [7]. Nevertheless it remains elusive how tau pathology propagation is associated with amyloid pathology. In this study, we hypothesized that there may be an association between pathological tau seeding and Aβ. We have previously shown that stereotaxic injection of PHF obtained from AD brain induced Argyrophilic grains composed of hyperphosphorylated murine tau in wild-type (WT) mouse brains [2]. In this study, we conducted stereotaxic injection of human PHF into the brains of WT and 5xFAD mice. Stereotaxic injection of PHF induced aggregation of endogenous murine tau into gallyas-positive grains in the cortex and corpus callosum of both WT and 5xFAD mice. This Argyrophilic grain pathology seems to be more important in 5xFAD compared to WT mice as the grains could be detected not only in the ipsilateral hemisphere (left) but also in the contralateral hemisphere (right) in 5xFAD mouse brains. These grains were never present when injected with human control material obtained from non-demented individuals. Finally, we carefully analysed the association of amyloid and tau pathologies in human post-mortem AD brains by CLARITY [1,5] and we found

that mature focal amyloid plaques contained more tau-positive dystrophic neurites than diffuse plaques. Taken together, this study shed lights on the acceleration of tau pathology propagation by amyloid pathology. Summary Alzheimer's disease causes progressive memory loss and behavioral changes, and it even causes many difficulties in daily tasks. It is characterised by the appearance of two abnormal lesions called amyloid plaques and tangles, responsible for the loss of neurons during the disease. Amyloid plaques are composed of a small protein called amyloid-beta, and tangles are formed by the aggregation of another protein called tau. Here we study how amyloid-beta affects tangle formation in animal models.

Résumé en français : La maladie d'Alzheimer est caractérisée par le développement de deux lésions cérébrales responsables de la mort des cellules nerveuses: les « plaques amyloïdes », composées d'une molécule appelée amyloïde-beta, et les « dégénérescences neurofibrillaires », composées d'agrégats d'une protéine appelée tau. Afin de comprendre si ces deux lésions interagissent lors de leur développement, nous avons injecté des protéines tau dans des souris développant ou non des plaques amyloïdes. Ces souris ont développé des agrégats de protéine tau, indiquant que les protéines tau anormales ont des capacités de propagation dans le cerveau dans la maladie d'Alzheimer.

Samenvatting in het Nederlands: De ziekte van Alzheimer wordt gekenmerkt door twee hersenletsels die verantwoordelijk zijn voor het afsterven van hersencellen: de "amyloïde neerslag" bestaande uit een molecule amyloïde-beta genoemd en de "neurofibrillaire degeneratie" bestaande uit aggregaten van een tau genoemde proteïne. Om te begrijpen of deze beide letsels interageren tijdens hun ontwikkeling hebben we tau proteïnes geïnjecteerd bij muizen die al dan niet een amyloïde neerslag ontwikkelden. Deze muizen maakten tau proteïne aggregaten aan, wat aantoont dat abnormale tau proteïnes zich in de hersenen kunnen propageren bij de ziekte van Alzheimer.

**Keywords: Sexual Violence, Domestic Abuse and the Approach of the Feminist Judge**

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# Stereotaxic injection of fibrillar PHF from Alzheimer brain induces propagation of argyrophilic grains constituted of murine tau in mouse brains

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Alzheimer's disease (AD) is neuropathologically characterized by two hallmarks: extracellular amyloid deposits and intracellular neurofibrillary tangles (NFTs) [7]. The level of NFTs better correlates with cognitive dysfunction than amyloid load. NFTs are composed of hyperphorylated tau proteins that forms paired helical filaments (PHF) [2]. Increasing evidences support the idea that misfolded pathological forms of tau proteins propagate in Prion-like manner [4-6,10]. Indeed in AD brains, tau pathology progression follows neuroanatomically connected pathways, implying cell to cell transmission of pathological tau species to seed endogenous "healthy" tau into pathological tau. But native PHF extracted from human AD brains had never been fully studied for its seeding effects and propagative propensity in vivo. In this study, we aimed to understand the transmission of human AD PHF in the mouse brains of wild-type and THY-Tau22 overexpressing double mutant human tau G272V/P301S [12]. PHF was extracted by sarkosyl fractionation method from an AD case [3,9]. 1 µg of AD PHF was stereotaxically injected into hilus of 3 month-old mice [1]. After 3 months of incubation, wild-type and THY-Tau22 mice developed an atrophy of the dentate gyrus accompanied with argyrophilic grain-like tau pathology characterized by Gallyas silver staining method [11]. Gallyas positive neuropil threads and oligodendroglial coiled bodies were also observed. These grains were mainly constituted of hyperphosphorylated murine tau proteins devoid of human tau. These grains are reminiscent of human argyrophilic grain disease (AgD): these grains were immunoreactive to 4R tau, ubiquitin and p62 [8,13]. These grains were observed in granule cells that extended in the hippocampal hilus and eventually away into the alveus, and the fimbria in injected wild-type and THY-Tau22 mice. Although local hyperphosphorylation of tau was increased in the dentate gyrus of PHF-injected THY-Tau22 mice, the development of NFTs made of mutant human tau was not accelerated in the CA region of hippocampus, indicating that wild-type human PHF were not efficient in seeding tau aggregates made of G272V/P301S mutant human tau. Taken together, stereotaxic injection of human AD PHF into mouse brains caused pathological transmission via

conferring wild-type murine tau into an argyrophilic 4R tau pathology [1]. Our data provide an interesting murine model independent of expression of a mutant tau protein.

Résumé: Selon une hypothèse, les dégénérescences neurofibrillaires (une des deux lésions caractéristiques de la maladie d'Alzheimer) composées de protéines tau anormalement phosphorylées et agrégées se propageraient de cellule à cellule. Notre étude a eu pour objectif d'étudier le développement et la propagation de ces lésions neurofibrillaires après injection de protéines tau insolubles provenant de patients Alzheimer dans le cerveau de souris sauvages et transgéniques. Nous avons pu observer, 3 mois après injection, une atrophie d'une partie de l'hippocampe, ainsi que des agrégats intracellulaires composés majoritairement par des protéines tau hyperphosphorylées et agrégées.

Samenvatting in het Nederlands: De hypothese bestaat dat de neurofibrillaire degeneraties (één van de twee letsels die typisch voorkomen bij de ziekte van Alzheimer) die uit abnormaal gefosforyleerde en geaggregeerde tau proteïne samengesteld zijn zich van de ene naar de andere cel verspreiden. Onze studie had tot doel de ontwikkeling en de verspreiding van deze neurofibrillaire letsels te bestuderen nadat onoplosbare tau proteïnes afkomstig van Alzheimer patiënten in de hersenen van wilde en transgenetische muizen geïnjecteerd waren. Drie maanden na de injectie hebben we een atrofie van een deel van de hippocampus vastgesteld alsook intracellulaire aggregaten aangetroffen die voornamelijk uit hypergefosforyleerde en geaggregeerde tau proteïnes samengesteld waren.

**Keywords: nFTS, PHF, Alzheimer's disease, Argyrophilic grains, tau propagation**

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# New therapeutic management of Alzheimer's disease: interest to a phospholipase A2-targeted peptide able to cross the blood-brain barrier through the LDL receptor

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Introduction Alzheimer's disease (AD) is one of the main causes of dementia in the elderly, being characterized by a progressive and irreversible loss of cognitive functions. Actual therapies are only symptomatic and the AD treatment is a real challenge. Indeed, AD is a multifactorial pathology triggered by hereditary (familial AD) and environmental (sporadic AD) factors, making its management especially challenging. Phospholipase A2 (PLA2) signaling pathway was recently revealed to be involved in this pathology [1] and the modulation of some isoforms was already attempted in the treatment of neurological disorders [2]. The goal of the present work is to target specifically a PLA2 isoform in order to develop a new therapeutic strategy for the AD patients, as an alternative of the existing molecules that are non-specific or irreversible. We have therefore identified a PLA2-targeted peptide (PL-P25) by phage display able to inhibit the PLA2 in preliminary in vitro tests. On the other hand, the blood-brain barrier (BBB), localized at the interface between the blood and the brain, protects the brain against xenobiotics and limits the access of most molecules, including potential therapeutic agents. The development of non-invasive BBB crossing strategies is thus crucial to accede to the central nervous system (CNS) without BBB disruption. LDL receptor (LDLR) seems to be an interesting target for drug delivery due to its involvement in LDL transcytosis [3]. A LDLR-targeted peptide (LR-P2) was identified in order to facilitate the access to the brain of our therapeutic peptide. Methods A randomized linear peptide library fused to the p3 coat proteins of the M13 bacteriophage was used to identify PLA2- and LDLR-specific peptides. Peptide specificity was evaluated by immunofluorescence on mouse brain slices or human brain endothelial cells (ACBRI376) by colocalization with their respective target. The inhibitory effect of PL-P25 (alone or multivalent by coupling to streptavidin), targeted to PLA2, was evaluated by the dosage of AA release from H2O2-induced human astrocytes (1321N1) and from glutamate-induced mouse neurons (N18TG2). The mechanism of transcytosis of LR-P2, targeting LDLR, was investigated by immunofluorescence allowing to observe its colocalization with caveolae or lysosomes. After LR-P2 coupling to iron oxide nanoparticles (USPIO-LRP2), its crossing over the BBB was evaluated after in

vivo injection of NMRI mice by nuclear magnetic resonance (NMR) relaxometry and Perls'-DAB histochemistry staining. Results and Prospects The PLA2-targeted peptide PL-P25, selected for its affinity among two others, showed a good colocalization with the target on mouse brain slices, confirming its specificity. Its inhibitory potential on the PLA2 activity was confirmed on 1321N1 and N18TG2 cells, with an effect that ranged from 20% to 60% compared to positive control (induced but non inhibited cells), and a more potent activity at low concentrations (20μM) and for the multivalent model. Concerning the BBB crossing strategy, LR-P2 has co-localized with LDLR expressed by human brain endothelial cells, while its endocytosis via a caveolae-mediated non-degradation pathway was confirmed on these cells. After in vivo administration, USPIO-LRP2 was found within the brain parenchyma of NMRI mice, around the 3rd ventricle and brain capillaries by iron staining. NMR relaxometry has also confirmed the presence of USPIO-LRP2 in the brain, whereas the control nanoparticles coupled to a non-specific peptide (USPIO-NSP) was not able to cross the BBB as proven by both methods. This peptide will be used as vector in order to facilitate the access to the brain of our therapeutic peptide described above.

Résumé en français: L'objectif de ce projet est le développement d'une nouvelle stratégie thérapeutique de la maladie d'Alzheimer (MA) par modulation d'une molécule connue pour son implication dans les déficits de mémoire et la neurodégénérescence, appelée phospholipase A2. Un peptide spécifique à cette molécule a été identifié et sera couplé à un peptide facilitateur ciblant le récepteur aux lipoprotéines de basse densité (LDL) dans le but de passer la barrière hémato-encéphalique protégeant le cerveau. Cette nouvelle molécule pharmaceutique multifonctionnelle ainsi développée devra permettre une thérapie plus efficace de la MA.

Samenvatting in het Nederlands: Dit project heeft tot doel om een nieuwe behandelingsstrategie voor de ziekte van Alzheimer te ontwikkelen, door modulatie van een molecule, phospholipase A2, waarvan geweten is dat ze te maken heeft met geheugenstoornissen en neurodegeneratie. Een voor deze molecule specifieke peptide werd geïdentificeerd en deze zal gekoppeld worden aan een faciliterende molecule die zich richt op de lipoproteïne receptor met een zwakke dichtheid (LDL) met als oogpunt de hemato-encefalische afscherming die de hersenen beschermt te doorbreken. De aldus ontwikkelde multifunctionele farmaceutische molecule zal een meer efficiënte behandeling van de ziekte van Alzheimer mogelijk maken.

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**Keywords: Alzheimer's disease, Phospholipases A2, Blood-Brain Barrier, phage display, Peptides**

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# Identifying a neuronal circuit determining C. elegans behavioural decline and contributing to its lifespan

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Behavioural alterations and a decline in cognition characterise the aged nervous system of several species. The recent analysis of the aged mammalian brain restricted the neuronal loss to pathological processes – like neurodegenerative diseases1. Instead, the normal ageing of the brain is rather associated with subtle cell biology changes1. The paucity of studies correlating the appearance of the cell-biology change with the behavioural alterations impeded the exploration of the causality chain. We approach this question in Caenorhabditis elegans, a genetic model organism whose short lifespan (3 weeks on average) and simple nervous system (302 neurons) capable of multiple behaviours represent unique advantages2. By quantitatively assessing the evolution of the avoidance response to two independent cues over the entire adulthood of normally ageing C. elegans, we highlighted behavioural declines early in adulthood. For both cues, this behavioural decline occurred before muscle degeneration starts and can be delayed or accelerated by mutations known to modulate the lifespan of C. elegans. Using tissue-type specific rescue of a conserved key lifespan modulating gene, we show that behavioural decline and animal's lifespan can be fully rescued through its re-expression only in the nervous system of mutant animals. In addition, a conditional rescue, we identified a temporal window for the requirement of this gene's function for its neuroprotective and lifespan effects. Following the same cell-specific gene rescue strategy we aim to further restrict the circuit involved in the observed behavioural decline, to explore the cell biology basis of neuronal ageing and to identify the downstream effectors of this gene in the neurons of interest. Significance of the work presented and the potential relevance for patient care: The cause of normal brain ageing remains a mystery. Recent progress shows that rather than neuronal loss, normal brain ageing is associated with subtle cell biology changes1. We approach the causality question in the nematode C. elegans whose short lifespan and simple nervous system present power model. Using behaviour as an entry point to nervous system function, we identified a neuronal circuit determining behavioural decline and lifespan. We aim to identify the cell biology changes (biomarkers) that initiate the decline of nervous system function. This will accelerate development of therapeutics to delay the ageing of the nervous system.

Résumé en français: Voilà déjà une quinzaine d'années, le rôle d'une protéine a été mis en évidence dans la maladie de Parkinson : son nom : l'alpha-synucléine. Sous sa forme agrégée cette protéine est toxique pour les neurones. Plus récemment, la démonstration a été faite que l'agrégation de l'alpha-synucléine se propage de proche en proche dans le cerveau, contribuant à la dégénérescence des circuits neuronaux. Les mécanismes permettant cette propagation restent cependant inconnus. Nous étudions les mécanismes de la toxicité et du transfert intercellulaire de l'alpha-synucléine. Un modèle innovant a été mis au point dans notre laboratoire qui, à terme, identifiera les gènes impliqués. Connaître les mécanismes de transfert intercellulaire d'alpha-synucléine ouvre la perspective d'empêcher la propagation de la maladie dans le cerveau.

Samenvatting in het Nederlands : Reeds 15 jaar werd de rol van een proteïne aangetoond bij de ziekte van Parkinson: de alfa-synucleïne. Deze proteïne is toxisch voor neuronen. In een meer recent verleden werd aangetoond dat het aggregaat van synucleïne zich verspreid in de hersenen, en leidt tot degeneratie van netwerken van neuronen. Het mechanisme dat deze verspreiding veroorzaakt blijft echter onbekend. Wij bestuderen de mechanismes van de toxiciteit en de intercellulaire overdracht van het alpha-synucleïne. In ons laboratorium ontwikkelden we een innoverend model dat op termijn de betrokken genen zal identificeren. De kennis van de mechanismen van intercellulaire overdracht van alfa-synucleïne zal perspectieven openen om de verspreiding van een ziekte in de hersenen te beletten

**Keywords: Brain, Genetics, Cell Biology, Neuroscience, Ageing**

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# Towards a ventriculo-venous shunt that works

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The clinical problem At present, hydrocephalus is being treated by ventriculo-peritoneal or ventriculo-atrial shunts. These shunts possess an essential disadvantage: they do not establish physiological intracranial pressures. In a sitting or standing position of the patient, the cerebrospinal fluid column within the catheter – through gravity – exerts suction (siphoning), leading to shunt-related intracranial hypotension.This siphon effect is only imperfectly counteracted by the resistance of the valve. Ventriculo-venous shunts In healthy individuals, the cerebrospinal fluid (CSF) is resorbed to the superior sagittal sinus, an intradural venous canal. Shunting to this natural resorption site reduces the risk of shuntfailure in several ways. First,overdrainage is prevented by preservation of the natural, self-regulating anti-siphon effect of the internal jugular vein. This vein constantly adapts its diameter and thus the resistance against drainage of blood and cerebrospinal fluid out of the skull in function of the patient´s position. Secondly, the shunt system is shorter, less complex and confined to the skull, which minimizes the risk of mechanical failure and infection. Ventriculo-sinus shunts have been used in both pediatric and adult patients (more than 150 patients in total) to treat high- and normal-pressure hydrocephalus. No complications and shunt responses similar to those of conventional shuntswere reported. One author reported an excellent long-term efficacy. However, in a small prospective trial at our hospital 80% of the sinus shunts occluded within 4 months after implantation, although we respected all technical recommendations of the literature. Furthermore it was striking to us that no new information was released concerning this technology despite the very promising results reported in the literature. Material and methods The authors developed a dural venous sinus access device (DVSAD) that has significant advantages compared to the currently used silicone catheter. First, the tip is secured in the center of the superior sagittal sinus and secondly the endovascular volume is minimized. In the center of the sinus the risk of clotformation is minimal because blood velocity is maximal, there is no contact with the endothelial vessel wall, and the concentration of platelets and clotting factors is lowest. The DVSAD is designed in such a way that the tip is always in the center of the sinus. Stabilization with an epidural baseplate and intravascular barb minimizes the risk of dislocation. A foreign volume in a blood vessel disturbs the blood flow. It creates zones of low, non laminar flow in which the risk of clot formation is high. The DVSAD has a volume of only 40mm$^3$, which is significant less than the conventional shunt (600mm$^3$). Results and Conclusion Prototypes were designed with computer-aided design software and manufactured by injection molding.

The prototypes were then evaluated in a cadaver study and in an in-vivo animal trial (goat model).The prototypes were correctly implanted in all animals. None of the animals developed venous sinus thrombosis, air embolism, or excessive intra-operative sinus bleeding. The application of a small prospective clinical trial on ten adult patients was recently approved by the local Ethics Committee of Ghent University Hospital.

Summary We present a new prototype of the ventriculo-venous shunt that minimizes the risk of shuntobstruction by clot formation in the venous vessel. If this prototype proves to be efficacious in humans, ventriculo-venous shunting will finally be possible. This might be a revelation in the treatment of hydrocephalus. Ventriculo-venous shunts, in contrast to the currently used ventriculoperitoneal shunts restore the physiological intracranial pressure. By consequence the risk of shunt failure, which is as high as 50% over two years in ventriculo-peritoneal shunts will be drastically reduced.

Résumé en français : Nous proposons un nouveau prototype de dérivation du liquide cérébrospinal entre ventricule et sinus veineux afin de réduire au minimum l'obturation de la dérivation par des caillots de sang. Si ce prototype devait également fonctionner correctement chez l'homme, il serait enfin possible de réaliser des dérivations ventriculo-veineuses, ce qui serait un progrès révolutionnaire pour le traitement de l'hydrocéphalie. De fait, les dérivations ventriculo-veineuses rétablissent la pression physiologique dans le crâne. Par conséquent, il est possible de réduire radicalement le risque d'un shunt ventriculo-péritonéal défectueux, lequel risque peut s'élever à 50% sur une période de deux ans.

Samenvatting in het Nederlands : We stellen een nieuw prototype hersenvocht-shunt tussen hersenkamer en sinusader voor, dat het risico op shuntverstopping door bloedklonters moet minimaliseren. Indien dit prototype bij mensen goed zou blijken te werken, wordt het eindelijk mogelijk ventriculo-veneuze shunts aan te leggen. Dit zou een omwenteling kunnen betekenen in de behandeling van hydrocefalie. Ventriculoveneuze shunts herstellen namelijk de fysiologische druk binnen de schedel. Bijgevolg kan ook het risico op shuntfalen drastisch verminderd worden, dat bij ventriculoperitoneale shunts tot 50% over verloop van twee jaar kan bedragen.

**Keywords: Hydrocephalus;, Ventriculo-venous shunt, overdrainage, Prototype, Animal Experimentation**

# Compromised order processing in Alzheimer's dementia demonstrated by cortical thickness, DTI and rsfMRI

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Executive dysfunction observed in patients with Alzheimer's dementia is commonly attributed to higher order working memory (WM) processes. The mental representation of serial order is one of the essential components represented in serial verbal WM and is crucial for smooth daily life functioning (e.g., cooking, memorising a phone number). It remains unclear to what extent these serial constructions are affected in Alzheimer's dementia and which brain structures are essentially involved to successfully represent order. The data of 18 patients diagnosed with Alzheimer's dementia and 13 healthy elderly controls was collected. They were submitted to a neuropsychological test battery containing control measures such as the Montreal Cognitive Assessment, Frontal Assessment Battery, math test, measure of pre-morbid IQ; and following WM tests: Backward Digit Span (BDS) task, Forward Digit span (FDS) and Corsi Block (CB) test, from which an order-measure was calculated to investigate order-specific regional alterations in the brain. The following brain measures were obtained: anatomical information by T1, white matter integrity using the fractional anisotropy (FA) maps of diffusion tensor images and connectivity measures derived from resting state networks. The analysis of the BDS indicated for impaired order representations a reduction of cortical thickness of left hemispheric cingulate regions, bilateral medial orbitofrontal, bilateral inferior parietal and right temporal pole. The CB demonstrated reduced right superior and left inferior parietal cortical thickness, while no significant effect was found for the FDS. Correlations between the FA-maps and order-measure for both BDS and CB demonstrated crucially reduced FA-values at the level of the superior longitudinal fasciculus and forceps minor. No significant results were found for the FDS. The order-measure for the BDS was used to investigate alterations in the functional connectivity for the default mode network and executive control network. The left executive control network demonstrated reduced positive coupling for the left medial and inferior frontal regions. Reduced functional connectivity was observed for the right medial and inferior frontal regions in the right executive network. The default mode network demonstrated altered functional connectivity for the right precuneus, bilateral medial orbitofrontal regions and part of the posterior cingulate. Overall, these results indicate order-specific representational problems within WM associated with Alzheimer's dementia. Moreover, order-problems within WM are associated with

specific alterations in the brain; mainly localised in frontal and parietal regions, and are more pronounced in the left hemisphere. Summary for lay people The current study demonstrates that patients with Alzheimer's' dementia struggle with the processing of serial order, a crucial component of working memory, required to function well in daily life. Using anatomical brainscans (T1), visualisation of white matter integrity (DTI) and the analysis of functional connectivity between brain regions (rsfMRI), we demonstrate the degradation of cortical regions and specific connections is related to working memory issues and order-specific symptoms during the course of the disease. The knowledge on the specificity of working memory problems in patients provides the possibility to implement goal-directed cognitive trainings during the monitoring of patients with Alzheimer's' disease.

Résumé en français : Ce travail s'intéresse à un des volets de la mémoire, la mémoire sérielle (ou en série) dont le bon fonctionnement est nécessaire pour mener une vie de qualité normale. Nous avons montré, en utilisant des techniques d'imagerie moderne accompagnées de l'analyse des réseaux de connexion entre les différentes régions du cerveau impliquées, que cette mémoire sérielle se dégradait lors du développement de la maladie d'Alzheimer. Ce résultat permet d'envisager des tests mieux ciblés à appliquer lors du suivi des patients Alzheimer.

Samenvatting in het Nederlands : Deze studie toont aan dat patiënten met Alzheimer dementie specifiek problemen vertonen bij het verwerken van volgorde, een cruciaal onderdeel van het werkgeheugen voor een vlot dagdagelijkse functioneren. Met behulp van anatomische hersenscans (T1), de analyse van de integriteit van witte stof banen (DTI) en de functionele connectiviteit tussen regio's (rsfMRI) tonen we aan dat de aftakeling van specifiek regio's en connecties verantwoordelijk is voor deze werkgeheugen gerelateerde en orde-specifieke symptomen tijdens de ziekte. De kennis over de specificiteit van werkgeheugen problemen in patiënten voorziet de mogelijkheid om doelgerichte ondersteunende cognitieve training te voorzien tijdens de opvolging van patiënten met de ziekte van Alzheimer.

**Keywords: Dementia, working memory, white matter, Grey Matter, connectivity**

# Control of GDNF expression by AD-related proteins and implications in neurodegenerative and neuromuscular diseases

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The physiological function of the Amyloid Precursor Protein (APP) remains poorly understood. APP has been extensively studied for its involvement in Alzheimer's disease (AD) since its amyloidogenic processing leads to the formation of beta-amyloid peptide (Aβ), the major constituent of senile plaques that are typical AD lesions. We contributed to a more comprehensive picture of APP regulatory network and physiological function by showing that APP-dependent regulation of the Glial cell line-Derived Neutrophic Factor (GDNF) drives the process of neuronal and muscular maturation involved in neuromuscular junctions (NMJs) formation (1). APP function is likely to rely in important part mainly to the regulation of APP target genes expression. So far, the identity of genes regulated by APP is intense matter of debate and, in addition, these genes are often difficult to relate to the phenotype observed in APP-deficient mice. APP-dependent GDNF transcription appears as critical for the muscular phenotype observed in APP null transgenic mice (APP-/-). Interestingly, our recent experiments indicated that Presenilins (PS) are also involved in the regulation of GDNF transcription. Presenilins-dependent γ-secretase activity generates the Aβ peptide and releases the APP intracellular domain (AICD), which was shown to be the transcriptionally active APP fragment. It is therefore of particular interest to understand the molecular mechanisms recruited by APP/PS to control the expression of GDNF, a neurotrophic factor fundamental for both central and peripheral nervous system (CNS, PNS), neuron survival and, importantly, altered in AD and amyotrophic lateral sclerosis (ALS). The goal of this study is to identify cross-disease pathways and evaluate the relevance of therapeutic approaches targeting the APP/PS/GDNF pathway. Summary: The major role of APP in the onset and progression of AD has been revealed almost three decades ago by the combination of genetic and biochemical approaches, leading to the amyloid cascade hypothesis. Nevertheless, APP and Presenilins (PS) physiological function(s) and how they relate to AD and other associated pathologies are not clearly understood. We recently showed that APP controls GDNF transcription and this has a pivotal role in the formation of NMJs. This is particularly relevant to APP function, because APP deficient mice show a neuromuscular phenotype. Pilot experiments indicated that GDNF expression is also directly controlled by PS activity. Knowing that GDNF

is a major neurotrophic factor, involved in PNS and CNS neuron survival, and that GDNF has been suggested marker of AD, it is of particular relevance to understand how APP and PS control GDNF expression, and how these pathways are related to AD pathological process.

Résumé en français: La protéine précurseur du peptide amyloïde (APP) joue un rôle majeur dans l'apparition et le développement de la maladie d'Alzheimer. Cependant, les étapes menant à sa dérégulation et son rôle physiologique est encore très mal connu. Nous avons montré récemment que l'APP contrôlait la production d'un facteur capable de réguler l'expression de nos gènes (facteur de transcription), le GDNF. Ce facteur joue un rôle très important dans la formation des jonctions entre les neurones et les muscles et dans la survie des neurones du système nerveux central et périphérique. Le but de notre étude est de mieux comprendre comment l'APP régule le GDNF et si cette régulation est perturbée au cours de la maladie d'Alzheimer.

Samenvatting in het Nederlands: Het voorloper proteïne van de amyloïde peptide (APP) speelt een belangrijke rol in het ontstaan en de verdere ontwikkeling van de ziekte van Alzheimer. Toch blijft veel onbekend over de verschillende stadia van zijn ontregeling en zijn fysiologische rol. Recent toonden we aan dat APP instaat voor de ontwikkeling van een factor die in staat is de uitdrukking van onze genen te regelen (transcriptie factor) GDNF genaamd. Deze factor speelt een zeer belangrijke rol in de verbindingen tussen neuronen en spieren en in het overleven van neuronen van het centrale en perifere zenuwstelsel. Het doel van onze studie is tot een beter begrip te komen hoe de APP het GDNF reguleert en of deze regulering verstoord wordt tijdens de ziekte van Alzheimer.

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**Keywords: Alzheimer's disease, gene transcription, Amyloid beta-Protein Precursor, Presenilins, Neuromuscular Diseases**

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# Reciprocal influence between APP expression and glucose metabolism in the hippocampus

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Nowadays, there is evidence that brain glucose metabolism and Alzheimer's disease (AD) are linked (1). Patients suffering from type II diabetes present a higher risk to develop AD while in AD patients but also in preclinical stage (MCI) the brain glucose metabolism is reduced, leading to a general hypometabolism (2). It is therefore very important to better understand the link between brain utilization of glucose and AD. On the other hand, while beta-amyloid aggregates are one of the principal hallmarks of the disease, all strategies targeting these aggregates have failed until now to prove their efficiency. Targeting the amyloid precursor protein (APP) itself and its role in brain metabolism could bring some new insights and lead to novel therapeutic strategies. Our hypothesis is that APP is involved in energy flux between the body and the brain. During ageing or in case of pathology such as AD (2, 3, 4), Down Syndrome (5) and insulin resistance (6), glucose availability can be reduced in the brain leading to a compensatory increase in the expression of APP. This compensatory increase could be the starting point of disruption of metabolic and neurotransmitter homeostasis leading to cognitive deficit (3, 4). The aim of this project is to better understand the link between APP expression and brain glucose metabolism and its impact on neuronal activity and synaptic connections. Three levels of APP expression are investigated thanks to APP WT, HT and KO mice. APP roles in metabolic pathways in the hippocampus is evaluated by 1H-NMR spectroscopy. The neurophysiological impact of the genotype, of the glucose restriction and of the interaction between these two parameters is studied by extracellular electrophysiological recordings of cell excitability and synaptic activity in acute hippocampal slices incubated in control condition (10mM), mild (5mM) and severe glucose restriction (2.5mM). Because APP KO mice are known to be at risk of developing seizures (7), we also studied the effect of disinhibition on electrical activity by adding the GABA-A receptor antagonist picrotoxin to the aCSF. 1HNMR spectroscopy showed a strong increase in glutamate abundance while GABA decreased in APP KO mice. These metabolic modifications are consistent with the literature and could explain the hyperexcitability reported in APP KO mice (7). This overage in glutamate seems to be converted in glutamine whose abundance was also increased. Cholinergic metabolism is also modified as there was less choline and phosphocholine in KO mice but more of their precursor: glycerophosphocholine. This dysregulation is important in AD (8). Moreover, APP role in ATP production at the level of the mitochondria

(9, 10) seems consistent with our observations as ADP/ATP ratio and AMP level were modified according to APP expression. Interestingly, HT mice presented an intermediate level of expression for every metabolite characterised, confirming the importance of the level of APP expression in metabolism regulation. In vivo hypoglycemia are currently carried out and 1HNMR spectroscopy will be performed to determine if the differences in metabolic profiles observed can be intensified when glucose is restricted. Electrophysiological recordings also highlighted electrical differences in sensitivity to glucose restriction between WT, HT and KO mice and susceptibility to hyperexcitability in KO ones. Here again, HT mice presented an intermediate phenotype, strengthening the working hypothesis. Firstly, glucose restriction reduced synaptic activity and excitability of a neuronal network in a concentration dependent way. This indicates that a more pronounced glucose hypometabolism has more deleterious consequences on neural viability. Then, we observed that fEPSP and fiber volleys were not different according to the genotype in the basal condition. However, when glucose supply was impoverished to 5mM or 2.5mM, differences in synaptic activities appeared. Indeed, glucose restriction induced a large decrease of fEPSP in WT mice while this decrease in synaptic activity was considerably attenuated in KO mice. On the opposite, neuronal excitability was not modified as action potentials propagation measured by the fiber volley were not different. Also, ageing had an effect on the hippocampus functioning as 6 month-old mice showed smaller synaptic activity and excitability compared to 6 week-old mice. This reduction was observed for slices perfused in the three conditions. The lack of APP could have an influence on ageing as fEPSP of 6 months-old KO mice were smaller in the glucose restriction conditions than the other genotypes. As 1HNMR spectroscopy and literature showed that GABA is modified in APP KO mice, we studied the epileptiform activity of polyphasic fEPSP induced by a GABA-A receptor antagonist: picrotoxin. We observed that the intensity of the epileptiform activity was higher in KO mice (10mM and 5mM) and when the glucose was reduced to 2.5mM, we observed an extinction of fEPSP in most of the WT slices but not in the KO slices, where epileptiform activity was still high. Moreover, the drug decreased synaptic activity in WT mice but increased it in KO mice and had no significant effect on HT fEPSP. The next step is to confirm that the reduction in glucose supply causes an increase in APP expression as described in the literature (11). If this hypothesis is validated, it could allow us to have a new level of APP expression: the overexpression one. This hypothesis is critical to determine if modifications observed in ex vivo glucose restrictions can be related to molecular changes found in AD and Down syndrome. Nevertheless, we can already conclude that APP expression and glucose metabolism are indeed linked in the hippocampus and that further investigations need to be conducted in the future to better understand this relationship. Numerous studies on Alzheimer's disease focus on its principal hallmark: plaques of amyloid peptides. Unfortunately, these plaques are only detectable at an advanced and incurable stage. A groundbreaking theory aims to study the molecular modifications that appear before first clinical stages as the slow-down of brain functioning due to a poor glucose use by neurons. Therefore, this project investigating the relationship between the Amyloid Precursor

Protein (APP) and the role of brain glucose would allow to establish an early diagnosis and a therapeutic strategy that will take action before the appearance of dementia.

Résumé en français: Beaucoup d'études consacrées à la maladie d'Alzheimer se sont concentrées sur une de ses principales caractéristiques, les plaques amyloïdes. Malheureusement, ces plaques ne sont détectables qu'à des stades avancés de cette maladie actuellement incurable. De nouvelles recherches se sont donc consacrées à l'étude des processus cellulaires et moléculaires qui seraient modifiés aux stades plus précoces de la maladie et qui seraient liés à une mauvaise utilisation du glucose par les cellules du cerveau. Dans ce cadre, ce projet vise à comprendre les relations entre la protéine précurseur du peptide amyloïde (APP), responsable de la production des plaques amyloïdes aux stades tardifs de la maladie, et le métabolisme du glucose afin d'explorer de nouvelles pistes thérapeutiques pouvant prévenir ou ralentir la progression de la maladie.

Samenvatting in het Nederlands: Heel wat studies gewijd aan de ziekte van Alzheimer concentreerden zich op een van de voornaamste kenmerken: de amyloïde plaatjes. Jammer genoeg zijn deze slechts zichtbaar in een gevorderd stadium van de nog steeds ongeneeslijke ziekte. Nieuwe studies richtten zich op de studie van de cellulaire en moleculaire processen die afwijkingen gaan vertonen in eerdere stadia van de ziekte, en die in verband zouden staan met een verkeerd gebruik van glucose door de hersencellen. Binnen dit kader probeert dit project inzicht te verwerven in de relatie tussen het proteïne voorloper van de peptide amyloïde (APP), dat verantwoordelijk is voor de aanmaak van amyloïde plaatjes in een later stadium van de ziekte, en het glucose metabolisme. Dit moet leiden tot nieuwe vormen van behandeling die in staat zouden moeten zijn de ziekte te voorkomen of te vertragen.

**Keywords: Hippocampus, glucose metabolism, APP mice, Electrophysiology, Spectroscopy and biological molecules, Glucose restriction**

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# Importance of the blood-cerebrospinal fluid barrier in Alzheimer's disease

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The blood-cerebrospinal fluid (CSF) barrier forms a unique interface between blood and brain. It consists of a single cell layer, called choroid plexus epithelium (CPE), situated at the interface of blood and CSF. The CPE forms a barrier to protect the brain from fluctuations in peripheral blood thereby assuring brain homeostasis, produces CSF and is responsible for the active removal of toxic molecules from the brain. In recent years, the blood-CSF barrier has gained increasing attention, especially its role in inflammatory and age-related diseases. We studied barrier integrity of the CPE during Alzheimer's disease. Aβ1-42 oligomers, a key player in the pathology of AD, were injected intracerebroventricular in mice to assess their impact on the blood-CSF barrier. The study revealed the induction of a cascade of detrimental events, associated with loss of blood-CSF barrier integrity. Administration of Aβ1-42 oligomers triggered an inflammatory response at the CPE cells and secretion of proinflammatory cytokines into the CSF. Furthermore, Aβ1-42 oligomers rapidly affected CPE cell morphology and induced a decrease in RNA and protein expression of tight junctions. Finally, using a broad-spectrum matrix metalloproteinase (MMP) inhibitor, we provide evidence for the essential role of MMPs in the Aβ1-42 oligomer-induced loss of blood-CSF barrier integrity. In conclusion, our results provide new insights in the toxicity of Aβ1-42 oligomers and point to new possible molecular targets to reduce neuroinflammation associated with Alzheimer's disease.

Résumé en français : Dans ce travail nous étudions la modification de l'intégrité de la barrière hémato-encéphalique provoquée lors de la maladie d'Alzheimer. Pour l'approche expérimentale, nous avons utilisé des souris et montré que l'injection de l'oligomère Aβ1-42, une molécule clé de la maladie d'Alzheimer, dans les ventricules du cerveau de souris induisait une réponse inflammatoire des cellules de la barrière, une modification de leur morphologie, un affaiblissement de leurs jonctions serrées et l'activation de protéases qui diminuent l'intégrité de la barrière.

Samenvatting in het Nederlands: Dit werk bestudeert de wijziging van de integriteit van de hemato-encefalische afscherming bij de Ziekte van Alzheimer. Ten experimentele

titel hebben we gebruik gemaakt van muizen bij wie we het oligomeer Aβ1-42, een sleutelmolecule bij de ziekte van Alzheimer, geïnjecteerd hebben in de hersenholtes van de muizen. Het leidde tot een inflammatoire reactie van de cellen, een wijziging van hun vorm, een verzwakking van hun nauwe verbindingen en een activering van de proteasen die de integriteit van de afscherming verminderen.

Samenvatting in het Nederlands :

**Keywords: Alzheimer's disease, Blood-Brain Barrier, Choroid Plexus, Matrix Metalloproteinases, Tight Junctions**

# DMRT transcription factors are required for cortical development

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Patterning the cerebral hemispheres and generating the neocortical area map depend initially on interplay between morphogens secreted by organizing centers and transcription factors expressed in gradients across the cortical primordium. One of the latter, Dmrt5/Dmrta2, a zinc finger doublesex and mab-3 related (Dmrt) gene, is expressed in mouse cortical progenitors in a high caudomedial to low rostrolateral gradient. Dmrt5 is required for the development of caudomedial cerebral cortex but its mode of action remains unclear. In constitutively Dmrt5 null mice, the Wnt-and Bmp-rich cortical hem is missing, suggesting that hem formation relies on DMRT5, and that deletion of Dmrt5 affects cortical patterning indirectly through loss of signalling from the hem (Saulnier et al., 2013). In a positive feedback loop however, WNT signalling upregulates Dmrt5 expression, suggesting a second, direct patterning role for DMRT5. Our recent data indicate that inactivating or overexpressing Dmrt5 conditionally in cortical progenitors close to midgestation still affect cortical patterning without disrupting the function of the hem and that mutation of a related gene, Dmrt3, with a similar expression pattern to Dmrt5, also caused similar, albeit milder cortical patterning defects than that observed in Dmrt5 mutants. Thus, Dmrt5 and Dmrt3 appears to have direct roles in cortical patterning, in addition to their prior role in the establishment of the cortical hem (De Clercq et al., 2015). Analysis of the cortex of Dmrt3-/-;Dmrt5-/- double knock-out mice reveals that the phenotype is more severe than in the single mutants, suggesting that the two genes cooperates to control cortical patterning. RNA-seq and ChIP-seq analyses are underway to identify their direct targets.

Résumé en français Des anomalies de développement du cortex cérébral sont à l'origine de nombreuses maladies neuropsychiatriques et neurologiques chez l'homme. Nos travaux ont montré que chez la souris, les facteurs de transcription Dmrt3/5 jouent un rôle essentiel dans le développement cortical, en particulier dans la formation des aires du néocortex. Chez l'homme, des mutations dans le gène DMRT5 ont été identifiées et associées à une microcéphalie. Le but de nos travaux est de mieux comprendre leur mécanisme d'action dans le contrôle de la prolifération et la différenciation des progéniteurs corticaux.

Samenvatting in het Nederlands: Ontwikkelingsstoornissen van de hersenschors liggen aan de basis van tal van neuropsychiatrische en neurologische ziekten bij de mens. Ons werk heeft aangetoond dat bij muizen, de transcriptiefactoren Dmrt3/5 een essentiële rol spelen in de ontwikkeling van de hersenschors, meer bijzonder in de vorming van de gebieden van de neocortex. Mutaties van het genDMRT5 bij de mens werden geïdentificeerd en geassocieerd aan een microcefalie. Het doel van ons werk is een beter begrip van hun werking in de controle van de verspreiding en de differentiering van de ontwikkeling van de hersenschors.

**Keywords: cortical hem, Hippocampus, Neocortex, primary visual area, Transcription Factors**

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Marc Keruzore and Sarah De Clercq contributed equally to the work

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# The multi-tasking protein FMRP wires mammalian brain

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Deficiencies in the fragile X mental retardation protein (FMRP) lead to the most frequent form of inherited intellectual disability and Autism Spectrum Disorders (ASDs), the fragile X syndrome (FXS), with symptoms manifesting during infancy and early childhood. Because FMRP is expressed at very early stages of embryonic development, we hypothesize that FXS is the result of complex regulatory mechanisms occurring prenatally and at early postnatal stages, when synaptogenesis occurs. FMRP regulates the positioning of neurons in the cortical plate during embryonic development, affecting their multipolar-to-bipolar transition (MBT). We identified a few FMRP-regulated targets crucial for MBT in embryonic brain, among them N-cadherin. Spontaneous network activity and high-resolution brain imaging at earlier postnatal stages revealed embryonic defects in the establishment of excitatory and inhibitory neuronal networks. At early post-natal stages the-amyloid precursor protein (APP), involved in Alzheimer's disease, plays a role in synapse formation, and is upregulated in FXS and other intellectual disabilities. In FXS, APP signals through the metabotropic receptor that, activating the MAP kinase pathway, leads to synaptic and behavioral deficits. Proper control of APP processing is crucial for healthy spine formation and function(s). We propose that the affected brain wiring in FXS is in part the result of dysregulated mRNA metabolism that starts during the first weeks of life and persists with remnants into adulthood.

Résumé en français: Autisme et Alzheimer Le syndrome du X fragile est une maladie génétique responsable de déficiences mentales et d'autisme. Les symptômes se manifestent pendant l'enfance. La protéine (FMRP), appelée Protéine du X fragile, est exprimée à des stades précoces du développement embryonnaire et est diminuée chez les enfants ayant le syndrome. Notre hypothèse est que ce syndrome apparaît dès la naissance, lorsque la synaptogénèse se développe. De manière analogue, il a été montré qu'une autre protéine l'APP (responsable de la formation des plaques amyloïdes) joue aussi un rôle dans la synaptogénèse, interagit avec la protéine du X fragile qui, lorsqu'elle est diminuée, altère la synaptogénèse.

Samenvatting in het Nederlands: Autisme en Alzheimer. Het fragiele X syndroom is een genetische aandoening die mentale achterstand en autisme veroorzaakt. De symptomen treden op tijdens de kinderjaren. Het proteïne (FMRP), ook proteïne van het fragile X syndroom genoemd, komt voor in vroegtijdige stadia van de ontwikkeling van het embryo, en vermindert dan bij kinderen met het syndroom. Onze hypothese is dat dit syndroom tot uiting komt bij de geboorte, bij de ontwikkeling van de synaptogenese. Analoog daarmee werd aangetoond dat een andere proteïne, APP (verantwoordelijk voor de vorming van amyloïde neerslag) ook een rol speelt in de synaptogenese, in interactie met het proteïne van het fragile X syndroom dat, wanneer dit vermindert, de synaptogenese aantast.

**Keywords: Fragile X Syndrome, Fragile X Mental Retardation Protein, synaptogenesis, amyloid precursor protein (APP), Autism Spectrum Disorders, Alzheimer's disease, Intellectual Disabilities**

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Renoux, Nicolas M. Carducci, Arya A. Ahmady, Peter K. Todd. Fragile X mental retardation protein expression in Alzheimer's disease. Abigail J. Front. Genet., 2014 Oct 21 2014; 5 : 360

# Implication of Importin-8 in mouse brain development

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Regulation of nucleocytoplasmic transport of proteins by the karyopherin superfamily is critical for cell physiology as it controls mainy fundamental processes such as division, differenciation, migration, adaptation to external environment etc. Beside this fundamental role, ß1 and ß2 members of this superfamily are also implicated in mitosis and ciliary entry respectively. Importin-8 (IPO8), a member of the β-karyopherin family, is reported to control the transport Ago-2, c-Jun and Smad-4 for example, three proteins important for brain development. First, we have verify the subcellular localisation of IPO8 in HEK and hTert cells. No colocalisation with either the mitotic spindle or the primary cilia could be observed. So it seems IPO8 only plays a role in nuclear transport of proteins. Then we have assessed the expression of IPO8 in mouse brain by In Situ Hybridization at various embryonic (E12, E14, E18) and post natal age (P5, P60). A strong expression was observed during embryonic stages, and especially in the ventricular zone and the cortical plate of the cerebral cortex and the ganglionic eminences both at E14. Therefore, the implication of IPO8 in the radial migration has been assessed by in utero electroporation of shRNA at E14. Three days after IUE, we observed that neuroblast accumulates in the Intermediate zone (IZ) and do not reach the cortical plate (CP) in constrast to the control condition. This effect can be corrected by coexpressing a form of IPO8 that is not targeted by the shRNA, demonstrating the specificity of the effect. In conclusion, regarding its role in transport, IPO8 could modulate neurons migration in the developing brain and could be also at the origin of some diseases associated with neurons migration defects.

Résumé en français: Lors du développement embryonnaire, les neurones migrent vers leur futur emplacement dans le cerveau. Lorsque cette migration est perturbée, il en résulte de graves problèmes neurologiques. Il est donc très important de comprendre les mécanismes et les protéines responsables de ce processus. Dans cette étude, nous mettons en évidence une nouvelle protéine, l'importine-8, qui semble jouer un rôle important dans la migration neuronale et qui pourrait donc être impliquée dans certaines maladies neurologiques développementales.

Samenvatting in het Nederlands: Bij de embryonale ontwikkeling van de hersenen migreren de neuronen naar hun toekomstige plek in de hersenen. Als deze migratie verstoord geraakt kunnen zich zware neurologische afwijkingen voordoen. Het is dus van belang om de mechanismen en de proteïnes die voor deze ontwikkeling verantwoordelijk zijn te begrijpen. In deze studie verwijzen wij naar een nieuwe proteïne, importine-8 of IPO8 die een belangrijke rol blijkt te spelen bij de neuronale migratie en die dus aan de basis zou kunnen liggen van bepaalde neurologische ontwikkelingsziekten.

**Keywords: IPO8, Brain Development, radial migration, RNA Interference, in utero electroporation**

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# Normal and Pathological Brain Phenotypes from Homeorhetic Epigenetic Landscapes in Natural drift

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The cortical dynamics and the brain phenotypes (normal or pathological) take place at large-scale. Thus, why must a single neuron or even a gene be relevant? Could any single gene or genetic network determine pathological brain phenotypes like schizophrenia? An explanation based on mechanisms from the 'sequence hypothesis' of the molecular biology 'dogma', or from the teleonomic computational system biology suggests that the brain phenotypes will derive from the 'information' in genes and/or from the self-organized connectome neural network dynamics respectively. Nevertheless, our explanatory approach rest on another kind of biological fundaments (Rosen 1991, Lovelock 1987, Maturana and Varela 1980) that allow us to identify the neuron as a systemic (M,R)-Gaia autopoietic unity that is embedded in an organizationally close, but dynamically open neural network. This entails three fundamental things: i) the genome is not a software or teleonomic program controlling or to be executed by a cytoplasmic hardware, but an aperiodic copolymer that exerts gravitational forces in the neuron cytoplasm dynamics, while the neuron metabolic molecular network exerts inertial forces on the genome; ii) any single neuron, no matter its neuronal network vicinity display cognition, noncomputability, autonomy, and photonic emission either in action potential or resting dynamics; iii) the activity of a neuron is not an input-output mechanisms, but rather depends of its metabolism beyond oxygen dependencies. In other words, the neuron is far from being a 'cupper transistor device' or just a self-organized collection of molecular fractions. This leads us to the next conclusions: i) if the brain has to be fractioned in order to determine the neuronal bases of its phenotype, the right level is not the molecular DNA sequence, the genetic network, or any brain statistical borderless region or module of interest, but rather the systemic neuron unity; ii) henceforth, any possible brain phenotype (normal or pathological) is of a systemic and relational nature in the sense of biological unities within biological unities; iii) the establishment of brain phenotype is, thus, determined by the recurrent behavioural pattern of the meta-embodied brain (neuron-organism-environment's structural coupling); iv) the recurrent behavioural pattern of the meta-embodied brain in natural drift (Maturana and Mpodozis 1992) can leads to specific or unique homeorhetic epigenetic landscapes (epigenetics landscape ≠ DNA marking) (Waddington 1940, 1957) of single neurons, hence determine its possible

connectivity and brain phenotype outcomes. These conclusions are of a preventive, personalized, predictive and participative P4-medicine interest. Short resume The brain functions and its implication in whatsoever we can call pathological or normal behaviour, isn't genetically determined but epigenetically. Epigenetics may not only be understood as DNA modification marking (methylation or acetylation), but fundamentally as a systemic and relational phenomenon that occurs from specific, repeated, and unique interactions of the individual with its environment (familiar, social and institutional systems). In a nutshell, a pathological environment can lead potentially to a pathological brain and thus to a pathological genetic profile. Therefore, there aren't genetically inherited brain diseases, but rather inheritance of pathological or normal behavioural relations between organism and environment.

Résumé en français: Les fonctions du cerveau et ses implications dans tout ce que nous pouvons appeler un comportement pathologique ou normal, ne sont pas génétiquement déterminées mais le sont épigénétiquement. L'épigénétique peut non seulement être comprise comme l'expression de la modification de l'ADN (méthylation ou acétylation), mais surtout comme un phénomène systémique et relationnel qui se produit à partir des interactions spécifiques, répétées et uniques de l'individu avec son environnement (systèmes familiers, sociaux et institutionnels). En un mot, un environnement pathologique peut conduire potentiellement à un cerveau pathologique et donc à un profil génétique pathologique. Par conséquent, il n'y a pas de maladies du cerveau génétiquement héritées, mais plutôt un héritage des relations de comportement pathologiques ou normales entre l'organisme et l'environnement.

Samenvatting in het Nederlands :Het functioneren van de hersenen en wat daarvan afhangt, in wat we pathologisch of normaal gedrag noemen , is niet genetisch bepaald, maar wel epigenetisch. Epigenetica kan niet alleen beschouwd worden als wijzigingen van het DNA (methylatie of acetylatie), maar is vooral een systemisch en relationeel fenomeen gebaseerd op specifieke, eenmalige of herhaalde, interacties van het individu met zijn omgeving.(op familiaal, sociaal en institutioneel vlak) Samengevat, een pathologische omgeving kan potentieel leiden tot pathologische hersenen en dus tot een pathologische genetisch profiel. Bijgevolg zijn er geen genetisch erfelijke hersenziekten, maar eerder erfelijkheid van pathologische of normale gedragingen in de interactie tussen het organisme en zijn omgeving.

**Keywords: (M.R)-system, Gaia, Autopoiesis, Homeorhetic Epigenetic Landscapes, Natural drift, Brain phenotypes, Biological fundaments**

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This work is dedicated to the memory of Benjamin Rothman.

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# Microdeletion of 7p21.3 and 12p13.32 in a female patient with severe microcephaly

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The present case was identified during molecular cytogenetic screening in individuals with unexplained intellectual disability. MR scan (3T, Magnetom TrioTim, Siemens) performed at the age of 36 showed a head circumference of 49,5 cm which fits to criteria of microcephaly. By arrayCGH analysis, we found 49 genes that had aberrant copy number. Out of all affected genes, the role in neurodevelopment is the most extensively studied for Cyclin D2 (CCND2, HGNC:1583, 12p13.32), that is necessary for geometric expansion of cellular output from the subventricular zone (SVZ). When CCND2 expression level is increased, cells are maintained in cycle increasing number of progenitors and vice versa: decreased CCND2 expression will enable cell cycle exit and decrease number of progenitors. Therefore, we hypothesize that haploinsufficiency of CCND2 (%HI 2.32) is a strong candidate for reduced brain size in presented case, although liaison or contribution of other genes to the phenotype cannot be ruled out.

Résumé en français: Cette recherche porte sur une étude des anomalies génétiques observées dans un cas de microcéphalie. La microcéphalie désigne la taille anormalement petite du crâne. La microcéphalie est souvent secondaire à un arrêt de développement du cerveau. Pour ce cas, nous avons utilisé une méthode de cytogénétique moderne : l'analyse chromosomique sur puce à ADN (CGH array) et montré que 49 gènes avaient une anomalie. En particulier le gène qui code pour une protéine : la cycline D2 (CCND2), responsable de la croissance d'une région du cerveau et de la prolifération de cellules neurales. Ce résultat indique que cette protéine lorsqu'elle est sous-exprimée peut être responsable de microcéphalie.

Samenvatting in het Nederlands: Deze studie betreft de genetische afwijkingen aangetroffen bij microcefalie. Microcefalie houdt in dat de omvang van de schedel abnormaal klein is. Microcefalie is meestal een secundair gevolg van een ontwikkelingsstoornis van de hersenen. In dit geval maakten we gebruik van een moderne cytogenetische methode: de chromosomische analyse van een DNA chip (CGH array) en konden

we 49 afwijkende genen aantonen. In het bijzonder het gen dat de proteïne cycline D2 (CCND2) codeert, en dat verantwoordelijk is voor de groei van een gebied in de hersenen en de verspreiding van neurale cellen. Dit resultaat toont aan dat dit gebrek aan deze proteïne kan leiden tot microcefalie.

**Keywords: Intellectual Disability, Brain MR, arrayCGH, Microcephaly, Cyclin D2**

# Automated brain region recognition based on elastic registration and atlas mapping

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Brain tissue slices represent physiologically faithful models for analyzing pathological features in an anatomically defined manner. With throughput getting larger, fully automated analysis has become essential. A bottleneck in this context is the automated recognition and annotation of selected brain regions. To meet this demand, we are developing an automated image analysis pipeline (schematically shown in Fig. 1) for contextual quantification in the brain by mapping anatomical information onto microtome-cut brain slices. To this end, DAPI-stained brain slices are shape-normalized to a reference section by means of an elastic registration algorithm that is constrained by both intensity and shape information. The reference slices in turn, are mapped to manually or automatically assigned brain regions; the latter using information extracted from the openly available Allen Brain Atlas. A robust error metric is conceived to estimate registration performance, to optimize its parameters, and to automatically remove badly cut slices. The quality of our method is quantified by comparing the automatically defined brain tissue regions to the ground truth (manually segmented brain slices) of both registered and reference slices.

Résumé en Français: Lors de l'étude des modèles animaux de maladies du système nerveux, l'analyse des modifications anatomiques induites par la pathologie est une étape importante mais fastidieuse car reposant sur l'observation de milliers de coupes et l'identification des structures anatomiques. Dans ce travail, nous avons mis au point un programme qui permet de déterminer de manière automatique toutes les structures anatomiques à partir de coupes histologiques. Grâce à un algorithme mathématique les coupes sont superposées à des coupes modèles annotées sur base d'un atlas référencé. Cette technique permettra d'analyser beaucoup plus rapidement les changements anatomiques induits par différentes pathologies du système nerveux.

Samenvatting in het Nederlands: In ziekten van het zenuwstelsel, zoals o.a. de ziekte van Alzheimer, treden "zieke" zenuwcellen veelal eerst op in bepaalde brein-regio's. Om deze ziekten te onderzoeken maakt men veelal gebruik van scans van de hersenen of hersencoupes. Hierbij is het correct detecteren en onderscheiden van de verschillende

regio's van de hersenen erg belangrijk. Verder is, met het oog op een snelle en objectieve analyse van dergelijke scans, een geautomatiseerde regio-herkenning wenselijk. In dit onderzoek hebben we zulke methode ontwikkeld, toegespitst op beelden van fluorescente hersencoupes van muizen. Deze methode laat toe de beeldanalyse in onderzoek op muizen te versnellen door dit knelpunt te verwijderen.

**Keywords: Brain Mapping, Elastic warping, Brain Atlas, brain tissue slices, segmentation, image analysis**

# Influence of topography on cortical interneuron migration in microstructured environments

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In the adult brain, the cerebral cortex contains pyramidal neurons which are output neurons and interneurons, mostly inhibitory. Convergent evidence shows that interneurons are essential for computation by cortical circuits. During brain development, interneurons migrate a long distance to reach the cerebral cortex. Their correct positioning is crucial for the normal control of cortical activity. In particular, abnormal development or function of interneurons is now considered as a major factor in psychiatric diseases such as schizophrenia or epilepsy. Migrating interneurons are highly polarized cells with a long leading process at the cell front that explores the environment and adheres to the substrate. As previously described for growing axons, the migration of cortical interneurons is influenced by chemotactic molecules distributed as gradients in the extracellular space and by adhesive molecules. These cues are transduced in the growth cone and influence cytoskeleton dynamics. Interestingly, physical cues such as topography or substrate stiffness have also been shown to guide axonal growth, a process that resembles leading process navigation. However, the contribution of physical cues in interneuron migration remains largely unknown. To further investigate the influence of topography on interneuron migration, we developed a migration assay using microfabrication tools, in which migrating interneurons from mouse embryonic brains are cultured on microstructured surfaces of PDMS coated with a mix of adhesion molecules. We observed that migrating interneurons are indeed sensitive to topography and respond differently to isotropic (forest of pillars) or anisotropic (grooves) topography. Interestingly, we observed that the leading process of interneurons migrating in isotropic topography was highly sensitive to the shape and spacing of structures. In particular, leading processes of interneurons migrating among square pillars presented a stereotyped alignment which was rarely observed on round pillars. Cytoskeleton organization and dynamics differed on these two substrates, showing that topographical cues in the cellular environment can greatly influence the morphology and dynamic properties of the leading process. By using these biophysical approach, we hope to shed light on the influence of a new class of guidance cues – physical guidance- on migrating neurons. In the highly controlled environment of microstructured surfaces, we should be able to precisely characterize the cellular defects altering the migration of interneurons with mutations involved in human cortical malformations and neurodevelopmental psychiatric disorders.

Résumé en français: Les défauts de positionnement des interneurones dans le cortex cérébral sont à l'origine de nombreuses maladies neurodéveloppementales et neuropsychiatriques. La mise en place de ces cellules a lieu pendant le développement embryonnaire, à la suite d'une très longue migration qui utilise les molécules présentes dans l'environnement comme signaux de guidage. La contribution des paramètres physiques de l'environnement reste méconnue. Pour étudier l'influence de ces paramètres sur la migration des interneurones, nous avons développé par microfabrication des substrats présentant un microrelief. Nous montrons que les interneurones, spécialement leur prolongement migrateur, sont très sensibles à la géométrie du relief.

Samenvatting in het Nederlands: Foutieve instelling van interneuronen in de hersenschors liggen aan de basis van heel wat neuro ontwikkelings- en neuropsychiatrische ziektes. Het plaatsen van de cellen gebeurt tijdens de embryonale ontwikkeling, op basis van een zeer lange migratie die gebruik maakt van de moleculen aanwezig in de omgeving als bakens. De bijdrage van fysieke parameters van de omgeving blijft miskend. Om de invloed van deze parameters op de migratie van neuronen te bestuderen, hebben we door microfabricatie substraten ontwikkeld met een micro reliëf. We tonen aan dat de interneuronen, en in het bijzonder het verderzetten van hun migratie, zeer afhankelijk zijn van de geometrie van het reliëf.

**Keywords: Cortical development, cortical interneurons, Migration, Cytoskeleton, topography, Physical guidance, Microfabrication techniques, Cell Culture Techniques**

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# Neural coding of viewpoint, posture and identity of bodies in the macaque midSTS body patch

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FMRI studies in primates show body-category selective activations in temporal cortex. Single unit studies in the midSTS body patch revealed greater activity to images containing bodies compared to faces and objects. However, these neurons showed a marked body image selectivity, responding only to some body images. The body image selectivity may reflect encoding of body identity, posture and viewpoint. Here we examined the selectivity of the fMRI-defined macaque midSTS body patch neurons for these three variables. We designed a novel computer-generated stimulus set (n = 120 stimuli) depicting monkeys that differ in anthropometric features (identity; fat, normal and thin), with 5 different postures (2 threat, 2 submissive and 1 neutral posture), rendered at 8 viewpoints (45 deg step; rotated around the vertical axis). The internal facial features of the head were removed. The images were presented for 200 ms each during passive fixation in 2 rhesus male monkeys. Single unit recordings showed a marked selectivity for viewpoint and posture. We employed linear Support Vector Machines with cross-validation to decode viewpoint, posture and identity from the responses of 88 midSTS neurons. First we asked whether we could decode one variable (e.g. viewpoint) when varying the two other variables (e.g. posture and identity). Decoding of identity and posture invariant viewpoint (classification accuracy: 73%; 200 ms binwidth; chance level : 12.5%) and viewpoint and identity invariant posture (50%; chance level: 20%) was well above chance (permutation test with shuffled stimulus labels; $p < 0.005$) but viewpoint and posture invariant identity decoding (36%) was barely above chance level (33%; $p = 0.04$). However, it was possible to decode identity for particular view and posture combinations (accuracy ranging between 57 and 95%; chance = 33%). Second, we asked how tolerant the decoding of one variable is for changes of the other variables by training e.g. viewpoint decoding at one particular identity and posture and testing at the same and other combinations of the latter two variables. This analysis revealed little generalization of posture and identity decoding with viewpoint, in line with the viewpoint selectivity of midSTS body patch neurons. Posture and viewpoint decoding generalized well across identities. Analysis of the viewpoint-dependent errors in pose encoding suggested an encoding of head orientation by these body patch neurons. Viewpoint decoding generalized across the threat but not across the other postures. These data suggest that the output of midSTS

body patch neurons are useful to decode the posture and orientation of bodies, with a role of head orientation.

Résumé en français : Le codage sélectif par des neurones individuels selon l'angle de vision, la posture et l'identité d'un corps au niveau de la région du Sulcus Temporal Supérieur chez le macaque. Nous avons pu montrer dans ce travail que chez l'humain et le singe se trouvent des groupes de neurones dans le cortex temporal qui répondent fortement à certaines caractéristiques comme la forme extérieure et la posture plutôt qu'au faciès. L'une de ces régions est appelée le midSTS body patch qui est une sous-région du "Sulcus Temporal Supérieur "(1). Afin d'analyser les informations codées par cette région, nous avons pu décoder les réponses des neurones de ce patch. Nous avons constaté que ces neurones réagissaient effectivement et de manière sélective à des informations sur la forme extérieure du corps, la posture et dans une moindre mesure l'identité. L'orientation de la tête est un autre paramètre important qui est codé par ces neurones.

Samenvatting in het Nederlands : De codering van gezichtspunt, lichaamshouding en identiteit in het midSTS lichaamsgebied van de makaak. Bij mens en aap vindt men gebieden in de temporale hersenschors die sterker reageren op visuele beelden van lichamen dan op die van voorwerpen. Een van deze gebieden is de midSTS "body patch". Om inzicht te krijgen in de informatie die gecodeerd wordt in dit gebied, hebben we de antwoorden van neuronen in die patch gemeten op beelden van lichamen. We vonden dat de midSTS body patch informatie geeft over het gezichtspunt waaronder men een lichaam ziet, de lichaamshouding en in mindere mate de identiteit. De orientatie van het hoofd was een belangrijke parameter die gecodeerd wordt door deze neuronen.

**Keywords: Macaca mulatta, body patch, midsts, bodies, fMRI BOLD, Electrophysiology, single unit recordings, neural decoding**

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# Personal neglect and tactile extinction involve early deficit in bilateral tactile novelty detection at the secondary somatosensory cortex

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BACKGROUND: Using magnetoencephalography (MEG), we assessed in patients with a non-dominant hemisphere stroke the pathophysiology of personal neglect (PN) and tactile extinction by searching for alterations in the spatio-temporal dynamics of the multilevel cortical processing of bilateral tactile novelty detection. METHODS: Somatosensory evoked fields (SEFs) were recorded with whole-scalp-covering MEG (Elekta Oy) in ten right-handed healthy adults (29 +/-3 years, 5 females) and five right-handed stroke patients (58 +/ 8 years, 3 females) with PN and tactile extinction but no apparent somatosensory deficits. Participants underwent a tactile oddball paradigm adapted from the auditory « local/global » oddball paradigm.

METHODS: Somatosensory evoked fields (SEFs) were recorded with whole-scalp-covering MEG (Elekta Oy) in ten right-handed healthy adults (29 +/-3 years, 5 females) and five right-handed stroke patients (58 +/ 8 years, 3 females) with PN and tactile extinction but no apparent somatosensory deficits. Participants underwent a tactile oddball paradigm adapted from the auditory « local/global » oddball paradigm (Naeije et al., 2016)

Standard stimuli were pneumatic tactile stimulations of the right index fingertip and deviant stimuli corresponded to similar tactile stimulation but at bilateral index fingertip. Differences in SEFs elicited by standards and (local or global) deviants were assessed at the sensor level using non-parametric cluster statistics. Neural sources of SEF differences were localized using conventional multidipole equivalent current dipole modelling. RESULTS: In healthy subjects, local deviants elicited 55-120 ms post-stimulus a significantly higher SEF amplitude compared with standards at bilateral secondary somatosensory cortex (SII). Compared to standards, global deviants elicited a significant P300 response involving the temporo-parietal junctions bilaterally and the supplementary motor area. In patients, stroke-related abnormal low-frequency magnetic activity impeded the investigation of SEF responses in the affected hemisphere. Importantly, no difference in SEFs elicited by standards and local or global

deviants was detected in the patients' unaffected hemisphere. CONCLUSIONS: This study suggests that, in stroke patients, PN and tactile extinction are related to an early deficit in bilateral tactile novelty detection at SII cortex.

Résumé en français: Cette recherche porte sur des patients qui, ayant subi un accident vasculaire cérébral (AVC), souffrent d'un défaut d'attention (négligence) et d'une extinction tactile. L'utilisation de la magnétoencéphalographie (technique de mesure des champs magnétiques induits par l'activité électrique des neurones du cerveau) nous a permis de montrer que ces déficiences sont en relation avec l'activité du cortex somatosensoriel secondaire bilatérale (Cortex SII). Cette région du cortex participe à l'intégration sensorimotrice. Elle permettrait de situer en 3D notre corps dans l'environnement suivant des informations visuelles et sensorielles.

Samenvatting in het Nederlands: Deze studie gaat over patiënten met een niet aangeboren hersenletsel (AVC) die lijden aan aandachtstoornissen (onzorgvuldigheid) en verlies van tastzin. Het gebruik van magneto-encefalografie (techniek waarbij de magnetische velden, opgewekt door elektrische activiteit binnen de hersenen gemeten worden) liet ons toe aan te tonen dat de gebreken in verband staan met de activiteit van de secundaire bilaterale somatosensoriele hersenschors (Cortex SII). Dit gebied van de hersenschors staat mee in voor de sensomotorische integratie. Zij zou het mogelijk maken dat wij ons lichaam in 3D kunnen situeren op basis van visuele en sensoriele informatie.

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**Keywords: Stroke, neglect, MEG, mismatch negativity (MMN), somatosensory**

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# Neurophysiological test battery to assess the role of primary auditory perception, gating and attention in the auditory perception of sound intensity: A pilot study

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Background – Parkinson's disease (PD) is a neurodegenerative disorder that has been associated with auditory intensity perception deficits (De Keyser et al., Accepted). To date, the audiological and neurophysiological background of these perception deficits is unknown, and it is unclear which levels of auditory intensity perception are involved. Our group aimed to develop a neurophysiological test battery to investigate the role of primary auditory perception, gating and attention in the auditory perception of sound intensity (De Keyser et al., 2016). Method – Intensity dependent auditory evoked potentials (IDAEPs) and auditory oddball tasks for intensity discrimination (MMN, P3) in both silence and in multitalker babble (MTB) noise were administered during EEG acquisition. Subjects included 7 healthy controls (HC) and one patient with idiopathic PD. Results – Higher N1/P2 amplitude values were evoked by increasing intensity levels in the IDAEP measurement (Beauducel et al., 2000). Stronger IDAEPs were found in the PD patient. Concerning MMN and P3, higher amplitude values and decreased peak latencies were found for the loud deviant (90 dB SPL) compared to the silent deviant (70 dB SPL) in the HC. Concerning MMN and P3 measurements in noise, a significant effect of MTB noise was found, leading to higher amplitude values and increased peak latencies in the HC (De Keyser et al., 2016). Conclusion – The currently used paradigms seem relevant for the neurophysiological evaluation of auditory intensity perception in PD. Further work needs to be done to establish whether an auditory perception deficit in patients with PD can be demonstrated neurophysiologically. Short summaries:

Résumé en Français :Cette étude contribue à l'élaboration d'un protocole utilisant des tests neurophysiologiques pour l'évaluation de la perception sonore. Grâce à ce nouveau protocole, les patients souffrant d'une perception auditive altérée peuvent être diagnostiqués de manière plus précise et bénéficier d'un meilleur suivi.

Samenvatting in het Nederlands : Dit onderzoek draagt bij tot de ontwikkeling van een systematisch protocol voor de evaluatie van geluidsperceptie. Patiënten bij wie

mogelijks sprake is van een gestoorde auditieve perceptie kunnen via dit protocol meer nauwgezet gediagnosticeerd en opgevolgd worden.

**Keywords: Parkinson's disease (PD), Auditory Perception, Gating, Attention, AEP**

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The article of Journal of Speech, Language, and Hearing Research is still in press. No further specifications could be added.

# Asymmetric spatial processing under cognitive load not only in neglect patients, but also in healthy participants

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The detection of hemispatial neglect, namely an awareness deficit for items presented in the contralesional hemispace, is modulated by task demands and thus greatly depends on the tasks adopted. Bonato et al. (2013) and Bonato (2015) have found that in chronic right hemisphere stroke patients, computer-based dual-tasks detect neglect much more sensitively than the conventional paper-and-pencil tests. We aimed to investigate the consequences of functional damage to the spatial attention network (Corbetta & Schulman, 2011) using the same task in the acute stage after stroke, before compensatory strategies can be learned. Twenty-five stroke patients (left or right hemisphere) were tested within the first week of lesion onset. Both standard neglect paper-and-pencil tasks as well as the above mentioned computer-based tasks were administered. The computer-based tasks included a spatial processing task where patients had to report the position of targets (single-task; presented either left, right, or on both sides) and the coupling of this task with a second response to a visual or auditory feature (dual-task). Results in this acute stroke population showed that the computer-based tasks were more sensitive in detecting contralesional omissions (neglect and extinction) compared to the clinical tests. In particular, increasing the attentional load, either by dual-task or by bilateral target presentation, negatively affected the processing of the contralesional space. Interestingly, deficient performance (for right hemispace) was also found in some patients with left hemisphere stroke. Following a similar approach, we also investigated the effect of cognitive load on normal spatial processing within young, healthy participants, using a more difficult variant of the task. The amount of cognitive load was manipulated in a letter recall task (Majerus et al., 2012), where participants had to memorize sequences of either 2 or 6 consonants. Spatial processing was measured by a detection task where participants had to press a button as soon as they saw a target, irrespective of its position (left, right or none). They performed this detection task while their working memory was loaded by the letter recall task. Results showed an interaction between load and position which indicated that cognitive load affects the processing of targets presented in the left hemispace more than those in

the right hemispace. In summary, we found that both brain-damaged patients and healthy participants may display an asymmetric attentional deficiency under cognitive load. It has been well established that the presence of neglect has a great influence on daily tasks. Therefore, using a sensitive measure for its detection is imperative. Present findings may have implications for everyday life, not only for neglect patients, but also for a healthy population, for example when driving a car. In this situation, where fast reaction times are crucial, we are frequently dual-tasking (e.g., having a conversation, listening to the radio) and consequently we may be subject to asymmetric spatial attention processing with a disadvantage for the left hemispace.

Samenvatting in het Nederlands Bij neglect patiënten is de spatiale aandacht aangetast, waardoor één kant van de ruimte (contralateraal van het hersenletsel) minder of geen aandacht krijgt. Neglect heeft een grote impact in het dagelijkse leven en is dus belangrijk om te detecteren. In deze studie werd een computertaak gebruikt waarbij de positie van een stimulus gerapporteerd moest worden, al dan niet gepaard met simultaan een tweede taak. Resultaten tonen dat wanneer de cognitieve belasting hoger was, patiënten meer en ernstiger neglect vertoonden. Met een gelijkaardige taak bij gezonde proefpersonen, vonden we dit effect weerspiegeld door tragere reactietijden voor linkse stimuli onder cognitieve belasting.

Résumé en français: Une lésion cérébrale dans l'hémisphère gauche ou droit peut provoquer un défaut d'attention pour des objets se trouvant dans le champ visuel gauche si la lésion est à droite: on parle de négligence hémispaciale controlatérale, laquelle constitue un handicap pour l'exécution de tâches quotidiennes chez des patients ayant eu un accident vasculaire cérébral (AVC). L'utilisation de tests de doubles tâches (conduire une voiture et écouter de la musique) monitorés par ordinateur est nettement plus sensible que les tests cliniques sur papier. Cette augmentation de sensibilité a permis de détecter des négligences hémispaciales aussi chez les sujets sains. Ainsi, en conduisant une voiture et en parlant, notre attention est moins performante, surtout pour notre hémichamp visuel gauche.

**Keywords: Unilateral spatial neglect, Cognitive Load, asymmetric attentional deficiency, detection task, spatial attention**

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# Rapid Categorization of Snakes in the Infant's Occipital Cortex: Evidence from Fast Periodic Visual Stimulation

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Snakes and primates share a long evolutionary existence, with snakes being the first of the major predators of primates (Isbell, 2009). Natural selection may have then fostered primates whose detection of snakes allowed a better defensive behavior. In line with this idea, recent studies have shown that humans are remarkable snake detectors: In visual search tasks, adults and children are faster to detect a picture of a snake in an array of flower pictures than vice versa (e.g., LoBue & DeLoache, 2008; Öhman, Flykt, & Esteves, 2001). These findings have been taken as evidence for the existence of a fear module that could have evolved in mammals' brain to assist them in responding adequately to recurrent survival threat (Öhman & Mineka, 2003). A stronger and more decisive argument for an inborn threat detection system (that would allow a rapid detection of snakes in particular) would come from infants who, as a matter of fact, do not know about the potential dangerousness of snakes. A few recent studies revealed, by examining infants' looking behaviors when presented with pictures of snakes, enhanced visual detection of snakes and preferential attentional allocation to these stimuli, as well as attentional broadening resulting from the detection of these reptiles (Bertels, Bayard, Floccia, & Destrebecqz, submitted; DeLoache & LoBue, 2009; LoBue & DeLoache, 2010). These findings may suggest an inborn mechanism for the rapid detection of snakes that most probably rely on the identification of low-level perceptual features, such as their coiled shape (Rakison & Derringer, 2008). We do not know yet whether the detection of these perceptual features allows infants to discriminate snakes from other animals and to generalize across snakes despite their lack of experience with these reptiles. The neural bases of such discrimination and/or generalization also remain unknown. To clarify this issue, we used a fast periodic visual stimulation approach in the context of an oddball paradigm in which we presented base and oddball stimuli at two different periodic frequencies (Liu-Shuang et al., 2014; Rossion et al., 2014). Periodic stimulation is known to generate steady-state visual evoked potentials in participants' brain that have the same fundamental frequency as the driving stimulus. When two frequencies are used, the brain will generate two periodic responses but only if it differentiates between base and oddball stimuli. This approach can therefore provide an implicit, objective, predictive and robust measure of stimulus categorization. It has recently proven its efficacy in infants with complex

visual stimuli (de Heering & Rossion, 2015). We recorded scalp electrical brain activity using a 32-channel Biosemi system in 7- to 13-month-old infants (N=15; 10 females). Infants were presented with 20-second sequences of pictures flickering at 6 Hz (i.e., 6 images per second, see Figure 1). A stimulation sequence contained repetitive series of four pictures of various animals in their natural background followed, every fifth stimulus, by a picture of a snake (in the 'snake' sequences), or by a picture of a frog (in the 'frog' sequences). The oddball stimulation frequency was therefore of 1.2 Hz (6 Hz/5). Within a sequence, all pictures were different and highly variable. They were however equalized in terms of contrast and luminance across the whole set. Snake and frog sequences were presented in alternation. Half of the infants were presented first with a snake sequence, the other half with a frog sequence. Each infant saw as many sequences as possible, with an average of 13 sequences per infant (min = 8, max = 18). EEG trials were averaged separately for each infant and Fourier transformed into the frequency domain. A sharp response was found over the medial occipital lobe exactly at the base stimulation frequency (6 Hz) in snake and frog sequences (averaged signal-to-noise ratio (SNR) at O1 in snake and frog sequences: 5.84 and 5.28, respectively, see Figure 2, left panel), demonstrating successful synchronization of the infants' brain to the visual stimulation. Crucially, there was a distinct response at the oddball frequency (1.2 Hz) in snake sequences, more pronounced at the left occipital electrode O1 (averaged SNR at O1: 1.63; see Figure 2, right panel). No significant response was observed at the oddball stimulation frequency in frog sequences. These results reveal infant's particular brain sensitivity to snakes. Specifically, they show that infants discriminate snakes from other animal images, and that they generalize across snake images despite their perceptual variance. We did not find similar results with frogs. We would therefore suggest that infants' brain considers snakes as belonging to a specific animal category although they do not have any experience or knowledge about these reptiles. Interestingly, the localization of the effect on the scalp suggests that it is driven by low-level rather than high-level visual areas. In infants, snake categorization might therefore be more perceptual than semantic, namely based on the detection of salient perceptual features shared by snakes such as their coiled shape. These findings go along with the idea that humans have an inborn predisposition to detect snakes rapidly, based on their perceptual features (Rakison & Derringer, 2008).

Résumé en français : Au cours de l'évolution, les êtres humains auraient développé une prédisposition innée à détecter les serpents particulièrement rapidement, ceux-ci étant un de nos plus anciens prédateurs. En accord avec cette idée, notre étude montre, chez des nourrissons de moins d'un an (sans expérience ni connaissance de la dangerosité potentielle de ces animaux), des réponses cérébrales liées à la présentation d'images de serpents, mais pas de grenouilles, lorsque celles-ci sont présentées parmi des images d'autres animaux. La localisation de ces réponses suggère que les nourrissons détectent les traits perceptifs saillants des serpents (comme leur forme enroulée).

Samenvatting in het Nederlands: In de loop van de evolutie zou de mens een natuurlijke aanleg ontwikkeld hebben om bijzonder snel slangen, onze oudste roofdieren, op te merken. Uitgaande van dit idee, toont onze studie aan dat bij zuigelingen van minder dan één jaar (die dus noch kennis noch ervaring kunnen hebben betreffende het gevaar dat deze dieren vormen) reacties in de hersenen voorkomen wanneer tussen afbeeldingen van andere dieren afbeeldingen van slangen getoond worden, en helemaal niet bij het tonen van afbeeldingen van kikkers, De lokalisatie van deze reacties suggereert dat de baby's de opvallende kenmerken van de slangen (zoals het opgerold zijn) herkennen.

**Keywords: Infancy, Snakes, SSVEPs, oddball paradigm, Categorization, threat detection**

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# Does maternal immune activation lead to neuropsychiatric disorders through altered brain development?

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Several studies have indicated that inflammation during pregnancy increases the risk for the development of neuropsychiatric disorders like autism and schizophrenia in the offspring. Morphological brain abnormalities and deviations in immunity can be observed in patients of both disorders. It has been suggested that the acute infection induces changes in maternal cytokine levels which in turn affects the fetal brain and results in the development of both neuropsychiatric disorders in the offspring. In this study, the poly (I:C) model was used to mimic viral immune activation in pregnant mice in order to study the effect of the maternal infection on the developing brain. We injected pregnant mice with poly (I:C) (i.p., 20 mg/kg) on E11.5 and/or E15.5. The concentration of IL-6 in the maternal serum was used as a measure for systemic inflammation in the mother. To investigate the effect of the maternal inflammation on embryonic microglia, the microglial cell density and activation level (Mac-2, iNOS and IL1β immunostainings) in the cortex and hippocampus of CX3CR1-eGFP +/- embryos was determined. For evaluation of the effect on developing pyramidal neurons, neuronal progenitors were fluorescently labeled using in utero electroporation and positioning in the cortical plate was analyzed.

Résumé en français: Plusieurs études ont montré qu'une infection contractée pendant la grossesse augmentait le risque pour l'enfant de développer des troubles neuropsychiatriques – autisme et schizophrénie notamment. L'hypothèse est qu'une infection entraîne des modifications des facteurs d'immunité de la mère qui, à leur tour, entraînent des mutations dans le cerveau en développement du fœtus. Dans cette étude nous avons mimé une infection virale chez des souris gravides afin d'en étudier l'effet sur les différentes cellules du cerveau.

Samenvatting in het Nederlands Verschillende studies hebben aangetoond dat infectie tijdens de zwangerschap het risico op het ontwikkelen van neuropsychiatrische stoornissen, waaronder autisme en schizofrenie, bij het kind kan verhogen. Er wordt geloofd dat een infectie veranderingen teweeg brengt in immuun factoren van de moeder die op hun beurt veranderingen teweeg brengen in het ontwikkelende brein van de foetus. In deze studie bootsen we een virale infectie na bij zwangere muizen om te onderzoeken wat het effect hiervan is op de ontwikkeling van de verschillende cellen in de hersenen.

**Keywords: maternal immune activation, neuropsychiatric disorders, Brain Development, Cortex, Microglia**

# SVZ-nested GBM-initiating cells are resistant to radiotherapy treatment, due to mesenchymal activation by SVZ-released CXCL12

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Summary This study shows that Glioblastoma (GBM) cells which migrate from the tumor mass to the Subventricular Zone (SVZ) acquire stem features. This acquisition is in fact induced by a chemokine, CXCL12, which is essentially founded in SVZ. CXCL12 is able to induce a transition from an epithelial state to a mesenchymal state, known as Epithelio-Mesenchymal Transition (EMT), via its receptor CXCR4. We finally showed that this transition is associated to radioresistance and that radiosensitization of GBM cells, nested in the SVZ, could be induced by AMD 3100, an antagonist of CXCR4. Introduction Patients with glioblastoma multiforme (GBM) have an overall median survival of 15 months despite multimodal therapy, due to systematic relapses. We previously demonstrated that GBM-initiating cells (GIC) are able to escape the tumor mass and specifically colonize the sub-ventricular zone (SVZ – one of the two neurogenic zone in adult brain, including in humans) after experimental striatal xenotransplantation. We also demonstrated that this specific and oriented migration of GIC is a consequence of the release of the CXCL12 cytokine by SVZ and that GIC expressed CXCR4, the CXCL12 receptor. Using the same approach, we demonstrated in vivo a higher survival rate of SVZ-nested GIC after irradiation and investigated the pathway implied. Materials and Methods We collected conditioned media (CM) of the SVZ from nude mice and from one human brain. We used three human GBM primary cultures and one human GBM cell line for in vitro radiotherapy tests, with or without exposition to SVZ-CM or human recombinant CXCL12. We performed Western Blot and quantitative RT-PCR after stimulation of human GBM primary culture and cell line by CXCL12, with or without the use of AMD 3100, the antagonist of CXCR4. Finally, we tested in vivo a potential radiosensitization of SVZ-nested GBM cells by AMD 3100. Results Exposition to human and mice SVZ-CM and to CXCL12, present in SVZ-CM, induces radioresistance in vitro, quantified by clonogenic assays, γH2AX and 53bp1 immunostainings. The use of CXCL12 blocking antibodies in SVZ-CM sensitizes GBM cells to irradiation in vitro. We report here that CXCL12 promotes in vitro the mesenchymal activation of GBM cells quantified by N-cadherin and vimentin expression. In vivo, SVZ-nested GBM cells exhibit also those mesenchymal markers.

Finally, we showed in vivo the radiosensitization of SVZ-nested GBM cells by a concomitant AMD3100 administration. Conclusion Mesenchymal activation of GICs, mediated by CXCL12 of the SVZ, promotes their resistance to radiotherapy. Therefore, we propose that a modulation of the CXCL12 signalization could be useful during radiotherapy in order to decrease the risk of the deadly GBM relapses.

Résumé en français: Le glioblastome est une tumeur dans laquelle on trouve des cellules mobiles et résistantes à la radiothérapie ce qui permet leur diffusion hors de la tumeur. Dans ce travail, nous montrons que ces cellules acquièrent cette radiorésistance par une transition vers un stade de cellules souches générées par une chemokine* la CXCL12. Cette chemokine est produite par les cellules épithéliales voisines. Pour exercer sa fonction la CXCL12 doit interagir avec un récepteur présent sur les cellules du glioblastome, le CXCR4. Nous avons aussi montré qu'en faisant agir un antagoniste qui bloque le récepteur CXCR4, les cellules perdent cette radiorésistance et redeviennent sensibles à la radiothérapie.
*Chemokine : facteur de différenciation de cellules, permettant notamment l'acquisition par la cellule stimulée de mobilité.

Samenvatting in het Nederlands: De gliobastoom of glioom is een tumor waarin radiotherapie-resistente mobiele cellen aanwezig zijn, waardoor ze ook buiten de tumor verspreid worden. In dit werk tonen we aan dat deze cellen resistent worden voor radiotherapie door omvorming naar een stadium van stamcellen die gegenereerd worden door een chemokine*: CXCL12. Dit chemokine wordt geproduceerd door de nabije epitheelcellen (dekweefsel). Om te functioneren moet de CXCL12 in interactie treden met een receptor aanwezig in het glioom, het CXCR4. We toonden ook aan dat het activeren van een antagonist die de CXCR4 blokkeert, de cellen hun radiotherapieresistentie verliezen, en opnieuw reageren op de radiotherapie.
* Chemokine is een factor die cellen differentieert, en die de gestimuleerde molecule mobiel maakt.

**Keywords: Glioblastoma stem cells, radioresistance, Epithelio-Mesenchymal Transition, CXCL12, suventricular zone**

# Attentional engagement towards disgusted faces in socially anxious children: an ERP study

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Social anxiety disorder (SAD) is the most common psychopathological condition in children [1,2]. Attentional biases (AB) have been highlighted in SAD and are characterized by an enhanced attentional engagement towards threatening stimuli [3,4]. The attention control theory (ACT) associates these biases to a deficit of attentional control leading to an imbalance between the engagement towards aversive stimuli and the disengagement from these. The aim of this study was to attest this deficit in SAD children. We compared 20 SAD children to 20 controls (age 8 – 12) using a modified dot-probe task in which participants had to detect a probe following an emotional (disgusted/happy) or a neutral face. ERPs components were recorded throughout the task. We hypothesized that SAD children would produce shorter reactions times for disgusted faces and larger P100 amplitudes an enhanced attentional engagement towards these stimuli. Results will be presented and we will discuss the applicability of the ACT in pediatric social anxiety.

Résumé en français: Titre: L'engagement attentionnel envers les visages exprimant le dégout chez les enfants souffrant d'anxiété sociale: une étude électro-physiologique. L'anxiété sociale résulte d'une peur persistante d'être observé par les autres et de se retrouver dans une situation embarrassante ou humiliante. Ce désordre est associé au fait que l'attention se porte de manière trop importante vers les informations à caractère menaçant. Notre cerveau possède un système de contrôle de l'attention dont l'activité peut être mesurée grâce à l'électro-encéphalographie. Dans ce travail, nous avons étudié l'activité cérébrale d'enfants atteints d'anxiété sociale et d'enfants contrôles pendant une tâche consistant à repérer une cible après avoir eu l'attention attirée par un visage neutre ou menaçant. Lorsque l'attention vers le visage menaçant est trop importante, la cible est moins vite repérée.

Samenvatting in het Nederlands: Titel: Het aandacht schenken aan gelaatsuitdrukkingen die afschuw uitdrukken bij kinderen die lijden aan een sociale angststoornis, een elektrofysiologische studie. De sociale angststoornis is het resultaat van een

doorgedreven angst om door andere geobserveerd te worden en zich daardoor in een vervelende of vernederende situatie te bevinden. Deze stoornis heeft te maken met het feit dat de aandacht teveel gericht wordt op bedreigende informatie. Onze hersenen beschikken over een controlesysteem waarvan de activiteit kan gemeten worden met elektro-encefalografie. In dit werk hebben we de hersenactiviteit bestudeerd bij kinderen met een sociale angststoornis en een controlegroep van kinderen tijdens het uitvoeren van een taak die erin bestond een doel op te sporen nadat hun aandacht gevestigd werd op een dreigend dan wel een neutraal gezicht. Nadat de aandacht voor een bedreigend gelaat belangrijker was, werd het vinden van het doel moeilijker.

**Keywords: Social Anxiety Disorder, Children, Attentional control theory, Attention bias, dot probe paradigm**

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# Subcortical involvement in body and mental action verb processing: an electrophysiological registration study

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Electrophysiological registration of semantic processing generally involves paradigms eliciting semantic violations on recognition memory or semantic judgement tasks. The N400, LAN (left anterior negativity) and P600 may be sensitive to semantic judgement alterations on cortical level (Olichney et al., 2008). The LAN is a negative-going peak around 400 ms after word presentation and is typically elicited by morpho-syntactic violations (Coulson 1998). In the context of semantic processing, P600 components have been observed in response to thematic and other semantic violations (Kuperberg 2003; Kim 2005). Although semantic effects of subcortical modulation have been well described, it is unclear if semantic related local field potentials can be elicited in the main subcortical nuclei. Direct registration of language elicited EEG in the deep brain nuclei is only possible in patients recruited for deep brain stimulation as a treatment for their illness and in the short period after the operation that the electrode leads are still externalized. The current research project focusses on the electrophysiological registration of semantics (body versus mental action verbs) within the thalamus, subthalamic nucleus (STN) and the pedunculopontine nucleus (PPN) within 1 week after DBS-implantation. Methods Patients The current study included 18 patients with deep brain electrodes within the STN (mean age 59 (45-71); 8 male/10 female), 2 patients with electrodes in the thalamus (mean age 64 (56-73)/1 male/1 female) and 1 male patient with PPN stimulation (age 50). The difference in number of patients corresponds with the prevalence of indications for DBS in the course of two years in our centre. All patients were right-handed and testing was performed with and without medication. Paradigms The event-related potential (ERP)-paradigms consisted of 30 body action verbs (e.g. to sew, to point) and 30 mental action verbs without manual action connotation (e.g. to leave, to develop). All verbs consisted of two syllables, matched with respect to word form frequency, and imageability (Duyck 2004). The body action and mental action verbs were shown consecutively in a randomized order, with a stimulus duration of 1s and without interstimulus interval. Materials An in-house made interface between the registration device (Neurosoft) and the STN/thalamus/PPN leads was connected to the bilateral (temporarily) externalized DBS-contacts. The leads are numbered from 0 to 3, with 0 as the most distal and 3 as the most proximal contact.

Data was collected using a 32-channel SynAmp (Neuroscan) amplifier. EEG analysis (ERP analysis and source localizing) was performed in all nuclei using BrainVision Analyzer 2 (Brain Products, Munich, Germany). ERP data were analysed using linear mixed models. All statistical analyses were performed using IBM SPSS Statistics 23. Results Within the left STN a source waveform for both body and mental action verbs is demonstrated in a time frame between 150 and 200ms post-stimulus at L0-L1/L1-L2 and L1-L2/L2-L3 (Fig. 1). Significantly higher amplitudes were elicited by body action verbs compared to mental action verbs. In the body action verbs a significant increase of the negative peak amplitude and a decrease of peak latency can be seen after dopaminergic administration, while no changes can be observed in peak amplitude and latency detection in mental action verbs. Within the PPN, bipolar analysis points to a clear source waveform in the right hemisphere in the OFF condition for making the 'difference between body and action verbs'. This source waveform can be seen between R0-R1 and R1-R2 in a time frame between 250ms and 450ms (Fig. 2). Within the thalamus a negative peak and clear lateralization effect in the left hemisphere (L1-L2/L2-L3) can be found with both stimuli (body and mental action verbs) and in a time window between 200-800ms (Fig. 3). In making the 'difference between both body and mental action verbs' a positive peak can be demonstrated between 200-500ms. Discussion This study reports on the involvement of the STN, PPN and thalamus in semantic processing. Direct registration of EEG activity within these three nuclei allows to bring the temporal characteristics of semantic processing into focus. The results indicate that the three grey deep brain nuclei are involved in semantic processing in a different way. Where the thalamus suggests a significant left lateralized activity between 200-500ms during 'making the difference between body and mental action verbs', the PPN seems to be activated for the same time window and demands in the right hemisphere. The lateralization difference suggests an involvement of the PPN in the decision aspect (which is a well-known function of the PPN) in making a semantic differentiation than in semantic processing itself (Gut 2016). The time window occurs prior to the cortical semantic processing (300-600ms). The STN demonstrates a source waveform for both body and mental action verbs in a time frame between 150 and 200ms, which suggests a language modality aspecific ('tool use' in this semantic tasks) involvement of the STN in semantic processing. The language modality specific and aspecific demands are both under the influence of dopamine. The current results and methodology encourages new research on the involvement of subcortical grey and white matter in language specific and aspecific language modalities.

Résumé en français: L'objectif de cette recherche est de créer ou de modifier des modèles de perception et de compréhension de la langue à partir de l'enregistrement de l'activité des noyaux cérébraux profonds chez des humains. Le but ultime étant d'utiliser ces modèles pour mieux comprendre les troubles de langage observés dans différents groupes de patients. L'examen direct des noyaux cérébraux profonds (le thalamus,

le noyau sous-thalamique et le noyau pédonculopontin) n'a été possible qu'avec l'aide de patients qui étaient traités par une stimulation cérébrale profonde.

Samenvatting in het Nederlands: De doelstelling van het overkoepelende project is het opstellen en/of modificeren van modellen van perceptie en taalbegrip, waarin de activiteit van de diepe hersenkernen wordt betrokken. Deze modellen kunnen dan aangewend worden om stoornissen bij verschillende patiëntengroepen beter te begrijpen. Direct onderzoek van de diepe hersenkernen is enkel mogelijk bij patiënten die diepe hersenstimulatie hebben ondergaan, de onderzoeksgroep is op dit vlak een pionier. Deze specifieke studie onderzocht de rol van diepe hersenkernen in de semantische verwerking van verschillende werkwoordtypes.

**Keywords: EEG, Deep Brain Stimulation, Language, semantics, Subthalamic Nucleus, Thalamus, pedunculopontine nucleus, event-related potential (ERP), Dopamine Agonists, Parkinson Disease**

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# Involvement of striatal interneuron populations in locomotion, limbic functions and drug addiction

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The basal ganglia system is made of interconnected subcortical nuclei involved in voluntary movement, motivational processes and some cognitive processes. Such a system is made of four main nuclei including the striatum. The striatum is composed of more than 90% of projecting GABAergic Medium-sized Spiny Neurons (MSN). The rest is made of interneurons providing a strong control on MSN or other interneurons excitability. The role of these interneurons in the striatum is still quite unknown. Regarding the major role of the striatum in those functions, the purpose of this project is to understand the involvement of population of interneurons in locomotor activity, limbic functions and drug addiction, by specifically targeting them in vivo. This will be done by performing ablation or by controlling the activity of these neuronal populations. For this purpose, the initial step of this project consisted in the characterization of a first BAC Cre recombinase driver line, in order to assure its specificity. Now, the phenotypical characterization of this line, where this specific neuronal population was ablated, is on-going by using different behavioural test.

Résumé en français: Titre: Implication de populations d'interneurones striataux dans la locomotion, les fonctions limbiques et la dépendance aux drogues. Cette recherche a pour but d'élucider le rôle d'une sous-population de neurones dans le striatum, région du cerveau impliquée dans le circuit de la récompense et la locomotion. Cette étude est réalisée grâce à une série de techniques permettant l'ablation, l'activation ou l'inactivation de cette population de neurones et ce grâce à une lignée de souris permettant de spécifiquement la cibler. L'effet comportemental de la perte de ces neurones dans le striatum est en cours.

Samenvatting in het Nederlands: Titel: Invloed van populaties van striatale interneuronen op de beweging, de limbische functies en de drugverslaving. Dit onderzoek heeft tot doel de rol te verduidelijken van een sub populatie in het striatum, het hersengebied dat bij beloning en beweging betrokken is. Deze studie kwam to stand dankzij een reeks technieken die toelieten deze neuronengroep te verwijderen, te activeren of te deactiveren en dit bij een nakomelingschap van muizen die toeliet zich daar specifiek op te richten. Het gedragsmatig effect van het verlies van deze neuronen in het striatum is aan de gang.

**Keywords: Striatum, Interneurons, Locomotion, limbic functions, drug addiction**

# Characterization of striatal afferents by monosynaptic retrograde tracing

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The basal ganglia system is composed of a set of subcortical nuclei mainly involved in the control and motor learning, but also in the reward system as well as emotions. Various neurodegenerative diseases lead to a dysfunction of this system. The largest and entry nucleus in the basal ganglia is the striatum. All striatal projection neurons are inhibitory GABAergic neurons, known as "medium spiny neurons" (MSNs). They are divided into two subtypes, striatopallidal and striatonigral neurons. These two neuronal populations can be targeted using transgenic mouse lines expressing Cre-recombinase in 2 types of striatal neurons (respectively A2A-Cre and D1-Cre). From a functional point of view, the striatum may be divided into a ventral and a dorsal portion. The first is mainly involved in motivational and rewarding processes as well as drug addiction. The latter is more implicated in the control of locomotor activity and habits learning. The dorsal striatum can be divided into an outer portion called dorsolateral striatum (DLS) and an inner portion, dorsomedial striatum (DMS). Here we used a combinatorial viral approach with engineered rabies virus glycoproteins to monosynaptically highlight the afferents to these neurons in regard of striatal subdivisions.

Résumé en français: Le système des neurones appelés ganglions de la base est principalement impliqué dans le contrôle et l'apprentissage moteur mais également dans le système de la récompense et des émotions. De nombreuses maladies neurodégénératives entrainent un dysfonctionnement de ce système. La structure d'entrée la plus importante de ce système des noyaux de la base est le striatum. Par l'utilisation d'une approche virale utilisant des virus de la rage modifiés, il a été possible de mettre en évidence les afférences (entées) au striatum provenant du thalamus et du cortex en fonction des subdivisions du striatum.

Samenvatting in het Nederlands : Het neuronensysteem genaamd basale glangia(of basale kernen) is vooral betrokken bij de controle en het aanleren van de motoriek maar eveneens bij emoties en beloning. Verschillende neurodegeneratieve aandoeningen vervlakken dit systeem. De belangrijkste input van deze basale kernen vindt plaats via het striatium. Door gebruik te maken van een virale aanpak waarbij het rabiësvirus

(rabiës = hondsdolheid) gebezigd wordt, is het mogelijk de betrokkenheid van het striatum in dit inputsysteem, vanuit de thalamus en de hersenschors, te markeren in functie van de onderverdelingen van het striatum (het striatum is opgebouwd uit verschillende delen).

**Keywords: Striatum, tracing, Rabies virus, Neuroanatomy, neural circuits, Medium Spiny Neuron**

# Involvement of efferent striatal neurons in sexual behavior

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Basal ganglia form a system of subcortical nuclei critically involved in motor control and motivational processes. The main input structure of this system is the striatum that is subdivided in a dorsal and a ventral part. The ventral striatum, also called nucleus accumbens (NAc), is essential for motivation and reward processes. This structure has been extensively studied for its function in drug reward and reinforcement but is also implied in natural reward processes such as feeding and sex. The striatum is mainly composed of GABAergic medium spiny neurons (MSNs), divided in two efferent neuronal populations, the striatopallidal and striatonigral neurons, which are equal in shape and number, mosaically distributed throughout the striatum and having a motor and reward antagonist effect. Many studies have been published past years about the involvement of the NAc on sexual behavior and have shown the facilitating role of this structure on male rat sexual behavior. Nevertheless, none of these studies targeted one of the specific neuronal populations in the NAc because of the lack of techniques allowing it. To address this problem, our laboratory generates transgenic mice expressing the Cre recombinase in specific striatal neurons population along with an inducible diphtheria toxin receptor. By performing stereotaxic diphtheria toxin injection, this system allows selective ablation of these neurons in the NAc. The aim of this study is to get a better understanding of the mechanisms involved in sexual behavior by using selective ablation of MSNs in ventral striatum.

Résumé en français: Implication des neurones du striatum dans le comportement sexuel. Le striatum, principale structure d'entrée des ganglions de la base, est impliqué dans l'apprentissage d'une tâche motrice ou d'une habitude. Il est également impliqué dans le système de la récompense et dans le contrôle des processus motivationnels. Le dysfonctionnement du striatum est à l'origine de pathologies telles que la maladie de Parkinson ou la dépendance aux drogues. Nous avons décidé d'étudier les rôles des populations neuronales du striatum dans le comportement sexuel, un comportement qui présente une composante motrice et motivationnelle, pour potentiellement éclaircir les causes de troubles sexuels.

Samenvatting in het Nederlands: Het belang van striatale neuronen bij seksueel gedrag. Het striatum, de voornaamste connectie van de basale ganglia, is betrokken

bij taakgerelateerde en automatische motoriek. Deze anatomische structuur is tevens geïmpliceerd bij belonings- en motivatieprocessen. Een verstoorde striatale werking uit zich o.a. in de ziekte van Parkinson en afhankelijkheid van genotsmiddelen. Wij hebben hier de rol van de striatale neuronen onderzocht bij seksueel gedrag, welke zowel motorische als motivatie componenten bevat. Dit zou mogelijks afwijkingen in seksueel gedrag kunnen verklaren.

**Keywords: Striatum, Sexual Behavior, Nucleus Accumbens, male mice, Medium Spiny Neuron**

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# Functional role of new striatopallidal or striatonigral specific genes in motor and motivational behavior

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Basal ganglia are a set of interconnected nuclei involved in motor control and motivation. The striatum is the main input of basal ganglia and is mainly composed of medium spiny neurons, subdivided into striatopallidal (STP) and striatonigral (STN) neurons. STP and STN neurons give rise respectively to the indirect and the direct pathways of basal ganglia, with opposite effects at both motor and motivational levels. Cellular mechanisms involving these pathways in disorders such as Huntington's and Parkinson's diseases and addiction, are still poorly understood. Our laboratory has previously identified gene expression profiles of STN and STP neurons using microarray (Ena et al., 2013). Our project consists in the study of STP or STN specific genes function in locomotor control and addiction behavior. We selected several genes according to their potential involvement in striatal pathways. Specific expression of these genes in STP or STN neurons has been validated by qPCR, in situ hybridization and/or immunofluorescence experiments. Finally, knockdown models showing specific repression in STP or STN pathway have been generated using floxed mice or shRNA interference mediated by lentivirus. Phenotypic analysis is then achieved through behavioral tests to assess the effect of gene deletion.

Résumé en français: Le striatum, une structure située sous le cortex du cerveau, est composé principalement de neurones épineux de taille moyenne (MSNs), subdivisés en 2 groupes de neurones : les striatopallidaux (STP) et les striatonigraux (STN). Ces deux populations neuronales sont respectivement à l'origine des voies indirecte (ou inhibitrice) et directe (ou activatrice) des noyaux de la base, présentant des effets opposés à la fois au niveau moteur et motivationnel. Le projet vise à identifier et étudier la fonction de gènes spécifiques de l'une ou l'autre de ces populations neuronales, impliquées dans différentes pathologies, telles que les maladies de Huntington et de Parkinson ou encore l'addiction.

Samenvatting in het Nederlands: Het striatum, een structuur die zich in de hersenen onder de hersenschors bevindt is hoofdzakelijk samengesteld uit middelgrote stekelige neuronen (MSNs), onderverdeeld in 2 groepen: de striatopaliden (STP) en de striatonigralen (STN). Deze twee neuronenfamilies liggen aan de bron van respectievelijk de

indirecte (of afremmende) en de directe (activerende) doorgangen van de knooppunten van basale cellen, met tegengestelde effecten enerzijds op motorisch of motivationeel niveau. Het project is gericht op het identificeren en bestuderen van de werking van de genen eigen aan de ene of de andere neuronenfamilie, betrokken bij verschillende ziekten, zoals de ziekte van Huntington, de ziekte van Parkinson of ook verslaving.

**Keywords: Striatum, striatonigral pathway, striatopallidal pathway, cell-type-specific gene, knockdown model**

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# Role of striatonigral and striatopallidal neurons in decision-making

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Decision-making is necessary to adapt to variable environment. The underlying cognitive and emotional mechanisms in this phenomenon depend, among others, on the striatum, basal ganglia's main structure. The striatum is divided in the dorsal striatum, responsible for motor and cognitive control and the ventral striatum which manages reward and the influence of motivation. However, the function of the different striatal neuronal populations is still not clear in decision-making. We propose to study the role of the striatum in these processes by means of behavioral tests simulating real-life decision-making and pharmacogenetics.

Résumé en français: La prise de décision est indispensable pour s'adapter à l'environnement variable. Ce phénomène est pourtant perturbé dans de nombreuses maladies neuropsychiatriques (comme la dépression ou la schizophrénie) ainsi que la dépendance aux drogues. Le Striatum est l'une des structures cérébrales qui est responsable des processus de prise de décision, mais le rôle précis des différentes populations neuronales dans chacune des sous-régions striatales est peu connu dans les processus décisionnels. Pour mieux comprendre leur rôle nous utilisons différents tests comportementaux simulant les prises de décision en activant ou inhibant ces populations grâce à des modèles murins.

Samenvatting in het Nederlands: Het nemen van beslissingen is onontbeerlijk om zich aan een wisselende omgeving aan te passen. Bij heel wat psychiatrische ziektebeelden (schizofrenie, depressie,..) en bij verslaving is dit fenomeen verstoord. Het striatum is een van de hersenstructuren die instaat voor het nemen van beslissingen, maar over de precieze rol van de verschillende neuronengroepen in elk van de sub regionen van het striatum in het beslissingsproces is weinig bekend. Om hun rol beter te begrijpen maken we gebruik van diverse gedragstesten bij muizen die het beslissen simuleren door activering of door afremming van deze populaties.

**Keywords: Decision-making and learning, Striatum, Medium spiny neurons, Behavior, circuitry**

# Depletion of the glycine alpha 2 receptor promotes reward-related behaviors

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The striatum integrates cortical sensory information with motivational inputs originating in the ventral tegmental area. This integration allows for adequate goal-directed behavior, and impaired neurotransmission can induce reward-related psychopathologies such as drug abuse and psychosis. Here, we investigated the role of the glycine alpha 2 receptor (GlyRa2) in reward-related behaviors. Glycine receptors are pentameric ionotropic receptors that are composed of alpha (a1-a4) and/or beta subunits that form an ionic pore permeable to chloride. During development, most GlyRs are homomeric, composed of alpha 2 subunits. Expression of alpha 2 rapidly declines postnatally, suggesting its importance during early development. Indeed, we have shown proliferation and migration defects in layer V of the cortex, an area that provides input to the striatum. Interestingly, and in contrast to the idea of a restricted role in development, we have recently identified GlyRa2 as the only functional glycine receptor in adult striatum. We therefore investigated striatum-mediated behavior in GlyRa2 KO mice. We show a sensitized response to amphetamine (5mg/kg, i.p.) in GlyRa2 KO mice in a horizontal locomotor task, yet no changes in baseline locomotor activity. Accordingly, we exhibit increased nose poke behavior in an appetitive conditioning task that is only apparent at higher order reward schedules, which indicates altered motivation. Yet, GlyRa2 KO animals reveal greater extinction on the first extinction trial. In order to elucidate whether enhanced nose poking indeed reflected enhanced motivation, we performed a T-maze task. We found increased motivation, enhanced associative learning, but no changes in the hedonic response. We conclude that GlyRa2 plays a major role in striatal reward-related behavior. These processes are implicated in major psychopathologies, including schizophrenia and drug abuse, and modulation thereof can prove to be highly beneficial towards the development of targeted treatment. The present research demonstrates a crucial role for the glycine alpha 2 receptor in processes that are disrupted in severe pathologies such as schizophrenia and drug abuse. While it was long thought that this receptor was transiently expressed during development, we now know that it is the only functional glycine receptor in brain areas that are key to the development of these pathologies. We thus reveal the glycine alpha 2 receptor as a novel, promising target in striatum-dependent pathologies.

Résumé en français: Le système de récompense passe par un réseau de neurones du striatum, région du cerveau impliquée dans le circuit de la récompense. Cette

communication passe par un réseau de contacts (synapses) entre neurones qui utilisent des récepteurs pour cette transmission. Dans ce travail, en utilisant des tests comportementaux, nous montrons qu'un des récepteurs, appelé « Récepteur a2 à la Glycine» joue un rôle important dans le comportement lié à la récompense. Sachant que des altérations de cette transmission sont à la base de pathologies comme l'addiction et les psychoses, on peut estimer que la découverte de nouvelles drogues ciblant ce récepteur constitue une nouvelle approche thérapeutique.

Samenvatting in het Nederlands: Het striatum is een structuur in de hersenen die een cruciale rol speelt in beloningsleren, en verstoorde informatieoverdracht hier kan leiden tot drugverslaving en psychose. Belangrijke modulatoren van de informatieoverdracht zijn neurotransmitter receptoren. Wij hebben de rol onderzocht van een dergelijke receptor, GlyRa2. Lang werd gedacht dat deze receptor enkel voorkwam tijdens de ontwikkeling. Nu weten we echter dat in het striatum, GlyRa2 ook op volwassen leeftijd een belangrijke rol speelt. In ons onderzoek tonen we dat beloningsleren inderdaad is aangetast in muizen waarin deze receptor ontbreekt. GlyRa2 belooft dus een interessante therapeutische target te zijn voor striatum-afhankelijke psychopathologie.

**Keywords: Striatum, Reward, glycine receptor, Schizophrenia, Behavior, Animal**

# Cognitive-emotions deficits and sex offending: Preliminary meta-analysis data

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Our master thesis consists on a meta-analysis concerning the effects of emotion and empathy deficits on sexual official offences. Our analyses included both self-reports and checklists measures of emotions. We assessed the discriminant validity of empathy and emotion deficits between adult and child victim sex offenders. We also compared the differential effects of cognitive and emotional empathy factors. We considered specific effects of fundamental positive and negative emotions. The results are discussed in light of literature on emotion responding among sex offenders and on current Risk-Need-Responsivity (Andrew & Bonta, 2015) versus "Good Life" (Ward & Beech, 2006) dominant therapy models.

Résumé en français: Titre: Analyse psychologique des délinquants sexuels. Afin d'améliorer l'efficacité des thérapies appliquées aux délinquants sexuels, il est important de mieux comprendre leurs déficits dans le traitement des émotions et de l'empathie. Ce travail vise à mesurer la capacité d'empathie et de traitement des informations émotionnelles par les délinquants sexuels.

Samenvatting in het Nederlands : Titel: Psychologische analyse van seksuele delinquenten. Om de doeltreffendheid van behandeling van seksuele delinquenten te verbeteren is het van belang te begrijpen waar het misloopt in de aanpak van emoties en empathie. Dit werk is gericht op het meten van de bekwaamheid tot empathie en het verwerken van emotionele informatie door seksuele delinquenten.

**Keywords: Sex Offenders, Emotions, Empathy, Emotions Deficit, Empathy Deficit, Adult victim, Child victim**

# Cue reactivity modulates proactive and reactive motor response inhibition in frequent gamblers

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We used functional magnetic resonance imaging to examine whether motivationally salient cues could impact differently on proactive (the restrain of actions in preparation for stopping) and reactive (outright stopping) inhibition. Seventeen high-frequent poker gamblers and 16 matched non-gambler controls performed a modified version of the stop-signal paradigm that required participants to inhibit categorizing poker or neutral pictures. The probability that a stop-signal occurs was manipulated across blocks of trials, as indicated by the color of the back screen. First, we observed that, when viewing poker cues versus neutral cues, gamblers showed higher brain cue-related reactivity than controls, including the frontal orbital cortex, insula, and amygdala. Second, gamblers showed reduced proactive inhibition, as compared to controls. Moreover, in gamblers, proactive inhibition towards neutral cues was associated with lower brain activation in the middle and superior frontal gyrus. Third, gamblers did not differ from controls on behavioral indices of reactive inhibition. Nevertheless, during reactive inhibition of poker pictures, gamblers exhibited higher brain activation in the medial, middle, and superior frontal gyrus. Taken together, these results suggest that gamblers rely more on reactive than proactive control in order to inhibit motor response involving salient-gambling cues. Implications of present findings are discussed.

Short abstract: This study highlighted that, at both neural and behavioral levels, high-frequent gamblers rely more on reactive (the restrain of actions in preparation for stopping) than proactive (a "late correction" process that results in the stopping of the ongoing action) control in order to inhibit their motor response toward salient-gambling cues. These findings confirm that investigating both reactive and proactive inhibition offer a fine-grained analysis of inhibitory control, and that fMRI is a good input for studying the dynamic underlining these two processes. This approach could also advance our knowledge on humans' ability to control hedonic habits.

Résumé en français: Cette étude souligne, à la fois sur le plan neuronal et comportemental, que les joueurs avérés utilisent plus un contrôle « réactif » (restreindre les actions pour se préparer à s'arrêter) qu'un contrôle « pro-actif » (corriger « tardivement

» pour inhiber une action en cours) pour inhiber leurs réponses motrices envers un indice de jeu pertinent. Ceci confirme que l'étude de l'inhibition réactive et proactive permet une analyse fine du contrôle inhibiteur de nos actions et que la résonance magnétique fonctionnelle est un bon outil pour étudier la dynamique des processus cérébraux qui les sous-tendent. L'approche utilisée peut faire avancer nos connaissances sur la capacité humaine de contrôler les habitudes hédonistes.

Samenvatting in het Nederlands : Deze studie toont aan dat frequente gokkers, zowel op neuronaal als op gedragsmatig niveau, eerder reactieve controle (het onderdrukken van een bepaalde actie ter voorbereiding tot het stoppen ervan) dan proactieve controle (een 'laattijdig correctief' proces dat resulteert in het onderbreken van een effectieve actie) om motorische processen te onderbreken naar gok-gerelateerde stimuli. Deze bevindingen bevestigen dat onderzoek van zowel reactieve als proactieve inhibitie van belang zijn in het onderzoek naar inhibitie processen. Tevens toont deze studie aan dat fMRI een valide techniek is om deze twee processen te bestuderen. Deze aanpak geeft ons meer inzicht hoe mensen hun hedonistische gewoontes trachten te controleren.

**Keywords: Proactive Inhibition, Reactive Inhibition, fMRI, Gambling, cue-reactivity, stop-signal task**

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# STN DBS for Parkinson's disease results from a series of ten consecutive patients implanted under general anaesthesia with intraoperative use of 3D fluoroscopy to control lead placement

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Background Deep brain stimulation (DBS) of the subthalamic nucleus (STN) is a recognised treatment for advanced Parkinson's disease (PD) (1,2). We present our results of 10 consecutive patients implanted under general anaesthesia (GA) using intraoperative robotic threedimensional (3D) fluoroscopy (Artis Zeego; Siemens®, Erlangen, Germany). Method Ten patients (nine men) with a mean age of 57.6 (range, 41–67) years underwent surgery between October 2013 and January 2015. The mean duration of PD was 9.2 [1–10] years. The procedure was performed under GA: placement of the stereotactic frame, implantation of the electrodes (Lead 3389; Medtronic®, Minnesota, MN, USA) and 3D intraoperative fluoroscopic control (Artis Zeego) with image fusion with the preoperative magnetic resonance imaging (MRI) scans. All patients were evaluated preoperatively and at 6 months postoperatively. Results The mean operative time was 240.1 (185–325) minutes. The mean Unified Parkinson's Disease Rating Scale (UPDRS) (3) II OFF medication decreased from 23.9 preoperatively to 15.7 postoperatively. The mean OFF medication UPDRS III decreased from 41 to 11.6 and the UPDRS IV decreased from 10.6 to 7. The mean preoperative and postoperative LDopa doses were 1,178.5 and 696.5 mg, respectively. Two complications were recorded: one episode of transient confusion (24 h) and one internal pulse generator (IPG) infection. Conclusions With improvement in preoperative MRI and the ability to control the position of the leads intraoperatively using Artis Zeego, we now perform this procedure under GA. Our results are similar to others reported. The significant decrease in the duration of surgery could be associated with a reduced rate of complications (infection, loss of patient collaboration). However, this observation needs to be confirmed.

Résumé en français: La stimulation cérébrale profonde (DBS) des noyaux sous-thalamiques est un traitement de choix de la maladie de Parkinson (PD) à un stade avancé. Nous présentons ici les résultats de notre série de 10 patients consécutifs ayant bénéficié d'une implantation d'électrodes sous anesthésie générale (AG) à l'aide d'une fluoroscopie peropératoire robotisée (Artis Zeego, Siemens®, Erlangen, Allemagne) en trois dimensions (3D). Avec l'amélioration de la qualité de l'IRM préopératoire

et la possibilité de contrôler la position des électrodes avec l'aide de l'Artis Zeego, nous réalisons actuellement cette procédure sous anesthésie générale. Nos résultats sont similaires à ceux rapportés dans la littérature. La diminution significative du temps opératoire pourrait être associée à une réduction du taux de complications (infection, perte de la collaboration du patient). Toutefois, ces observations devront être confirmées.

Samenvatting in het Nederlands: Diepe hersenstimulatie (DBS) ter hoogte van de nucleus subthalamicus is een eerstekeuzebehandeling voor de ziekte van Parkinson (PD) in een gevorderd stadium. We stellen hier de resultaten voor van onze reeks van 10 opeenvolgende patienten waarbij de elektroden onder algemene anesthesie en met behulp van intraoperatieve gerobotiseerde driedimensionele fluoroscopie (Artis Zeego, Siemens®, Erlangen, Duitsland) werden geimplanteerd. De verbeterde kwaliteit van de preoperatieve MRI en de mogelijkheid om de elektrodenpositie met het Artis Zeego-systeem te controleren, lieten ons toe deze procedure heden onder algemene anesthesie te verrichten. Onze resultaten zijn vergelijkbaar met die uit de literatuur. De significant verkorte operatieduur kan worden geassocieerd met een verminderd aantal complicaties (infectie, verlies van samenwerking van de patient), maar dit vereist nog bevestiging.

**Keywords: Artis Zeego, Deep Brain Stimulation, Parkinson Disease, general anaesthesia, Neurosurgery**

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# Generation of an in vitro model of human nigrostriatal pathway

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Parkinson's disease (PD) dynamics are still elusive, especially the first steps that trigger dopaminergic neurodegeneration. To date the only available models for PD are in vitro cell population in 2D culture, which does not represent the complexity of the brain, and in vivo animal models, do not fully recapitulate the disease symptoms. Indeed human brain sensitivity to neurodegenerative insults is much higher than the one showed by rodent brain. Therefore we need to have a faithful human neurodegeneration model. This can be achieved by generating in vitro human neuronal pathways by combining cell reprogramming and bioengineering approaches. Since the groundbreaking discovery of induced pluripotent stem cells (iPSCs) in 2006 (Takahashi and Yamanaka, 2006) we can stemmness transcription factors to reprogram adult human somatic cells to a pluripotent stage. Therefore we can use personalized human ESCs in order to generate patient-matched neuronal cells with specific neurotransmitter phenotype such as GABAergic, glutamatergic, dopaminergic, ecc... The future challenge is combine these different neuronal populations in order to recreate in vitro neuronal pathways that could be then tested for neurodegenerative challenges. In the case of PD we can hypothesize to combine iPSC-derived mesencephalic neurons and their natural target, GABAergic medium spiny neurons in order to create a model of nigrostriatal pathway whose degeneration is the main cause of parkinsonian motor dysfunctions. Recent bioengineering technologies can be used to reach this goal by used defined 3D microenvironments (Caiazzo et al., 2016) that can be enriched with axon path-finding molecules in order to drive the correct synaptic connections between the projecting and target neuronal populations. Once we have available such in vitro models of human neuronal pathways, we can hypothesize to study the early steps of human neurodegeneration that nowadays is still a black box, and then potentially to identify early detection diagnostic markers and potentially new therapeutic targets.

Résumé en français: La compréhension de la genèse d'une maladie neurodégénérative comme la maladie de Parkinson nécessite de pouvoir travailler sur des modèles qui reproduisent non seulement les différents types cellulaires mais aussi la circuiterie qui relie les neurones entre eux. Les découvertes récentes de reprogrammation des cellules (i.e. de fibroblastes en neurones) et de bioengineering permettent aujourd'hui de reconstituer un microenvironnement à 3 dimensions contenant les faisceaux de

prolongements neuronaux et leurs terminaisons aboutissant sur les populations de neurones cibles. L'obtention de tel modèle permettra de détecter les premières étapes de la neurodégénérescence qui est encore un mystère et ensuite d'identifier des marqueurs précoces et de nouvelles drogues.

Samenvatting in het Nederlands: Het begrijpen van het ontstaan van een neurodegeneratieve ziekte, zoals de ziekte van Parkinson, is slechts mogelijk wanneer kan gewerkt worden met modellen die niet alleen verschillende types van cellen weergeven maar ook de netwerken die de neuronen onderling verbinden. Recente ontdekkingen op het vlak van de herprogrammering van cellen (fibroblasten en neuronen) en de bio-engineering laten ons nu toe een 3 dimensionele reconstructie te maken van de micro omgeving, met daarin de bundels neuronale verlengstukken en hun uiteinden die leiden naar de doelneuronen. Het bekomen van dergelijk model zal toelaten de eerste fases van de neurodegeneratie op te sporen, wat tot nu toe een mysterie blijft, zal toelaten de voortijdige kenmerken te identificeren en zal het mogelijk maken nieuwe geneesmiddelen te ontwikkelen.

**Keywords: cell reprogramming, Dopaminergic Neurons, Parkinson Disease, Bioengineering, Hydrogels**

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# A genetic model of alpha-synuclein spreading

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Parkinson's disease affects approximately 1% of the population over 65 years. The role of a specific protein was highlighted in several neurodegenerative diseases including Parkinson's disease: its name is alpha-synuclein. Alpha-synuclein aggregation is the molecular signature of Parkinson's disease, both in its sporadic and family forms. Alpha-synuclein in its aggregated form is toxic to neurons. A more recent development of research on alpha-synuclein is the demonstration that the aggregation of alpha-synuclein is spreading in the brain, contributing to the degeneration of neural circuits. The mechanisms for this propagation from cell to cell, however, remain unknown. The nematode C. elegans is an important model in biology. Its genetic power, the genome sequencing and the associated techniques have brought C. elegans in the post-genomic era well before its vertebrates competitors. Genetic analysis of this animal is ideal for molecular and cellular exploration of poorly understood phenomena such as the spreading of alpha-synuclein. In addition to these genetic advantages, C. elegans remains probably the best described organism in physiological and anatomical terms: in particular, all the connections (synapses) between each of its 302 neurons are mapped. Using this model, we study the mechanisms of toxicity and the transmission of alpha-synuclein. Recently, an innovative model was developed in our laboratory, which will identify the genes allowing the transfer of alpha-synuclein between neurons. Knowing the transmission mechanisms of alpha-synuclein opens the perspective of preventing the spread of the disease in the brain.

Résumé en français: Voilà déjà une quinzaine d'années, le rôle d'une protéine a été mis en évidence dans la maladie de Parkinson : son nom : l'alpha-synucléine. Sous sa forme agrégée cette protéine est toxique pour les neurones. Plus récemment, la démonstration a été faite que l'agrégation de l'alpha-synucléine se propage de proche en proche dans le cerveau, contribuant à la dégénérescence des circuits neuronaux. Les mécanismes permettant cette propagation restent cependant inconnus. Nous étudions les mécanismes de la toxicité et du transfert intercellulaire de l'alpha-synucléine. Un modèle innovant a été mis au point dans notre laboratoire qui, à terme, identifiera les gènes impliqués. Connaître les mécanismes de transfert intercellulaire d'alpha-synucléine ouvre la perspective d'empêcher la propagation de la maladie dans le cerveau.

Samenvatting in het Nederlands: Reeds sinds een vijftiental jaren wordt de rol van een proteïne bij de ziekte van Parkinson erkend, het betreft de alfa-synucleïne.

In geaggregeerde vorm is deze proteïne toxisch voor neuronen. Onlangs werd duidelijk gemaakt dat deze geaggregeerde alfa-synucleïne zich stap voor stap verspreid in de hersenen, en aldus bijdraagt tot de ontaarding van zenuwnetwerken. Het mechanisme waardoor deze verspreiding gebeurt blijft echter onbekend. Wij bestudeerden de mechanismen van toxiciteit en intercellulaire overdracht van deze proteïne. In ons laboratorium werd een innovatief model uitgewerkt, dat, op termijn, de betrokken genen zal identificeren. Het leren kennen van de intercellulaire overdracht mechanismen bij alfa-synucleïne opent perspectieven om de verspreiding van de ziekte in de hersenen te beletten.

**Keywords: genetic model, alpha-synuclein oligomers, Parkinson Disease, Prion Diseases, Cell Biology**

# Resting state connectivity shows differential effects for subsets of the mesocorticolimbic circuit during drug addiction in rats

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Objective: Cocaine addiction is a major health concern, for which currently no approved treatment exists. This is largely due to the neurobiological complexity of addiction, with several neurotransmitter systems and different brain regions involved in different phases of the addiction process. Preclinical studies have shown that altered resting state functional-MRI (rs-fMRI) can be shown in the mesocorticolimbic circuit (MLC), involved in the acquisition of reward memory differences during cocaine self-administration in rats. Therefore, we followed up animals subjected to cocaine self-administration in a longitudinal design to investigate the temporal dynamic of the intrinsic neurobiological interactions between the different brain regions involved in this circuit. Methods: Eighteen male adult Wistar rats were surgically implanted with an IV-catheter before training self-administration with oral sucrose rewards. Hereafter baseline measurements were performed before starting two cocaine exposure weeks, and 2 weeks of withdrawal. Additionally, animals regained access to cocaine for 1 week to mimic a relapse. During the drug-exposure and relapse phase animals had free access to cocaine via lever presses in 3 hour sessions. Animals were followed up with weekly rs-fMRI scans while being anesthetized with 1.5% isoflurane. As previously described, data were obtained with an EPI sequence on a 9.4T Bruker scanner. Obtained scans were motion corrected using FSL FEAT, spatially smoothed and normalized. Hereafter, brains were extracted and registered to the Schwarz rat atlas, before being subjected to temporal band-pass filtering. Included a priori regions of interest were the left and right (L/R) striatum (S), nucleus accumbens (NAC), prefrontal cortex (PFC) and cingulate cortex (CC). To obtain the most sensitive signal, clusters of 200 voxels were identified within these ROIs with a homemade R script that iteratively calculated the cluster with maximal inter-voxel correlations. Reported correlations were calculated between each ROI's cluster. Associations between cocaine self-administration and functional connectivity between regions were assessed with linear mixed models allowing random intercepts per animal. Results: Animals readily self-administered cocaine when available. Additionally, during two drug-exposure weeks high inter-subject variability was observed. Mean correlation between assessed parts of the MLC showed an increase

of functional connectivity during drug-exposure (7.5±3.1%, $p = 0.03$) and withdrawal (15.0±3.7%, $p < 0.001$) as compared to baseline. However, no difference between relapse and baseline was found. Furthermore, within the MLC, 4 connections showed significant association with self-administration; CC – RS, PFC – LS, RS * RNAC, and LS * RS. Interestingly, the negative relation between prefrontal regions (PFC & CC) and LS suggests an inhibitory effect on self-administration. Conclusions: Cocaine was readily and increasingly self-administered over time, as has been reported before. Using an iteratively selected cluster, we could show that the average functional connectivity between MLC clusters increased during drug-exposure and withdrawal, but normalized in relapse. This could be suggestive for the self-medicating hypothesis of addiction, but needs to be substantiated in future studies. Furthermore, the connection between the CC and PFC, and the LS and RS respectively was negatively associated with cocaine self-administration. Conversely, the connectivity between the RS * RNAC, and the LS * RS was positively associated with drug intake. These findings corroborate the inhibitory influence of the prefrontal brain regions over the reward-oriented structures, such as the nucleus accumbens and striatum.

Résumé en français: L'addiction à la cocaïne est un problème majeur de santé publique pour lequel aucun traitement satisfaisant n'existe. Afin de mieux comprendre les mécanismes de l'addiction, des modèles animaux ont été développés. Ces modèles basés sur l'auto-administration de drogue chez le rat, permettent d'étudier les différentes régions du cerveau impliqués dans ce processus. Dans ce travail, la technique d'imagerie fonctionnelle par résonance magnétique (IRMf) permet d'analyser les régions du cerveau dont l'activité est corrélée à la prise de drogue et à la privation de cette même drogue et à la rechute. Les résultats ont montré qu'une région située à l'avant du cerveau, dans le lobe préfrontal, inhibent les centres cérébraux impliqués dans la prise de substances addictives.

Samenvatting in het Nederlands: Cocaïneverslaving is een belangrijk probleem voor de volksgezondheid waarvoor nog geen afdoende behadeling bestaat. Om de verslavingsmechanismen beter te begrijpen, werden proefdiermodellen ontwikkeld. Deze modellen zijn gebaseerd op zelfvoorziening van de drug door de rat, om na te gaan welke hersengebieden bij dit proces betrokken zijn. In deze studie laat de functionele beeldvorming met magnetische resonantie (iMRF) toe deze hersengebieden te analyseren waarvan de activiteit verband houdt met het gebruik van de drug, het niet meer gebruiken van de drug en het hervallen. Het onderzoek toonde aan dat een gebied, vooraan in de hersenen in de prefrontale lob, een belemmering vormen voor hersencentra die verband houden met inname van verslavende middelen.

**Keywords: Addiction, fMRI BOLD, animal model, Cocaine, Substance Abuse, Intravenous**

# Dead men talking? Europe's largest psychiatric brain bank

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Recently, the University of Antwerp gained ownership of what probably is the largest psychiatric brain bank in Europe. Interestingly, the earliest brains in the collection date from the early 50's, a period were psychopharmacological drugs were still in development and therefore scarcely administered to patients. This exceptional feature enables us to excavate the influence of psychiatric disorders themselves on the brain, bypassing the need for caution in interpreting post-mortem data from medicated patients as is the case with most if not all post mortem studies available today. As our research group investigates the role of neuroinflammation in psychiatric disorders, we aim to relate our clinical patient data to findings in post mortem tissue. Specifically, we will try to determine whether pro-inflammatory cytokines and kynurenine metabolites found in the peripheral blood of patients are also present in the brain; this via both histology and state-of-the-art liquid chromatography–mass spectrometry (LCMS). Since this collection harbours a treasure of information due to its uniqueness, we would also like to make a warm appeal to fellow researchers interested in collaborating with us on this rare but valuable collection.

Résumé en français : Récemment, l'université d'Anvers a acquis ce qui est probablement la plus grande banque de cerveaux en Europe. Une des particularités et richesse de cette acquisition est que les premiers cerveaux de cette collection datent des années 50, période à laquelle peu de drogues de type psychopharmacologique existaient, et donc vierge de tout traitement par ces drogues administrées des années plus tard aux patients et qui compliquent l'analyse des données collectées. Cette collection constitue un trésor d'information et nous sommes prêts à ouvrir cette collection et collaborer avec les chercheurs qui ont un projet innovant nécessitant l'utilisation de ces cerveaux.

Samenvatting in het Nederlands: Onlangs verwierf de universiteit van Antwerpen de wellicht grootse verzameling hersenen in Europa. Een van de bijzonderheden en ook de waarde van deze aanwinst is dat de oudste exemplaren dateren van de jaren 50, een periode waarin nog bijna geen psychofarmocologische medicatie bestond, en de hersenen dus niet blootgestaan hebben aan psychofarmacologische behandeling

zoals die in de daaropvolgende jaren gebruikt werd, en die de analyse van de gegevens bemoeilijkt. Deze verzameling vormt een schat aan informatie en we zijn bereid de collectie open te stellen en samen te werken met onderzoekers die aan een project werken waarvoor deze hersenen nuttig kunnen gebruikt worden.

**Keywords: Brain, postmortem, Schizophrenia, Depression, Inflammation**

# White matter tract dissection in the non-human primate

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Introduction: Brain connectivity has been extensively studied in non-human primates (NHPs) using tracer techniques. Imaging studies using diffusion tensor imaging in NHPs are scarce but suggest similar white matter connections compared to the human brain. Our group recently studied commissural motor fibers in the human brain (Naets et al., 2015) and showed that leg motor connections are running in the posterior part of the corpus callosum. To our knowledge, post-mortem anatomical dissection studies of white matter tracts in the NHP brain have not been performed. In this study we discuss the course and topography of the different white matter tracts in the NHP. Methods: 8 hemispheres were dissected according to Klingler's fiber dissection technique (Klingler et al., 1935). Results: Major white matter tracts could be demonstrated using both a mesial and lateral dissection. These fibers include the corona radiata, the uncinate fasciculus, the anterior commissure, the corpus callosum, the stratum sagittale, the cingulum and the fornix. Conclusion: The major white matter tracts in NHPs were found to be similar to human white matter tract anatomy. In particular, callosal fibers connecting the primary motor cortex invariably ran through the posterior corpus callosum, which contrasts to older anatomical studies (Pandya et al., 1971).

Résumé: Analyse de la dissection de la substance blanche du primate non-humain. On ne sait pas si les faisceaux de fibres dans le cerveau des primates non-humains sont identiques à ceux du cerveau humain. Dans cette étude les auteurs ont disséqué plusieurs cerveaux de singes après autopsie. Les faisceaux de fibres identifiés sont semblables à ceux décrits dans le cerveau humain, en particulier les fibres inter-hémisphériques connectant le cortex moteur passant par la portion postérieure du corps calleux contrairement à ce que l'on pensait auparavant.

Samenvatting in het Nederlands: Het is momenteel niet goed geweten of de witte stofbanen in de hersenen bij de aap vergelijkbaar zijn aan deze bij de mens. In deze studie hebben de auteurs verschillende apen hersenen onderzocht bij autopsie. De ligging van de grote witte stofbanen is vergelijkbaar aan deze bij de mens. Zo is de ligging van de interhemisferische vezels in het corpus callosum gelijkaardig als bij de mens, waarbij de motorische vezels steeds in de posterieure helft lopen, in tegenstelling tot wat er eerder werd gedacht.

**Keywords: white matter anatomy, Corpus Callosum, Klingler's technique, non-human primate, Dissection**

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# Evoked motor potentials in genetically proven Friedreich's ataxia patients

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Introduction: Friedreich's ataxia (FA) is the most prevalent recessive cerebellar ataxia. Pathophysiology implies an intronic triplet expansion that alters the expression of a mitochondrial protein, the frataxin, involved in the mitochondrial iron-sulfur complex. Clinical hallmark of the disease is afferent ataxia caused by prominent involvement of the medullary dorsal columns and the dentate nuclei of the cerebellum. Corticospinal tracts are also involved and motor central conduction time (MCCT) on evoked motor potentials (MEPs) has been found to correlate with the duration of the disease. However, MEPs in FA patients evaluated with objective clinical scores and accurate genetic analysis are scarce. Methods: 9 FA patients [5 females, mean age: 36 years (range 23-51), mean scale for the assessment and rating of ataxia (SARA): 25 (range 14-37,5), mean duration of disease 20 years (range 9-38), mean triplets expansion on shortest allele (GAA1): 623 (range 280-850)] participated in the study. EMPs were measured at first dorsal interosseous of the right hand and evoked with 9 cm Circular coil (Magstim 200 Co Ltd, Whitland, Dyfed, UK) placed over the location around the central location (Cz) that led to the best evoked motor responses. Motor threshold was assessed and 3 to 6 MEPs were recorded with a stimulation intensity corresponding to 140% of the motor threshold at determined cortical location and at a cervical location above C5-C6 as cervical location. Spearman's correlation test was used to test for significant correlations between subjects and MEPs parameters. Results: Mean amplitude of MEP after cervical stimulation was 6,8 mV (range 0,4-18,2mV, normal values: 8,1+/- 4,9 mV) with a mean MEP length of 16,2ms (range 9-24 ms) while cortical stimulation led to a mean amplitude of MEP of 0,47mV (range 0,005-1,45 mV, normal values: 4,9 +/-2,4 mV) and mean MEP length of 22,4ms (range 14-33ms). Mean cervical latency was 15,21ms (range 14-18ms, normal value <19,2ms) and mean MCCT was 16ms (range 9-20ms, normal value: 9,15ms). Significant correlation was found only for disease duration and MCCT. Conclusions: FA patients display altered MEPs with loss of amplitude and prolonged conduction time partly due to desynchronized responses along the corticospinal tracts. Interestingly MCCT correlates only with disease duration and not with clinical scores or the triplet size expansion.

Résumé en français: L'ataxie de Friedreich (FA) est une maladie neurodégénérative d'origine génétique caractérisée par un déséquilibre et une perte de coordination des

mouvements. Ces symptômes sont dus à la dégénérescence des neurones transmettant l'information sensorielle vers les centres du contrôle du mouvement mais l'information descendant des centres de contrôles vers les muscles est également perturbée au cours de la pathologie. Dans ce travail, nous avons mesuré l'activité électrique déclenchée par la stimulation du cortex moteur ainsi que le temps de conduction de l'information motrice du cortex vers la périphérie. Nous avons montré que les patients FA présentaient des activités électriques de plus faible amplitude et des vitesses de conduction plus lentes en corrélation avec la durée de la maladie.

Samenvatting in het Nederlands: De ataxie van Friedreich (FA) is een genetische neurodegeneratieve ziekte, gekenmerkt door het verlies van het evenwicht en de coördinatie van de bewegingen. Deze symptomen worden veroorzaakt door de aftakeling van neuronen die essentiële sensoriele informatie doorsturen naar het controlecentrum van de bewegingen, en ook het versturen van informatie vanuit deze controlecentra naar de spieren is verstoord. In deze studie maten we de elektrische activiteit die ontstaat door het stimuleren van de motorische hersenschors, en maten we de tijd nodig om deze informatie vanuit de hersenschors naar de omgeving te versturen. We toonden aan dat bij FA patiënten de omvang van de elektrische activiteit minder was, en de snelheid verminderde in de loop van de ziekte.

**Keywords: Friedreich Ataxia, motor evoked potential, Cerebellum, Electrophysiology, follow-up**

# Decreased corticokinematic coherence in patients with Friedreich's Ataxia

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Friedreich's ataxia (FA) is a genetic neurodegenerative disorder characterized by a progressive loss of spino-cortical and spino-cerebellar proprioceptive neurons leading to limb ataxia and gait instability. This pilot study investigates if the corticokinematic coherence (CKC) method can be used as a potential marker of spino-cortical proprioceptive pathways degeneration in FA. CKC, which indexes the coupling between cortical magnetoencephalography (MEG) signals and movement kinematics, is indeed mainly driven by proprioceptive afferents to the primary sensorimotor (SM1) cortex. CKC was evaluated using whole-scalp-covering MEG (Elekta) in 11 right-handed FA patients (8 females, mean age: 29 y (range: 9-46 y), mean SARA score: 21.7 (range: 14-30.5)) and five healthy adult subjects (3 females, mean age 34 (range: 30-43 y)) performing active right index finger flexion–extension. Index finger acceleration was monitored with a 3-axis accelerometer. Coherence was used to index the coupling between cortical activity and movement kinematics. Coherent brain areas were identified using dynamic imaging of coherent sources. Non-parametric permutation statistics was used to assess the statistical significance of local coherence maxima. Movement frequency (F0) was significantly different between patients and controls (Patients, 1.4 ± 0.5 Hz; controls 2.4 ± 0.5 Hz). In both groups, significant coherence was found at movement frequency (F0) and its first harmonic (F1) in the left SM1 cortex (coherence, patients: 0.8 (F0) and 0.17 (F1); subjects: 0.22 (F0) and 0.31 (F1)). Coherence values were significantly lower in patients than in healthy subjects. This pilot study demonstrates lower CKC levels in FA patients compared with healthy subjects. CKC might therefore represent an interesting method to evaluate spino-cortical proprioceptive pathways degeneration in FA.

Résumé en français: L'ataxie de Friedreich (FA) est une maladie neurodégénérative d'origine génétique caractérisée par une perte progressive des neurones essentiels au contrôle des mouvements des membres. Cette dégénérescence est responsable de l'apparition de troubles de l'équilibre et d'une perte de coordination des mouvements.

Dans cette étude, nous avons étudié l'activité électrique du cortex somatosensoriel et l'avons corrélée à la cinétique des mouvements afin de voir si une discordance pouvait être mise en évidence chez les patients FA. Nous avons mis en évidence une cohérence entre la cinétique des mouvements et l'activité cérébrale chez les patients mais moins importante que celle observée chez les volontaires sains. Cette technique d'enregistrement pourrait permettre de diagnostiquer la neurodégénérescence des voies sensorielles responsables du contrôle du mouvement.

Samenvatting in het Nederlands: De ataxie van Friedreich (FA) is een neurodegeneratieve genetische ziekte gekenmerkt door een voortschrijdend verlies van neuronen essentieel voor de beweging van de ledematen. Dit verval is verantwoordelijk voor evenwichtsstoornissen en verlies van coördinatie van de bewegingen. In deze studie onderzochten we de elektrische activiteit van de somatosensoriele hersenschors en vergeleken we die met de kinetiek van de bewegingen, om na te gaan of er een verband kan gelegd worden bij patiënten die lijden aan FA. We vonden een verband tussen de kinetiek van de bewegingen en de hersenactiviteit bij patiënten, minder belangrijk dan bij gezonde vrijwilligers. Deze registratietechniek moet het mogelijk maken de aftakeling van de sensoriele paden die verantwoordelijk zijn voor de controle van de bewegingen beter te diagnosticeren.

**Keywords: Friedreich Ataxia, Magnetoencephalography (MEG), coherence analysis, somatosensory, Cerebellum**

# Friedreich's ataxia affects the cortical processing of tactile novelty detection

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Background : The mismatch negativity (MMN) is a novelty detection response evoked by deviant stimuli embedded in sequences of standard stimuli. In the somatosensory system, the MMN is generated at contralateral secondary somatosensory cortex (cS2). Previous studies have shown that the cerebellum is part of the somatosensory novelty detection network and that patients with unilateral cerebellar lesion have no or weaker somatosensory MMN (sMMN). Friedreich's ataxia (FA) is a genetic disorder associated with a selective atrophy of the cerebellar dentate nucleus, which is a key node of the efferent dentatothalamic tract. So, FA may prove a good model to study the role of the cerebellum in tactile novelty detection. Methods : 13 right-handed FA patients (9 females; 29 years (range 9-46), mean scale for the assessment and rating of ataxia (SARA): 23 (range 14-37.5), shorter allele (GAA1) mean expansion 688 (range 280-100) and 8 right-handed healthy adults (3 females, 29 years (range 23-45)) were measured with whole-scalp-covering magnetoencephalography (MEG, EleKta Oy) while they underwent an tactile oddball paradigm. Standard stimuli were pneumatic tactile stimulations of the right index fingertip and deviant stimuli corresponded to similar tactile stimulation simultaneously applied to the first two phalanxes of the index finger. Mismatch negativity responses associated with deviants were investigated at the sensor level. sMMN responses were assessed in the sensor space using non-parametric cluster statistics. sMMN neural sources were localized using conventional equivalent current dipole modeling. Post-hoc paired t-tests were applied on the corresponding sources intensity (standards or deviants) at the timing of maximum source amplitude within the time frame disclosed by sensor-level analyses. Results: In healthy subjects, standard stimuli evoked responses at contralateral primary somatosensory cortex (cSI) with a mean latency of 23.75ms (range 15-33ms) and mean amplitude of 9 mA (range 6-16mA) and contralateral secondary somatosensory cortex (cSII) with a mean latency of 48.7ms (range 55-80 ms) and mean amplitude of 9.44 mA (range 7-19mA). Deviant stimuli led to a mean amplitude at cSI of 11.7 mA (range 9-19 mA) and mean amplitude at cSII of 23,7 mA (range 8,5-37 mA) leading to significant msMMN only at cSII. In FA patients, standards stimuli evoked at cSI in 12/13 patients with a mean latency of 65 ms (range: 50-80ms) for a mean amplitude of 7,4 mA (range 1,8-15,1mA) and cSII in 11/13 FA patients with a mean latency of 120ms (range 74-149 ms) and a mean amplitude of 6,2 mA (range 0,6-11 mA). Deviant stimuli led to a mean amplitude of 13,4 mA (range

4,4-34,4 mA) at cS1 and of 15,2 mA (7,2-31,4 mA) leading to a statistically significant mismatch both at cSI and cSII. Latency of cortical responses were significantly longer in FA patients. Still there was no correlation between SARA scores, GAA1 expansion, the latency or the amplitude of SEFs. Conclusions: Somatosensory evoked fields are reliably found at cSI and CSII cortices with MEG in FA patients. Cerebellar dorsal nuclei atrophy in FA affects the cortical processing of tactile novelty detection with an increase is cSI and a decrease in cSII cortical responses.

Résumé en français: Lorsque le système tactile est stimulé, des ondes électriques peuvent être mesurées au niveau du cortex somatosensoriel situé dans la partie haute et centrale de notre cerveau. L'arrivée de l'information tactile est détectée au niveau du cortex primaire tandis que des informations plus complexes génèrent une activité électrique dans le cortex secondaire. Dans cette étude, nous nous intéressons à la détection de nouveauté, c'est-à-dire la détection d'une stimulation tactile différente dans une suite de stimulations standards. Cette nouveauté induit une activité électrique dans le cortex secondaire qui peut être mesurée par magnétoencéphalographie. Des études précédentes ont suggéré que le cervelet pouvait jouer un rôle dans notre capacité à détecter la nouveauté. Afin de confirmer cette hypothèse, nous avons étudié ce phénomène chez les patients atteints d'ataxie de Friedriech car ils présentent une atrophie du cervelet et avons mis en évidence des modifications de la réponse électrique à la nouveauté chez ces patients.

Samenvatting in het Nederlands: Wanneer het tastsysteem geprikkeld wordt, kunnen elektrische golven gemeten worden op de somatosensoriele hersenschors, centraal aan de bovenzijde van de hersenen. Deze tast-informatie is aanwijsbaar op het primaire niveau van de hersenschors, meer complexe informatie veroorzaakt een elektrische activiteit in de secundaire hersenschors. In deze studie richtten we ons op het ontdekken van nieuwe, anders dan gewone tastzinprikkels, binnen een reeks van standaardprikkels. Deze nieuwigheid leidt tot een elektrische activiteit in de secundaire cortex die kan gemeten worden door middel van magneto-encefalografie. Eerdere studies suggereerden dat de kleine hersenen een rol kunnen spelen in het ontdekken van nieuwigheden. Om deze hypothese te toetsen hebben we dit fenomeen bestudeerd bij patiënten die lijden aan de ataxie van Friedreich, gezien ze een atrofie van de kleine hersenen vertonen en we konden bewijzen dat er een wijziging is in de elektrische reactie op nieuwigheden bij deze patiënten.

**Keywords: Magnetoencephalography (MEG), mismatch negativity (MMN), somatosensory, Friedreich Ataxia, Cerebellum**

# TGF-ß receptor I promotes early microglia development in zebrafish embryo

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Because neurodegenerative diseases are often associated with neuroinflammation, microglia, the resident macrophages in the central nervous system, represent a potential target for the development of novel therapeutic approaches. Our research aims at characterizing microglia ontogeny, an important aspect of microglia biology that remains poorly understood. Because the first steps of microglia ontogeny occur early during development, transparent transgenic zebrafish embryos offer great opportunities to characterize these processes in a non-invasive way. In an effort to characterize the molecular signature of zebrafish microglia, we have generated novel transgenic fluorescent reporter lines allowing for the prospective isolation of a pure population of microglial cells from the adult brain. Through microarray and whole transcriptome high-throughput sequencing (RNA-seq), we identified, among others, TGF-ß receptor I as a candidate for further investigations into microglia biology. The results we present here demonstrate that, as previously shown in mice, TGF-ß receptor signaling acts as an essential positive regulator of microglia ontogeny in the zebrafish embryo. Exploiting the strenghts of the zebrafish model, we further demonstrated that this signaling pathway seems to be specifically required during the last steps of microglia differentiation. These preliminary observations have opened the door to future investigations aiming at characterizing the precise molecular mechanisms underlying TGF-ß receptor-mediated activity. Furthermore, these results validate the feasibility of our approach for providing novel insights into the genetic control of microglia ontogeny and differentiation. This could ultimately open new directions for the generation of microglia cells in vitro, a goal that has been largely unmet so far.

Résumé en français: Lors d'un traumatisme cérébral, un réseau de cellules de défense du cerveau (cellules de l'immunité) est activé : la microglie. La microglie est également activée dans les maladies neurodégénératives où elle génère une neuro-inflammation permanente. Elle peut donc être vue comme une cible potentielle pour le développement de nouvelles approches thérapeutiques. Nous travaillons sur le poisson zèbre qui, grâce à sa transparence et à la facilité d'obtenir des colonies de poissons présentant des cellules de la microglie fluorescentes, forme un modèle qui nous permet de suivre en temps réel le développement de cette microglie et les facteurs génétiques qui gouvernent

son développement. Un tel modèle devrait ouvrir de nouvelles voies dans le traitement de la neuro-inflammation et par là des maladies neurodégénératives.

Samenvatting in het Nederlands: Bij een hersentrauma wordt een netwerk van verdedigingscellen (immuun cellen) van de hersenen geactiveerd: de microglia. Deze microglia wordt eveneens geactiveerd bij neurodegeneratieve ziekten waarbij die een permanente neuro-inflammatie opwekt. De microglia kan dus beschouwd worden als een mogelijke doelstelling voor de ontwikkeling van nieuwe behandelingsvormen. Wij werken met zebravisjes die door de doorzichtigheid en het gemak waarmee kolonies vissen tot stand komen met fluorescente microgliacellen, een diermodel vormt dat ons toelaat de ontwikkeling van de microglia in real time te volgen, evenals de genetische factoren die van belang zijn voor de ontwikkeling ervan. Dergelijk model zou nieuwe wegen moeten openen voor de behandeling van neuro inflammatie en bijgevolg ook van neurodegeneratieve ziekten.

**Keywords: Microglia, Myeloid Cells, RNA sequencing, TGFbeta signaling, Zebrafish**

# Towards the clinical use of tolerogenic dendritic cells in multiple sclerosis by applying the immunomodulatory effects of 1,25-dihydroxyvitamin D3

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We first investigated the effect of 1,25-dihydroxyvitamin D3 (vitD3) on monocyte-derived DC (mo-DC) from healthy controls and MS patients. VitD3 treatment of mo-DC resulted in a maturation-resistant phenotype and anti-inflammatory cytokine profile as compared to conventional immunogenic DC, in both healthy controls and MS patients. Importantly, vitD3-treated DC induced T cell hyporesponsiveness, as demonstrated by a reduced ability to induce interferon-γ secretion by allogeneic peripheral blood lymphocytes stimulated with vitD3-treated DC as compared with conventional DC. We also investigated the influence of cryopreservation on the phenotype and allogeneic T cell stimulatory capacity of vitD3-treated DC. Following a freeze-thaw cycle, vitD3-treated immature DC could be recovered with a 78% yield and 75% viability. Cryopreservation did not affect the expression of DC membrane markers by vitD3-treated DC nor their capacity to induce T cell hyporesponsiveness in an allogeneic mixed leukocyte reaction. The T cell hyporesponsiveness induced by vitD3-treated DC is antigen-specific since T cells retained their capacity to respond to an unrelated antigen, i.e. cytomegalovirus pp65-derived peptides, while being unresponsive to myelin-derived peptides following tolerization to a myelin oligodendrocyte glycoprotein (MOG)-derived peptide pool and a myelin basic protein (MBP)-derived peptide pool. Furthermore, these T cells did not reactivate upon rechallenge with fully mature conventional DC, demonstrating that this induced T cell hyporesponsiveness was robust. Based on our observations, it can be concluded that vitD3 treatment of DC results in the generation of highly potent toleranceinducing DC (tolerogenic DC (tolDC)). Importantly, we demonstrate the feasibility of cryopreservation of these tolDC. In this perspective, our results contribute to large scale production and preservation of tolDC and further underscore their potential clinical applicability in order to correct the immunological imbalance in auto-immune disease in general and in MS in particular. These data suggest that DC play an important role in MS and that it can be envisaged to develop a new form

of immunotherapy for this disease, using tolerogenic DC. Currently, we are initiating a phase I dose escalation clinical study to assess the feasibility and safety of administering myelin-derived peptide-pulsed tolDC in patients with MS. Short summary Cell therapy is rapidly gaining momentum as a clinical option for several diseases. In particular, the clinical benefit and safety of dendritic cell-based immunotherapy has been well-documented in numerous clinical trials in patients with cancer and infectious diseases. More recently, tolerance-inducing or tolerogenic dendritic cells (tolDC) have also become a promising immunotherapeutic tool for restoring immune tolerance in autoimmune diseases, including multiple sclerosis (MS). While Our recent observations indicate that dendritic cells (DC) play a central role in the pathogenesis of multiple sclerosis (MS), their modulation with immunoregulatory agents provides a prospect as disease-modifying therapy.

Samenvatting in het Nederlands: Het gebruik van lichaamseigen cellen als medicijn zou heel wat mogelijkheden kunnen bieden voor de behandeling van ernstige en slopende aandoeningen. In het bijzonder, zou vaccinatie met dendritische cellen die de afweerreactie kunnen onderdrukken - tolerogene dendritische cellen - in de nabije toekomst een belangrijke piste kunnen worden in de aanpak van multiple sclerose. Dendritische cellen (DC) zijn een gespecialiseerde populatie van witte bloedcellen en functioneren als de aan- en uitzetknop van het afweersysteem. Gebaseerd op onze eerdere bevindingen zullen we voorlopercellen van de dendritische cellen isoleren uit het bloed van de patiënt. Deze cellen worden daarna gekweekt met vitamine D in een steriele en ultramoderne laboratoriumomgeving. Vervolgens kunnen ze terug aan de patiënt worden toegediend. Deze behandelingsstrategie zal bij een eerste groep van patiënten met multiple sclerose getest worden.

Résumé en français: La thérapie cellulaire (remplacement/modification de cellules malades par des cellules souches) est une voie thérapeutique nouvelle prometteuse. Dans le cas de la sclérose en plaques (SP), une maladie auto-immune (maladie où les cellules de défense attaquent les cellules du SOI), il a été récemment découvert que de telles cellules, appelées dendritiques, étaient une cible thérapeutique car responsables de cette auto-immunité. Dans ce travail, notre équipe a montré que les cellules dendritiques de patients atteints de SP pouvaient être modifiées (rendues tolérantes) grâce à un traitement à la vitamine D.

**Keywords: tolerance, Dendritic Cells, Multiple Sclerosis, cell therapy, Vitamin D**

# Generating myelin-expressing tolerogenic dendritic cells using mRNA electroporation: potential players for a cellular vaccine for the treatment of multiple sclerosis?

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BACKGROUND: In the pursuit of re-establishing tolerance in autoimmune disorders such as multiple sclerosis (MS), the use of antigen-loaded tolerogenic dendritic cells (tolDC) is a promising strategy to restore immune balance in a disease antigen-specific manner. For antigen loading of tolDC, electroporation with mRNA encoding full-length proteins might offer the potential to prevent epitope spreading by inducing presentation of all myelin epitopes in a non-HLA-restricted manner. OBJECTIVES: To generate antigen-expressing human tolDC using mRNA electroporation. METHODS: In this study, 1-alpha,25-dihydroxyvitamin D3-treated monocyte-derived tolDC were electroporated with mRNA encoding enhanced green fluorescent protein (eGFP) and myelin oligodendrocyte glycoprotein (MOG). To validate the electroporation procedure, mRNA and protein levels were analyzed in function of time following electroporation using qRT-PCR and flow cytometry or Western blot. RESULTS: Flow cytometric analysis of eGFP expression revealed a transfection efficiency of 68.6% (SD ±3,4%) for immature tolDC (itolDC) and 54.4% (SD ±29.7%) for cytokine-activated tolDC, demonstrating the feasibility to electroporate tolDC. MOG protein expression was detectable until at least 72 hours after mRNA electroporation as evidenced by Western blot analysis. Importantly, no difference in the expression of the costimulatory markers CD80 and CD86 by electroporated (i)tolDC nor in their capacity to induce T cell hyporesponsiveness in an allogeneic mixed lymphocyte reaction was found following mRNA electroporation when compared to mock electroporated and/or unelectroporated (i)tolDC.

CONCLUSION: Our results demonstrate that mRNA electroporation of tolDC effectively induces MOG expression without affecting their tolerogenic properties. The capacity of mRNA-electroporated tolDC to present antigen in a specific manner is

currently being assessed by evaluating their modulatory effect on myelin-specific T cell responses. Ultimately, therapeutic vaccination with tolDC electroporated with mRNA encoding full-length myelin-derived proteins may lead to a more effective therapy for MS by induction of T cell tolerance in a myelin-specific manner.

Résumé en français: Le progrès des connaissances sur les causes de maladies auto-immunes tels que la sclérose en plaques (SEP) permet de développer de nouvelles stratégies thérapeutiques prometteuses basées sur la thérapie cellulaire. Les cellules dendritiques (CD) sont des globules blancs spécialisés qui permettent de susciter ou de réprimer des réactions de défense. Les CD dites "tolérogènes" qui répriment les mécanismes de défense forment une population de cellules intéressante dans la thérapie cellulaire de la SEP. Dans cette étude nous avons étudié une technique spécifique qui permet de charger les CD tolérogènes avec des protéines contre lesquelles est dirigée la réaction auto-immune dans la SEP. De cette façon nous essayons de mettre au point une thérapie cellulaire, une sorte de vaccin, qui permettra d'inhiber de manière ciblée la réponse immune chez des patients atteints de SEP.

Samenvatting in het Nederlands: Naarmate de basismechanismen achter autoimmuunziekten zoals multiple sclerose (MS) verder ontrafeld worden, vormen celgebaseerde therapieën een veelbelovende strategie voor de behandeling van deze aandoeningen. Dendritische cellen (DC) zijn gespecialiseerde witte bloedcellen die afweerreacties kunnen aansturen dan wel onderdrukken. De afweeronderdrukkende of zogenaamde tolerogene DC vormen een interessante celgroep in het onderzoek naar celbehandelingen voor MS. In deze studie onderzochten we een specifieke techniek voor het opladen van tolerogene DC met de eiwitten waartegen de afweerreactie bij MS gericht is. Op die manier trachten we een celgebaseerde behandeling te ontwikkelen die de immuunrespons bij MS-patiënten op een gerichte manier kan onderdrukken.

**Keywords: Dendritic Cells, MRNA electroporation, Multiple Sclerosis, antigen-specific tolerance induction, cellular therapy**

# Remyelination if the adult Central Nervous System: A possible role for Elongator

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Multiple sclerosis (MS) is a chronic inflammatory demyelinating disease of the central nervous system (CNS) in which subsequent remyelination can spontaneously take place but often fails or is incomplete, for yet unknown reasons. Among others, one strategy to treat MS is to stimulate the recruitment and the activation of resident oligodendrocyte precursor cells (OPCs) to differentiate them into functional myelinating cells. In this context, we are studying the role of the Elongator complex in the adult CNS demyelination and remyelination processes. Elongator is a highly conserved six subunits complex (Elp1 to Elp6) known to be involved in the modulation of neuronal migration and differentiation, in specific tRNA modifications and in the development of some cancers. Its enzymatically active Elp3 subunit has an acetyl transferase activity that could prevent OPCs to differentiate into mature oligodendrocytes. This hypothesis is supported by the presence of higher acetylated protein levels in the white matter of MS affected brains compared to unaffected ones. We thus investigated the in vivo implications of Elongator using a new conditional knockout mouse (cKO) model (PLPCreERT2:Elp3floxed) in which Elp3 is specifically invalidated in the oligodendroglial lineage after tamoxifen treatment. We then compared wild type (WT) and cKO mice using the cuprizone model, a toxic experimental model of demyelination characterized by a reproducible demyelination in certain regions of the CNS followed by a spontaneous remyelination after the end of the treatment. Preliminary results based on MBP and MAG immunostaining quantifications in the corpus callosum of cuprizone-treated mice seem to indicate a faster remyelination in the cKO mice compared to the WT. Interestingly, we also showed that Elp3 mRNA expression is increased at the end of the cuprizone treatment in the corpus callosum of WT animals. In vitro, we analyzed cAMP-induced differentiation of Elp3-depleted OPCs by measuring the expression of myelin protein genes and we observed an increased expression of MAG and MOG in Elp3-depleted cells compared to control cells. Taken together, our results suggest that Elongator could play a role in the differentiation of OPCs and thus act as a negative regulator of the remyelination. We are now trying to confirm those observations and to elucidate the molecular pathways triggered by Elongator in this process.

**Keywords: myelin, oligodendrocytes, cuprizone mouse model, Central Nervous System, Elongator**

## Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

# TNF-alpha mediated effect on the synaptic plasticity of controlled neuronal networks

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Neuroinflammation is well established as a key secondary injury mechanism after TBI [1], [2] and it has been long considered to contribute to the damage sustained following brain injury [3]. However, neuroinflammation after TBI can have both detrimental and beneficial effects, and these likely differ in the acute and delayed phases after injury. The compression and stretching of brain tissues that occur during a traumatic injury leads to the activation of glial cells that release inflammatory molecules such as the cytokines and interleukins. At the early stages, inflammatory molecules are globally neurotoxic, whereas they have been reported to enhance the neuronal regeneration at long terms. As a consequence, the key to developing future anti-inflammatory based neuroprotective treatments for TBI is to minimize the detrimental and neurotoxic effects of neuroinflammation while promoting the beneficial and neurotrophic effects, thereby creating optimal conditions for regeneration and repair after injury. In this context, we have studied the impact of tumor necrosis factor alpha (TNF-alpha) on the neuronal plasticity of neuronal networks of controlled architectures [4]. A small amount (10 ng/ml) of TNF-alpha was added to cortical neuronal networks at early (DIV4 to DIV13) and late (DIV7 to DIV13) growth stages. Our results demonstrate that TNF-alpha promote tubulin polymerization but decreases the synapse density. To get insight into this mechanism, we have quantified in control neuronal networks the ratio between TNFR1 and TNFR2 TNF-alpha receptors, whereas TNFR1 is predominantly associated with neurodegeneration, TNFR2 is involved in tissue regeneration and neuroprotection. Our results show that the ratio between TNFR1 and TNFR2 is statistically similar at the early growth stages, whereas the balance is in favour of TNFR2 at later growth stages. These preliminary results suggest that detrimental and beneficial effects of TNF-alpha can be associated to the balance of TNFR1 and TNFR2 receptors during acute and delayed phases after injury.

Résumé en français: Titre: Étude du rôle de l'inflammation dans les traumas crâniens. Les traumas crâniens légers concernent 1,4 million de personnes chaque année en Europe. Ils peuvent être causés par une chute, un accident de voiture ou survenir suite à la pratique d'un sport de contact. Lors des traumas crâniens, le cerveau subit une forte accélération suivie d'une décélération brutale. Ces mouvements rapides de la tête

conduisent à la formation de lésions au niveau des neurones qui composent le cerveau et à l'activation des cellules gliales, autres cellules composant le cerveau qui libèrent alors des molécules inflammatoires. Dans ce travail, nous nous sommes intéressés à l'effet d'une molécule inflammatoire, le facteur de nécrose tumorale (TNF-alpha), sur l'organisation des connexions au sein de réseaux de neurones (plasticité synaptique). Nos premiers résultats montrent un impact à la fois bénéfique sur la croissance neuronale mais néfaste sur la densité de connexions.

Samenvatting in het Nederlands: Titel: Studie over de invloed van ontstekingen bij hersentrauma's. In Europa worden jaarlijks 1,4 miljoen mensen geconfronteerd met een licht hersentrauma. Deze kunnen het gevolg zijn van een val, een auto-ongeval, of ontstaan tijdens het beoefenen van een contactsport. Bij een hersentrauma ondergaan de hersenen een plotse versnelling, gevolgd door een brutale afremming. Deze snelle bewegingen van het hoofd veroorzaken letsels op het niveau van de neuronen die de hersenen vorm geven en activeren de gliale cellen, andere hersencellen die dan ontstekingsmolecules afgeven. In dit onderzoek gaat onze interesse naar de effecten van een ontstekingsmolecule, de TNF-alpha - een afsterffactor van tumoren - op de organisatie van verbindingen binnen netwerken van neuronen (synaptische plasticiteit). Onze eerste resultaten tonen aan dat enerzijds de aangroei van neuronen verbetert, maar dat anderzijds de dichtheid van de netwerken afneemt.

**Keywords: Traumatic brain injury (TBI), Neuroinflammation, Neuronal Plasticity, TNF alpha, TNFR1, TNFR2**

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# Neuroinflammatory processes induced during EAE also affect the hippocampus and its associated cognitive processes

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Recent scientific advances have clearly demonstrated the important role of the immune system in the central nervous system (CNS). Specific immune responses take place within the brain and are not only involved in pathological events but also in normal brain functioning. An integrative network develops between neurons, glia and peripheral immune cells which actively interact to regulate many neuronal functions and ensure the proper brain functioning. This complex neuroimmune crosstalk is particularly implicated in the remodeling of synaptic circuits contributing to synaptic plasticity and memory function. Microglial cells represent the resident immune cells of the CNS, protecting the brain against various pathological insults. Their immune responses are tightly regulated to maintain CNS homeostasis and limit neurotoxic processes. However, under diseased conditions, the delicate balance between neuroprotective and neurodegenerative effects of immune responses can be rapidly disrupted due to an excessive or prolonged activation of immune and glial cells. This can result in the delivery of damage signals and the propagation of neuroinflammation leading eventually to neuronal alterations(1). Synaptic plasticity is the ability of neurons to modulate the strength of their synaptic transmission. Different forms of synaptic plasticity exist such as the long-term potentiation (LTP). This process enables to modify neural circuits dynamic and ensures memory consolidation in the hippocampus. Immune processes are directly implicated in learning and memory and play a dual role. In the healthy brain, time-controlled immune responses including glial cells activation and cytokine production exert a positive effect on neural plasticity by increasing neuronal excitability. However, an excessive brain immune activation can induce a neuronal hyperexcitability state which is associated to disturbances in synaptic plasticity and memory(2). Cognitive impairments are very common in many neuroinflammatory disorders. However, the mechanisms involved are still poorly understood because of the large diversity and complexity of immune responses that can be engaged(3). This project aims to study the effects of neuroinflammation on neuronal network activity and synaptic plasticity in mouse hippocampus and to highlight the molecular and cellular inflammatory actors related to cognitive disorders. We are particularly interested in inflammatory processes developed during experimental autoimmune encephalomyelitis (EAE), a mouse model of multiple sclerosis induced by a specific autoimmune reaction against myelin sheaths of neurons leading to demyelination and

motor disorders. We use EAE as a model of CNS chronic neuroinflammatory disease to analyze the possible implication of the NFκB pathway and glial cells in synaptic plasticity dysfunctions and in neuronal network functioning during neuroinflammatory diseases. The chronic course of EAE allows us to dissociate the different inflammatory steps of the disease (relapsing versus remitting stage) and to analyze more precisely their impact on cognition. Only few studies using EAE as an experimental model have analyzed the hippocampal integrity and they show conflicting results(4–6). Hippocampal synaptic plasticity was analyzed during the course of EAE by ex vivo electrophysiological recordings (LTP) made on acute hippocampal slices from EAE mice. LTP measurements showed that the level of potentiation is higher at the peak of EAE but progressively decreases during the remission phase when motor symptoms improve. This suggests a time dependent impairment of hippocampal plastic potential during the EAE remission stage. A cognitive impairment was also demonstrated in vivo during this remission stage by evaluating the learning and memory capacities of remitting mice with contextual fear conditioning. Although myelin is the main target of the immune reaction during EAE, no modification of MBP expression was found by western-blotting and immunohistochemistry in mouse hippocampus at any stage of EAE. Besides the lack of demyelination, the structural integrity of the hippocampus was also unaffected during EAE as no atrophy, inflammatory infiltrates or dendritic area modification were found. However, our immunostainings and ELISA experiments revealed a higher glial activation and a production of inflammatory factors like IL1β or TNFα in the hippocampus of EAE mice. The number of both astrocytes and microglial cells follows the disease progression as it enhances at the peak of the disease and then decreases during the remission stage. So, although motor impairments are the main symptoms of EAE, we demonstrated that immune responses and neuroinflammation associated to EAE can also affect cognitive structures like hippocampus and can lead to cognitive impairments during the course of the disease. Taken together, our results suggest that, as no demyelination occurs, activated microglia and astrocytes could be linked to modifications of hippocampal synaptic plasticity during EAE and could therefore be important actors implicated in cognitive disorders related to neuroinflammation. The next step of the project will be to investigate the implication of the NFκB signaling pathway in the hippocampus during EAE thanks to an inducible adenoviral vector system which will allow us first to visualize the hippocampal NFκB expression and then to induce a negative feedback to inhibit its own activity.

Summary for lay people Many neuroinflammatory diseases are characterized by cognitive impairments which are now a major problem in our society, becoming more and more common and disabling. This study will help to better understand how neuroinflammation can affect our memory processes. We will attempt to identify some inflammatory factors which could play a key role in cognitive disorders related to neuroinflammatory diseases. These one could then be the subject of further investigations

for the development of new therapeutic approaches aimed at improving cognition in patients suffering from neuroinflammatory disorders.

Résumé en français: Le cerveau comme les autres organes de notre corps est soumis à l'inflammation. Cette neuro-inflammation peut provoquer des troubles de la mémoire, une atteinte débilitante de plus en plus répandue. Ce travail a pour but de mieux comprendre comment la neuro-inflammation attaque les mécanismes cellulaires qui nous permettent d'acquérir cette mémoire. Le but ultime étant de trouver de nouvelles approches thérapeutiques permettant si pas d'améliorer, de ralentir cette perte de mémoire.

Samenvatting in het Nederlands : Zoals de andere organen van ons lichaam zijn de hersenen vatbaar voor ontstekingen. Deze neuro-ontstekingen kunnen geheugenstoringen veroorzaken, een steeds meer voorkomende aandoening. Dit werk heeft tot doel beter te begrijpen hoe neuro-ontstekingen een aantasting vormen voor de cellulaire mechanismen die ons geheugen vorm geven. Het ultieme doel is nieuwe therapeutische benaderingen te vinden die toelaten deze vorm van geheugenverlies te verminderen of te vertragen.

**Keywords: Experimental autoimmune encephalomyelitis, HIPPOCAMPUS AND MEMORY, Neuroinflammation, Synaptic plasticity (LTP/LTD), Microglia, nfkb pathway**

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# Electrophysiological evidence for a deficit in distinguishing facial expressions of contempt and disgust in social anxiety disorder

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Social anxiety disorder (SAD) is associated with an altered processing of social threat, notably emotional facial expressions (EFE) signaling disapproval or hostility. Recent results suggest that disgust may have a negative value for individuals with SAD. Originally a food-related expression, it may also be interpreted as a form of social disapproval, close to the concept of contempt. The aim of this study was to investigate whether the ability to distinguish disgust and contempt EFE is disturbed in SAD. To this aim, 15 individuals (mean age 20,84 ± 1,52) with high social anxiety (HSA) and 15 with low SAD score (LSA) performed an oddball paradigm with EFE displaying sadness, disgust or contempt. Behavioral results showed a main effect of anxiety, HSA subjects producing more correct responses suggesting an enhanced ability to discriminate EFE. Regarding ERPs, we evidenced enlarged amplitudes of P1 and N170 in SAD. Finally, results showed an N2b-P3a complex negatively modulated by social anxiety suggesting lower detection of EFE deviance in HSA subjects. In conclusion, SAD isn't only characterized by a distinction between disgust and contempt, while the subjects discriminate both EFEs.

Résumé en français: Titre: Mise en évidence d'une incapacité à distinguer les visages exprimant le mépris des visages exprimant le dégout chez les patients souffrant d'anxiété sociale. L'anxiété sociale résulte d'une peur persistante d'être observé par les autres et de se retrouver dans une situation embarrassante ou humiliante. Ce désordre est associé à une mauvaise interprétation des expressions faciales à connotation émotionnelle négative, comme le dégout, la colère, le mépris, la peur ….. Dans notre étude nous avons mis en évidence une perturbation de la capacité à distinguer les visages exprimant le dégout de ceux exprimant le mépris chez les patients souffrant d'anxiété sociale. Cette perturbation pourrait être à l'origine d'un sentiment de détresse lors des interactions sociales.

Samenvatting in het Nederlands: Het aantonen van de onbekwaamheid tot onderscheiden van gezichten die misprijzen uitdrukken van gezichten die afschuw uitdrukken bij patiënten met een sociale angststoornis. Sociale angststoornis berust op een doorgedreven angst door anderen bekeken te worden en in een vervelende of

vernederende of situatie terecht te komen. Deze afwijking heeft te maken met een verkeerde interpretatie van gelaatsuitdrukkingen met een negatieve emotionele connotatie zoals daar zijn: misprijzen, woede, afschuw, angst… In onze studie hebben we aangetoond dat het mogelijk is niet in staat te zijn gelaatstrekken die afschuw uitdrukken te onderscheiden van misprijzende gelaatsuitdrukkingen, bij patiënten die lijden aan sociale angststoornis. Deze stoornis zou aan de oorsprong kunnen liggen van een gevoel van onmacht binnen sociale interacties.

**Keywords: Social Anxiety Disorder, disgust, Contempt, Event Related Potentials, emotional facial expressions**

# Neural mechanisms of encoding and maintenance of emotional faces in social anxiety disorder : An ERP study with an N-back task

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Social Anxiety Disorder (SAD) is associated with an automatic orientation of attention towards emotional stimuli. This phenomenon called attentional bias could reduce the available resources for more complex cognitive processes. In this study, we tested the impact of these attentional biases on the perceptual processing (reflected by the P100, N170 and P200) of angry faces and on the working memory abilities (reflected by the P300) in anxious individuals. To this aim, we compared 24 SAD and 25 control individuals during emotional N-Back tasks where participants were asked to remember either the identity or the emotion (angry, happy or neutral) of three different faces. ERPs potentials were recording during the tasks. Results showed enhanced P200 amplitudes in SAD group in both emotional and identity conditions, in the one and two-back tasks. However, no differences were noticed between both groups regarding to behavioural responses, despite the improved perceptual treatment recorded for all faces. Further studies are needed to clear up the dissociation between ERPs modulations and behavioural responses in SAD population.

Résumé en français Titre: Le fonctionnement cérébral impliqué lors de la mémorisation de visages émotionnels dans l'anxiété sociale : une étude par électro-encéphalogramme Les patients présentant de l'anxiété sociale (AS) orienteraient automatiquement leur attention sur les éléments émotionnels de leur environnement, phénomène appelé « biais attentionnel «. Dans cette étude, nous avons testé l'impact de ce biais sur les différentes étapes du processus de mémorisation à court terme de visages "menaçants". Pour cela, nous avons comparé les performances de sujets présentant une anxiété sociale à 25 sujets contrôles lors d'une tâche de mémoire à court terme. Les résultats ont mis en évidence un fonctionnement cérébral différent lors de la perception des visages chez les sujets AS, sans que ceci n'influence leurs performances.

Samenvatting in het Nederlands: Titel: Het functioneren van de hersenen bij het memoriseren van emotionele gelaatsuitdrukkingen bij sociale angststoornis: een studie op basis van elektro-encefalogrammen. Patiënten die lijden aan sociale angststoornissen richten hun aandacht automatisch op emotionele elementen in hun omgeving, dit

fenomeen heet "aandachtsafwijking". In deze studie hebben we de impact van deze afwijking getest tijdens de verschillende stappen binnen het proces van memorisatie op korte termijn van bedreigende gelaatsuitdrukkingen. Daartoe hebben we een vergelijking gemaakt tussen de resultaten van personen met een sociale angststoornis en een controlegroep van 25 personen tijdens een korte termijn geheugentest. De resultaten toonden aan dat bij personen met een sociale angststoornis de hersenen anders functioneren bij de perceptie van gelaten, echter zonder invloed op hun prestaties.

**Keywords: Event-related potentials, Social Anxiety Disorder, working memory, Perceptual bias, attentional bias, facial expressions, emotional faces, n-back task**

# Effect of anxiety on inhibition processes and emotional judgment of social and non-social stimuli in a non-clinical sample using antisaccade and decision tasks

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Abstract: Cognitive biases have been highlighted in anxiety disorders impairing inhibition function and emotional evaluation. This study aimed to provide evidence for these deficits in a non-clinical sample. 118 students (aged 18 to 31) were submitted to an anti-saccade task to assess inhibition processes (Derakshan et al., 2009). They had to focus their attention toward a target according 3 conditions: non emotional stimuli (white ovals), emotional social stimuli (emotional facial expressions, EFE) or emotional non social stimuli (animal/plant eliciting positive/negative valence). Correct answers' (CA) rates and reaction times (RT) were recorded. According to Eysenck's attentional control theory (Eysenck et al., 2007), we hypothesized an effect of anxiety on efficiency (longer RT) but not on effectiveness (CA). To assess emotional evaluation, participants had also to perform a decision task by identifying emotion, valence and arousal of displayed EFEs. In the line of Mogg and Bradley studies (Mogg & Bradley, 1998), we expected a negative interpretation of neutral stimuli and an enhanced evaluation of negative ones. Results will be presented and discussed based on literature findings.

Résumé en français: Les troubles anxieux sont caractérisés par des dysfonctionnements cognitifs et émotionnels. Notre étude investigue ces processus chez 118 étudiants à travers des tâches qui consistaient à porter leur attention vers des cibles non émotionnelles (formes géométriques), socio-émotionnelles (visages exprimant une émotion) ou émotionnelles mais non sociales (animaux provoquant des émotions positives ou négatives) et à identifier l'émotion des visages présentés. Nous attendons une réactivité plus lente et des interprétations plus négatives des expressions faciales émotionnelles en lien avec l'anxiété. La mise en évidence de tels résultats permettrait d'établir des programmes pour ré-entraîner ces fonctions et aider à pallier ces difficultés.

Samenvatting in het Nederlands: Effect van de angst op aandachts- en emotionele processen bij willekeurige studenten. Angststoornissen zijn gekenmerkt door cognitieve en emotionele functiestoornissen. Onze studie onderzoekt dit proces bij 118 studenten die taken kregen waarbij hun aandacht gericht werd op niet emotionele (geometrisch

vormen), socio-emotionele (emotie uitdrukkende gelaatstrekken) of emotionele maar niet sociale (dieren die positieve of negatieve emotie uitlokken) aandachtspunten. En waarbij de emotie van het voorgesteld gelaat diende geïdentificeerd. Wij verwachten een kortere reactietijd en meer negatieve interpretaties bij de emotionele gelaatsuitdrukkingen die met angst te maken hebben. Als dit kan aangetoond worden zouden programma's kunnen ontwikkeld worden om deze functies te herstellen en de moeilijkheden te lenigen.

**Keywords: Inhibition processes, attentional bias, Emotional judgment, antisaccade paradigm, Attentional control theory, Efficiency, effectiveness, Anxiety, emotional facial expression**

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# Neuropsychology and psychopathy: study on a sample of Belgian forensic patients

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Studies investigating the intelligence construct revealed an association between psychopathy and the Intellectual Quotient (Hampton, Drabick, Steinberg, 2014). To our knowledge, no study has yet measured the intellectual profile of patients with the Comprehensive Assessment of Psychopathic Personality (Cooke, Hart & Logan, 2004). Indeed, the CAPP-IRS offers a new perspective with the development of the cognitive aspect. We administered the Wechsler Adult Intelligence Scale – IV among 20 male adult forensic patients (Wechsler, 2008). The results will be discussed in light of the international literature on psychopathy.

Résumé en français Titre: Psychopathie et intelligence vont-elles de pair ? La psychopathie est un trouble de la personnalité caractérisé par un comportement antisocial et une absence de remord. Certaines études ont montré que les psychopathes avaient un quotient intellectuel élevé. Notre étude a pour but d'étudier cette relation entre psychopathie et intelligence en analysant le niveau d'intelligence de patients placés en psychiatrie médico-légale.

Samenvatting in het Nederlands:Titel: Gaan psychopathie en intelligentie samen? Psychopathie is een persoonlijkheidsstoornis gekenmerkt door antisociaal gedrag en gebrek aan schuldgevoel. Bepaalde studies toonden aan dat psychopaten een hoog intelligentiequotiënt hadden. Onze studie heeft tot doel deze relatie tussen psychopathie en intelligentie te bestuderen, door analyse van het intelligentieniveau van patiënten die in forensische psychiatrische instellingen verblijven.

**Keywords: psychopathy, Intelligence, Forensic Psychiatry, Neuropsychology, CAPP-IRS**

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# Implementation of Miyake task in psychopathic forensic sample: an exploratory research

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Much research has been done on the links between the concept of psychopathy and executive functions. However, to our knowledge, study has yet assess the executive function tasks among psychopathic population using Miyake Task. The Miyake's model evaluates three executive functions: shifting, updating, and inhibition (Miyake, & al, 2000). We administered the computer battery and the PCL-R (Hare, 2003) to forensic male inpatients (N = 20). We hypotheses that facets 3 and 4 of the PCL-R is more related to the tasks performances than the two others facets. We will discuss the implementation of Miyake's tasks among the forensic population.

Résumé en Français: Titre: Analyse des capacités mentales de haut niveau des patients psychiatriques médico-légaux. Les fonctions exécutives sont des fonctions mentales de haut niveau permettant d'organiser, de planifier et d'élaborer des stratégies. Ces fonctions peuvent être testées en mesurant la flexibilité, c'est-à-dire la capacité de changer de stratégie, la mise à jour, c'est-à-dire la capacité d'intégrer des nouvelles informations dans sa réflexion et l'inhibition, c'est-à-dire la capacité de retirer de son processus de réflexion des informations non pertinentes. L'ensemble de ces fonctions sont étudiées chez des patients placés en psychiatrie médico-légale afin de mieux comprendre leurs dysfonctionnements.

Samenvatting in het Nederlands: Titel: Analyse van de hogere mentale vaardigheden bij forensische psychiatrische patiënten. Uitvoeringsfuncties zijn mentale functies van hoog niveau die toelaten strategieën te organiseren, plannen en uit te werken. Deze functies kunnen getest worden door het meten van de flexibiliteit, t.t.z. de bekwaamheid om van strategie te veranderen, om een strategie bij te sturen op basis van nieuwe informatie, en de vaardigheid om in het denkproces niet pertinente informatie uit te sluiten. Het geheel van deze functies werden onderzocht bij patiënten opgenomen in de gerechtspsychiatrie, teneinde beter inzicht te krijgen in hun disfunctioneren.

**Keywords: psychopathy, Executive Function, Miyake Task, Forensic Psychiatry, PCL-R**

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# Psychopathy and cognition: deficits in contextual information processing

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Psychopathy is characterized by a particular processing of peripheral information that is secondary to the primary focus of attention (Vital et al, 2015). Authors have highlighted that psychopaths would not pay attention to the contextual information which could modulate their behaviour (Hallé, Hodgins & Roussy, 2000). This deficit is situation-specific (Newman, Schmitt & Voss, 1997). Our study evaluates the processing of contextual information in relation to attentional components among forensic population. The participant' performances would be negatively correlated to the PCL-R. The results will be discussed in light of the international literature.

Résumé en français: Psychopathie et cognition : déficits en traitement de l'information contextuelle. La psychopathie est caractérisée par un traitement particulier des informations périphériques au centre d'attention premier. Des auteurs ont mis en évidence que les individus psychopathiques ne traiteraient pas les informations contextuelles qui permettent de moduler leur comportement. Ce déficit se limite à certaines situations. Notre étude vise l'évaluation du traitement des informations contextuelles, en lien avec les composantes attentionnelles, chez des internés. Les performances de ces sujets seraient négativement corrélées à l'échelle de psychopathie de Hare. Les résultats seront discutés au regard de la littérature internationale.

Samenvatting in het Nederlands: Psychopathie en cognitie: gebreken in de verwerking van contextuele informatie. Psychopathie wordt gekenmerkt door een bijzondere verwerking van perifere informatie binnen de primaire aandacht. Bepaalde auteurs hebben erop gewezen dat psychopathische individuen contextuele informatie die het gedrag regelen niet verwerken. Dit gebrek uit zich enkel in bepaalde situaties. Onze studie richt zich op de evaluatie van contextuele informatieverwerking, in samenhang met de aandachtscomponenten, bij geïnterneerden. De prestaties van deze personen zouden een negatieve correlatie hebben met de psychopathieschaal van Hare. De resultaten zullen bediscussieerd worden, rekening houdende met de internationale literatuur

**Keywords: psychopathy, Contextual Processing, Attention, Forensic Psychiatry, Information Processing**

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# Extracellular vesicles from the choroid plexus propagate a pro-inflammatory message in the CNS upon peripheral inflammation

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Introduction. It is still unknown how the periphery communicates with the central nervous system (CNS) and vice versa, in normal as well as pathophysiological conditions. Here, we hypothesized that the choroid plexus epithelium (CPE), a unique single layer of epithelial cells situated at the interface of the blood and the cerebrospinal fluid (CSF) which forms the blood-CSF barrier, might be equipped to do this. In recent years, the blood-CSF barrier has gained increasing attention, especially its role in inflammatory and neurodegenerative diseases. Our lab recently showed that systemic inflammatory conditions such as sepsis compromise blood-CSF barrier functionality in vivo, allowing components of the blood to gain access into the CNS via the CSF. Methods: Several high-throughput technologies, such as NanoString and advanced mass spectrometry (MS) were used, together with in vitro and in vivo qPCR, western blot, and immunohistochemistry analyses. Results: Here, we found that systemic inflammation induced a fast decrease in miRNA expression levels in the CPE and this was inversely correlated with increased miRNAs levels in the CSF, such as the pro-inflammatory miRNAs miR146, miR155, miR9 and miR1a. This was linked with an increase in amount of extracellular vesicles (EVs) in the CSF. Using transmission electron microscopy (TEM), we also observed in the CPE cells a time-dependent increase in multivesicular bodies (MVBs) filled with EVs, called exosomes, upon inflammatory stimulation in vivo. In vitro and in vivo studies revealed that these secreted EVs are taken up by brain parenchymal cells and are able to transfer a message from the blood to the CNS. Proteomic analysis of EVs isolated from the CSF revealed that they also carry proteins that act as key signaling molecules in the recipient cells. In vitro and in vivo pharmacological inhibition of the exosome production reduced the inflammation-induced exosome release by the CPE cells, resulted into accumulation of several miRNAs in the CPE cells and had an anti-inflammatory effect on the recipient brain cells. Conclusion. In conclusion, we identified CPE-derived EVs as a new mechanism of blood-CNS communication upon peripheral inflammation by transferring a pro-inflammatory message from the blood-CSF barrier to recipient brain cells.

Résumé en français: La barrière hémato-encéphalique isole, mais de manière sélective, le cerveau du sang et de ses constituants, éventuellement toxique. Cette barrière est

constituée de cellules tapissant l'interface entre le sang et les ventricules du cerveau. Dans les cas de maladies neurodégénératives, caractérisées par une neuro-inflammation chronique, cette fonction d'isolement sélectif est altérée. Dans ce travail nous mettons en évidence, par des techniques modernes telles que le haut débit et la spectrométrie de masse avancée la propagation de vésicules extracellulaires (exosomes) qui transmettent des messages pro-inflammatoires des cellules de la barrière vers les cellules du cerveau.

Samenvatting in het Nederlands: de hemato-encefalische afscherming isoleert, op een selectieve wijze, het hersenbloed van de eventueel toxische stoffen die het bevat. Deze afscherming bestaat uit cellen die een beschermlaag vormen tussen het bloed en de hersenholtes. Bij neurodegeneratieve ziektes, gekenmerkt door chronische ontsteking, is deze isoleringsfunctie verstoord. In dit werk tonen we aan, door gebruik te maken van moderne technieken als daar zijn breedband en geavanceerde massa spectrometrie, dat extracellulaire blaasjes (exosomen) zich verspreiden en de ontstekingsboodschappen van de cellen uit de beschermlaag naar de hersencellen overbrengen.

**Keywords: Sepsis, Blood-Brain Barrier, Choroid Plexus, extracellular vesicles, miRNA**

# Proteins and neurodegenerative diseases

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Most neurodegenerative diseases are characterized by the accumulation of proteins in the CNS. For unknown reasons, the proteins which form part of the CNS start to accumulate, aggregate and become "toxic" for the brain. Depending on the region of the brain in which this accumulation takes place, we see the appearance of different neurodegenerative diseases. Why do these "good" proteins become killers? Are all these diseases different or are they just different clinical expressions of the same neuropathological process? Can understanding this pathological process lead us towards a treatment one day? The answers in the years to come…

Résumé en français: La plupart des maladies neurodégénératives sont caractérisées par l'accumulation de protéines dans le cerveau. Pour des raisons encore mal comprises, des protéines utiles pour notre cerveau commencent soudainement à s'accumuler et à devenir toxiques. En fonction de la protéine concernée et de l'endroit du cerveau où elle s'accumule, différentes maladies neurodégénératives apparaissent. Le Dr Elosegi reviendra sur ces différentes pathologies et sur toutes les questions qui restent aujourd'hui sans réponses et qui font l'objet de recherches intenses au sein des laboratoires belges et internationaux.

Samenvatting in het Nederlands: De meeste neurodegeneratieve ziektes zijn gekenmerkt door de opeenstapeling van proteïnes in de hersenen. Omwille van redenen die nog altijd niet goed gekend zijn, beginnen nuttige proteïnen zich plots op te stapelen en toxisch te worden. In functie van de betrokken proteïne en de plaats in de hersenen waar ze zich opstapelt, ontstaan diverse neurodegeneratieve ziektes. Dr. Elosegi zal terugblikken op deze diverse pathologieën en op alle onbeantwoord blijvende vragen die momenteel in Belgische en internationale laboratoria onderzocht worden.

**Keywords: Neurodegenerative Diseases, toxit proteins, neuropathological process, Alzheimer Disease, Parkinson Disease**

# Non-drug therapies for Alzheimer's patients

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The main objective of our talk will be to present principal, recent, non-drug therapies which are currently proposed in order to reduce behavioural and cognitive difficulties encountered by Alzheimer's disease patients. In synthesis, four approaches can be isolated: -Person-centered therapies - interventions on informal caregivers - interventions on formal caregivers' interventions - Context and environment-centered therapies We will present some results concerning each of these approaches and we will conclude by promoting more integrated, articulated interventions.

Résumé en français: Traitements non médicamenteux pour la maladie d'Alzheimer. L'objectif principal de notre intervention sera de présenter les principaux traitements récents non médicamenteux proposés dans le but de résoudre les difficultés comportementales et cognitives que connaissent actuellement les patients Alzheimer. Pour résumer, on peut distinguer quatre approches : les thérapies centrées sur la personne – les interventions auprès des aidants naturels – les interventions relatives aux interventions des aidants naturels – Les thérapies centrées sur le contexte et l'environnement. Nous présenterons des résultats concernant chacune de ces approches et conclurons en faisant la promotion des interventions mieux articulées et intégrées.

Samenvatting in het Nederlands: Het hoofddoel van ons gesprek is het toelichten van de belangrijkste en meest recente behandelingen zonder medicatie die momenteel worden voorgesteld om gedrags- en cognitieve problemen bij alzheimerpatiënten te verminderen. Samengevat kunnen vier benaderingen worden onderscheiden: -cliëntgerichte therapieën - interventies bij informele mantelzorgers - tussenkomst bij interventies van formele mantelzorgers - contextuele en omgevingsgerichte therapieën. We bespreken enkele resultaten van elke benadering en besluiten met het aanbevelen van meer geïntegreerde, duidelijk uitgesproken interventies.

**Keywords: Alzheimer, therapies, Psychology, Caregivers, Cognition Disorders**

# Personalised Medicine for Brain Disorders

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Personalised medicine holds the promise for prediction, prevention and treatment of illness that is tailored to individuals' needs. The vision is that the therapeutic paradigm will shift from 'one-fits all' and 'trial-and-error' prescription to a personalised concept of prevention and treatment targeted to the unique characteristics of an individual. In recent years, the advent of new technologies for detailed biological profiling of individuals at the molecular level have been crucial in opening the way toward personalised medicine and in many contexts, we have already started to move from treating diseases to treating individuals. In the context of brain disorders, which comprise a variety of complex diseases of the nervous system, including psychiatric, neurological, and neurosurgical conditions, the availability of more detailed information, obtained from 'omics' data has offered the possibility of dissecting the genetic or epigenetic components of these disorders and opened the door to personalised medicine. For instance, in the area of neurological disorders such as Huntington's disease, new interventions are being developed targeted to lower the levels of the abnormal huntingtin protein, which causes the disease. In the field of neuro-oncology, the identification of a number of mutations allows an elaborated genetic analysis of brain tumours and opens the door to individualised therapies. Finally, in psychiatry, new approaches are being developed to leverage genetic information to predict patients' responses to treatment for psychiatric disorders. Despite these exciting developments, the discipline is facing some formidable scientific, policy, and ethical challenges that need to be addressed in order to translate the scientific discoveries responsibly into clinical applications for the benefit of patients [1]. Personalised neuro-medicine, with its promise of better and more precise treatment for individuals, calls for increased support for curiosity-driven research into the mechanisms of normal brain functioning as well as challenging adaptations of health care and research infrastructures, encompassing legal frameworks for analysing large amounts of personal data, a flexible regulatory framework for correlating big data analyses in cooperative networks between academia and the drug development industry, and finally new strategies for brain banking in order to increase access to brain tissue samples.

Résumé en français: La médecine personnalisée, également appelée médecine de précision, a pour but d'améliorer la performance des soins, d'éviter des traitements inutiles et d'améliorer la qualité de vie des patients. La mise en place d'une telle médecine dépend de l'accès à des données de type génétique comme le séquençage du génome

de chaque patient mais aussi de données sur l'environnement et le mode de vie. En neuro-médecine, qui couvre un large spectre de maladies du système nerveux, incluant les maladies mentales, neurodégénératives et neurologiques, les progrès sont plus lents qu'en oncologie. Néanmoins, le challenge est relevé et l'espoir de voir de meilleurs traitements s'appliquer permettra d'affronter avec flexibilité les obstacles éthiques, ceux de la coopération entre le monde académique et industriel et le difficile passage de la recherche vers la clinique.

Samenvatting in het Nederlands: Gepersonaliseerde geneeskunde, ook precisiegeneeskunde genoemd, heeft tot doel de zorg te verbeteren, onnodige behandelingen te vermijden en de levenskwaliteit van de patiënt ten goede te komen. Het invoeren van dergelijke geneeskunde is afhankelijk van het beschikbaar zijn van genetische informatie (het genoom van elke patiënt) maar ook gegevens uit de omgeving en betreffende de levensstijl. In de neurogeneeskunde, die een breed spectrum aan ziektes van het zenuwstelsel, inclusief geestelijke, neurodegeneratieve en neurologische ziektebeelden, verloopt deze evolutie trager dan bij de oncologie. De handschoen wordt echter opgenomen en het vooruitzicht betere behandeling te kunnen bieden moet ons in staat stellen op een flexibele manier de ethische bezwaren aan te pakken, evenals obstakels tot samenwerking tussen de academische wereld en de industrie uit de weg te ruimen om te komen tot een vlotte doorstroming van onderzoek naar klinische praktijk.

**Keywords: Brain Disorders, neuro-medicine, individualized therapy, Brain banking, Genetic Markers**

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# Demonstrating the 'Value of Treatment' for brain disorders in Europe

Vinciane Quoidbach[1], Giovanni Esposito[1] and Frederic Destrebecq[1*]

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In recent years, greater emphasis was provided on the importance of mental and neurological disorders. Conditions such as depression, stroke, dementia, schizophrenia or anxiety affect at least one in three people during their lifetime – currently 165 million people in Europe[1]. And the global burden of brain disorders is rising, accounting for 35% of the burden of all diseases in Europe [2]. According to WHO estimates, major depression is projected to be the leading cause of disease burden worldwide by 2030 [3]. Besides, studies have demonstrated that there is a considerable treatment gap with only about a third of cases receiving the therapy or medication needed [4]. Reducing the burden of these disorders requires better-targeted interventions such as timely diagnosis and treatment. Amidst the current reforms of European health systems and the challenges related to the management of chronic conditions, new models of care -that include a societal benefits approach- are being considered by national governments with the ultimate aim to ensure a better coordination of care. It is within this context that the EBC current project on the Value of Treatment (2015-2017) is aiming at identifying (cost)-effective interventions in clinical practice; developing and validating an overarching model of care for brain disorders, and; providing policy recommendations for the adoption and implementation of a patient-centred and sustainable coordinated care model for brain disorders in Europe. Demonstration of the need for such new paradigm was provided on the occasion of a workshop organised in the Belgian Parliament on 11th April 2016 under the auspices of Ms Yoleen Van Camp MP and Mr Damien Thiéry MP to focus on the particular case of drug-resistant epilepsy. The treatment gap for such condition rises to 80% and more should be done to address the high number of people who live with ongoing seizures. The conclusions of EBC's study are much awaited to optimise the model of care for epilepsy in Belgium and in other countries. Analysis of data to pull out the evidence are being conducted by experts within the network of EBC member organizations in coordination with relevant local stakeholders such as patient groups, professional societies and health authorities. In this respect, the role of National Brain Councils will appear to be crucial in the implementation of these recommendations.

Résumé en français: Titre: Démontrer la 'valeur du traitement' pour les désordres cérébraux en Europe Jusqu'à récemment, une attention plus grande a été accordée à l'importance des désordres mentaux et neurologiques. Au-delà de leur prévalence forte (1 personne sur 3, soit 165 millions de personnes en Europe), les troubles liés

au cerveau connaissent un écart de traitement (« treatment gap ») assez important selon de récentes études. Cet état de fait incite à une réflexion plus profonde quant au modèle de soins, au sein du processus actuel de réforme des systèmes de soins de santé. C'est dans ce contexte que le « Conseil européen du Cerveau » (« European Brain Council » - EBC) a lancé un projet d'étude sur la valeur du traitement (2015-2017) afin d'identifier les interventions cliniques efficientes ; de développer et valider un modèle de soins pour les troubles cérébraux, et ; fournir les recommandations politiques pour la mise en œuvre d'un modèle de soins soutenable, coordonné et centré sur le patient pour les troubles cérébraux en Europe. Ce nouveau paradigme a été présenté à l'occasion d'un atelier organisé au Parlement belge le 11 avril 2016 sous les auspices de Madame la députée Yoleen Van Camp et Monsieur le député Damien Thiéry sur le cas particulier de l'épilepsie pharmaco-résistante. De même et afin d'exploiter au mieux les conclusions de cette étude, des experts de tout bord au sein des organisations membres d'EBC ainsi que des organisations nationales –tels que les conseils nationaux pour le cerveau (« National Brain Council » - NBC) et le conseil belge en particulier (« Belgian Brain Council » - BBC)- seront amenés à apporter leur contribution.

Samenvatting in het Nederlands: Titel: Het "belang van het behandelen" van hersenziektes binnen Europa aantonen. Tot voor kort werd steeds meer belang gehecht aan de geestelijke en neurologische ziektes. Ondanks het feit van de toenemende prevalentie (1 persoon op 3, of 165 miljoen Europeanen) blijkt uit recente studies dat de behandeling van hersenziektes ontoereikend is (treatment gap). Deze feitelijke vaststelling brengt ons bij de meer fundamentele bedenking over ons zorgmodel, binnen de huidige hervorming van de gezondheidszorgsystemen. Het is binnen deze context dat de Europese Vereniging voor de Hersenen (European Brain Council - EBC) een studieproject opzette over het belang van het behandelen (2015/2017) om efficiënte klinische aanpak te identificeren, een zorgmodel voor hersenproblemen te ontwikkelen en te valideren en om beleidsaanbevelingen te geven tot het uitbouwen van een werkzaam gecoördineerd zorgmodel in Europa. Dit nieuwe paradigma werd voorgesteld tijdens een werkvergadering in het Belgisch Parlement op 11 april 2016, georganiseerd met de steun van parlementsleden Yoleen Van Camp en Damien Thiéry, en was specifiek gericht op medicatie resistente epilepsie. Om de resultaten van deze studie beter bekend te maken wordt beroep gedaan op deskundigen binnen de European Brain Council evenals binnen de diverse Nationale Brain Councils (NBC's).

**Keywords: Brain Disorders, ebc, Value of Treatment, Treatment gap, National Brain council**

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# Addressing brain health in a holistic, integrated and collaborate manner

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Contemporary public health challenges are complex and interlinked. Addressing them at all levels – individual, institutional, community, local or national – requires strategic and coordinated initiatives to integrate and ensure coherence between the many different sectoral policies, which are relevant to keep individuals and populations healthy. This is particularly true in the field of brain health. Brain disorders will affect at least one in three people during their lifetime – 179 million people in Europe, they cost Europe around € 800 billion every year. WHO concluded that they account for 35% of the burden of all diseases in Europe and are predicted to become the major medical need of the 21st century. Recognizing these challenges, the closing conference of the European Month of the Brain in May 2013 delivered a call to "develop, or refine, national strategies on brain research and healthcare within an overarching European context". Building on this, the European Brain Council launched in 2015 a Call to Action, calling for development of National Brain Plans (NBP), brought under the umbrella of an EU-wide plan addressing brain health in a comprehensive, integrated and collaborative way. A NBP is a public health programme bringing together multiple stakeholders in the national brain space such as scientific community, patient organizations, research funding agencies, national governments, academic institutions, advocacy organizations and health providers in order to streamline and optimize the use of existing resources, coordinate sectoral policies and address horizontal thematic areas such as prevention, stigma, treatment, research, support for carers, health economics or education. This approach can help properly address the huge challenges posed by brain disorders by streamlining and making the best use of available resources. The ultimate goal of NBPs is reducing the burden of brain disorders through improving the quality of life of those living with a brain disorder. A Taskforce of National Brain Councils task was established by EBC to foster the development of NBPs in interested countries. EBC study Value of Treatment, scheduled to be published in 2017, will provide further evidence supporting the Call by generating evidence on the socio-economic benefits of healthcare interventions through analysis of case studies in order to build towards closing this treatment gap and develop a workable model of care for brain disorders in Europe.

Résumé en français: Titre: S'attaquer à la santé cérébrale de manière holistique, intégrée et collaborative Les défis de santé publique actuels sont complexes et intimement liés. Afin de s'y attaquer, une approche à tout niveau –individuelle, institutionnelle, locale

ou nationale- est nécessaire afin de maintenir la santé des personnes et d'assurer coordination et cohérence entre les différentes politiques sectorielles nécessaires à cette fin. Ce besoin est particulièrement criant dans le domaine de la santé cérébrale. C'est dans ce cadre que le « Mois européen du Cerveau » organisé en mai 2013 avait appelé au développement de stratégies nationales sur la recherche et la santé cérébrales dans un cadre européen. Sur cette base, le « Conseil européen du Cerveau » (« European Brain Council » - EBC) a lancé un appel au développement de plans nationaux pour le cerveau (« National Brain Plans ») sous l'égide d'un plan européen, afin d'adresser les problématiques liées à la santé cérébrale de manière complète, intégrée et collaborative et de réduire le fardeau lié aux multiples désordres cérébraux.

Samenvatting in het Nederlands: Titel: Een holistische, geïntegreerde en collaboratieve aanpak van de cerebrale gezondheid. De uitdagingen waarmee de volksgezondheid thans geconfronteerd wordt zijn complex en staan in nauw verband met elkaar. Om deze uitdagingen het hoofd te bieden is er nood aan een aanpak op alle niveaus – individueel, institutioneel, lokaal of nationaal- teneinde de gezondheid te waarborgen en teneinde beleid in diverse sectoren op elkaar af te stemmen. Dit is uitermate nodig binnen de gezondheid van de hersenen. Binnen dit kader werd in 2013 tijdens de" maand van de hersenen" een oproep gedaan om in een Europees kader nationale strategieën te ontwikkelen betreffende het onderzoek en de cerebrale gezondheid. De Europese Hersenvereniging (European Brain Council EBC) lanceerde een oproep tot het ontwikkelen van Nationale actieplannen voor de hersenen (National Brain Plan), onder de vleugels van een Europees plan, om aldus de problemen van de hersenen op een complete, geïntegreerde collaboratieve wijze aan te pakken en de last die vele van deze hersenziektes met zich mee brengen te lenigen.

**Keywords: National Brain Plan, Brain health, European Union, European Month of the Brain, European Brain Council, National Brain Councils, Value of Treatment**

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# Ten good reasons to invest in the Belgian brain council … and the brain !

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1. The BBC is a unique national platform that federates scientific societies, patients associations and industry representatives with one common objective: make all efforts to reduce the burden of brain disorders. 2. The biennial Belgian Brain Congress organized by the BBC is unique in its format because it gathers stakeholders from different disciplines (neurobiologists, neurologists, psychiatrists…) and various societal groups (patients associations, industry partners, paramedical associations). Thanks to renowned national and international lecturers, this allows discussing in a transdisciplinary strategy available and missing data on various brain disorders and their management including medico-social aspects. 3. The brain is our most precious patrimony. It makes us "human" and determines our personality. It is at the origin of any past, present and future achievement of mankind be it in medicine, economics, engineering, arts or philosophy. 4. The brain is our most complex organ. Despite great advances in the knowledge on how it is wired and functions, much more research is needed to fully understand the brain's development as well as its organisation and plasticity in health and disease. 5. It is thus of no surprise that the disorders of the brain are more complicated to analyse, diagnose and treat than other disorders. Although their management is progressing constantly, the holy grail of a cure is still out of reach for most of them. 6. Brain disorders causing neurological or psychiatric diseases (to name the most frequent ones: depression, Alzheimer's, schizophrenia, stroke, migraine, epilepsy, Parkinson's, sleep disorders, chronic pain syndromes, addiction to alcohol or other substances) cause 35% of the total disability due to human diseases. They cost as much as 45% of the annual health budget of Europe (i.e. 800 milliards € as compared to 170 milliards € for cardiovascular diseases). The cost in Belgium is estimated at 18 milliard 396 million € per year (i.e. 1672€/inhabitant/year). 7. The total cost of brain disorders steadily increases with increasing longevity of the population: it rose by 42% between 2004 and 2010 in Belgium. A common denominator of brain disorders is that, for physical and/or mental reasons, they disconnect the sufferer from social, professional and/or family life and cause enormous individual disability, economic impact and societal burden. 8. Contrasting with 35% of overall disability caused by brain disorders, public funding in Belgium of research on the brain and its disorders hardly reaches 15% of the total money spent for medical research. Despite these limited resources, Belgian brain researchers are highly productive and cost- effective (2673€ per publication) compared to other countries. 9. An American study of NIH-sponsored

trials in stroke found that 1$ invested in research is worth 5€ in disability reduction after 10 years, i.e. a yearly return of 50%! Investing in brain research is thus highly profitable, the more so in Belgium where neuroscientists are international frontrunners. 10. Healthy brains are of crucial importance for the future of human societies; challenges ahead are huge and resources have to be prioritised in order to respond to present and future needs. The planning of brain research must therefore empower patients, caregivers and as many stakeholders as possible in the society. You are part of the latter and should not stand aside!

Résumé en français: Cet article explique clairement en 10 points la vertigineuse ascension des maladies du cerveau et son impact sur la santé publique et son budget national et en quoi une association faîtière comme le Belgian Brain Council peut modifier cette «descente aux enfers» qui touche un belge sur quatre au cours de sa vie.

Samenvatting in het Nederlands: In dit artikel wordt, in 10 punten, de duizelingwekkende stijging van de hersenaandoeningen, hun impact op de publieke gezondheid en het nationaal budget uitgelegd en hoe een overkoepelende vereniging zoals de BBC deze "afdaling naar de hel", die 1 Belg op 4 treft gedurende het leven, kan beïnvloeden.

**Keywords: Belgian Health Poiicy, Belgian budget for brain diseases, National Brain Plan, Belgian Brain Council, patients associations**

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# A lack of new permanent positions threatens the future of neuroscience

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In this contribution we will discuss the drop in the number of new permanent positions available in academia and public research centers which is affecting several European countries. Our reasoning builds upon the data we mentioned in a recent letter to a leading neuroscientific journal [1]. While this problem is very well known by young researchers we have the feeling that, overall, the scientific and political communities are not fully aware of the current situation and of the dramatic long-term consequences this "downsizing" in the recruitment process will have. The duration of this severe recruitment freeze has been so long that we think it is posing a serious threat for young neuroscientists: those who are the future of neuroscience. Where does this situation come from ? Several players are involved, some of which are doing their duties and some of which are not. The good ones first: local and European institutions are regularly funding the best research projects in Europe and universities are doing their job in preparing new researchers. The responsibility of the situation can be then attributed to those countries which, by prioritizing budget over people, regrettably started to neglect their crucial role: regularly hiring new scientists with permanent or tenure track positions. The presence of funding for research in the absence of new permanent positions is resulting in a perfect storm. More and more (over)qualified researchers cannot obtain any security for their future after having contributed to several, often successful, scientific projects. This is especially worrisome for the domain of neuroscience due to its high number of temporary jobs (i.e., research projects usually involving several doctoral students and postdocs). The reduction in the offer for permanent positions is very short-sighted. It temporarily allows research activities to continue but worsens the problems on the long term. We maintain that the updated societal long-term investment in Neuroscience is better mirrored by the number of new positions opened every year rather than by the overall funding. This number plummeted, in the last years, in at least three main European countries (Italy, France and Spain). -Italy- Recruitment for new personnel in Italy (tenure-track research positions in public universities) across all disciplines decreased from more than 1500 per year between 2005 and 2010, [2] to less than 300 per year after 2010 [3]. With a retirement age at 70, the average age of a full professor in Italy is now about 60 years. In several countries there is a limit (either explicit or implicit) on the number of years a researcher can spend as a postdoc and therefore for many

the only option left is to leave the country. The outcome of the Marie Curie mobility fellowships [4] confirms this trend. Between 2007 and 2014, 615 Italian researchers moved to another European country to start their research projects, while only 147 foreign researchers moved to Italy. The best index of the ever increasing number of young Italian researchers leaving the country is probably the fact that two thirds of the young Italian researchers obtaining the prestigious starting grant from the European Research Council (ERC) are already operating abroad or in the process of moving their research abroad [5]. -Spain- Also worrisome is the fact that 703 Spanish researchers moved to another country after gaining Intra European Fellowships, while only 277 foreign researchers decided to undertake their research in Spain. Spain has lost about 14 000 researchers from 2010–2014, and 3000 in 2014 alone [6,7]. Moreover, the research and development funding in the Spanish national budget for 2016 has been reduced by 34% in comparison to 2009. As a result Spain became the European country with the major descent within the OECD [8]. Only 11% of the planned hiring of CSIC researchers between 2010 and 2013 has been fulfilled [9]. The speed by which Consejo Superior de Investigaciones Científicas (CSIC), the major public conjoint of Spanish research centers, is getting old has never been so dramatic (e.g. average age of researchers is more than 53 years) [9]. -France- Also the number of entry-level permanent positions in France (Maître de conférences) significantly decreased from a rather stable average of 2000/year (2004-2010), [10] to 1444 in 2014 and to 1299 in 2015, [11] when the minimum number of new openings in the last fifteen years has been reached. Candidates for the positions are increasing: only in 2016 the number of researchers with a PhD who qualified for participating (qualification requires a competitive CV and lasts for several years) was as high as 6000. In a nutshell, in the last few years at least three major European countries have dramatically reduced the number of researchers hired every year with permanent jobs in universities and research centers. This means that fewer and fewer young researchers have the possibility to start an independent lab and to focus on long-term achievements. This also means that these researchers will be unharmed in case of potential further cuts while society will not benefit from the professional skills they acquired thanks to past public expenditure. This situation is relevant for the Belgian context for at least two reasons. First, many foreigners researchers are now applying for scientific positions (permanent and non-permanent) in Belgium. While the advantages of an international research environment are clear this new scenario is posing a number of new challenges because foreigners do not always master French or Dutch sufficiently well to teach, help with paperwork or perform clinical activities. Second, due to the lack of positions before mentioned a Belgian researcher will regrettably have difficulties in finding a job in academia in several European countries. Finally, it would be interesting to compare the high number of temporary contracts in Belgium with the comparatively limited opportunities offered by universities and research institutions.

**Keywords: Research Policy, budget impact, Neuroscience, Science, Academies and Institutes, Universities, Research**

**Acknowledgements**

**Summary**

Science is made for people and by people. Due to dramatic budget cuts young researchers from Italy, France and Spain are not being hired by universities & public research centers with permanent contracts but only with temporary ones. The almost total absence of possibility to secure a permanent position in Academia before the age of 40 made many young and promising researchers leave their country or look for another job. It is important to raise awareness on this problem.

Résumé en français : la science est faite pour et par les gens. Les restrictions budgétaires forcent les universités et centres de recherche publics à ne plus engager de jeunes chercheurs italiens, français et espagnols sur base d'un contrat permanent mais à ne leur proposer que des contrats temporaires. La quasi-totale impossibilité de décrocher un poste académique permanent avant 40 ans a poussé de nombreux jeunes chercheurs prometteurs à quitter leur pays ou à chercher un autre emploi. Il est important d'attirer l'attention sur ce problème.

Samenvatting in het Nederlands: Wetenschap wordt door en voor mensen gemaakt. Omwille van budgettaire beperkingen kunnen de universiteiten en openbare onderzoekscentra jonge Franse, Spaanse of Italiaanse wetenschappers niet langer een lange termijn contract aanbieden, maar worden alleen korte termijn contracten voorzien. De onmogelijkheid om, voor men veertig wordt, een vaste academische positie te verwerven doet heel wat veelbelovende onderzoekers hun land verlaten of naar een andere job uitkijken. Het is belangrijk hierop de aandacht te vestigen.

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Our association was created in 2009. It brings together young neuropsychologists who come together to facilitate a space for learning, exchange and reflection but also to register neuropsychologists in a more political dimension to a recognition of the profession. Our association is changing every year since its creation and it applies to always offer more events: conferences, Intervisions Cognition and Aging and Intervisions Cognition and Children. It also attempts to respond to current events and does not hesitate to take sides openly through articles. His involvement with the advisory bodies of the health sector grows. One of our working groups involved in the movement of current changes in clinical psychology to the field of neuropsychology is not forgotten. In order to more transparency and more efficient communication, it offers a website for the month of January 2015 in which you can check out his agenda. This agenda will resume, in addition to its own activities, the conferences organized by other organizations in order to promote continuing education of psychologists specialized in neuropsychology. A directory is also available. We will rely more members, the more we can continue to invest and we will be representative from the governing bodies. The ABPN consists of several working groups and still many projects (a group of standardisation of neuropsychological test, aid for representation in the bodies ...). In conclusion, the BBC is an opportunity for our association to come forward and reach more neuropsychologists.

Résumé en français: L'ABPN aide les psychologues spécialisés en neuropsychologie à se connecter. Elle évolue depuis 2009 pour proposer des débats, conférences, intervisions et partage d'informations utiles dans ce domaine. Depuis quelques années, elle s'implique aussi dans un rôle politique afin de favoriser la reconnaissance de notre profession et de potentialiser nos actions en ce sens. Par nos différents activités, nous nous engageons à favoriser la reconnaissance de notre profession et à promouvoir la spécificité du psychologue spécialisé en neuropsychologie auprès des organes consultatifs du secteur de la santé mais aussi à offrir aux neuropsychologues et aux autres professionnels de la santé toujours plus de rencontres dans un espace d'échanges et de réflexions autour de thèmes riches et diversifiés.»

Samenvatting in het Nederlands: De ABPN (de Belgische Vereniging van Psychologen gespecialiseerd in Neuropsychologie) brengt de psychologen gespecialiseerd in

neuropsychologie samen. Sinds 2009 worden debatten, conferenties, intervisiegroepen en informatie uitwisselingen georganiseerd. Sinds enkele jaren is de vereniging ook beleidsmatig actief en streeft ze naar de erkenning van het beroep, daartoe wordt ook actie gevoerd. Door deze activiteiten wordt niet alleen aandacht gevraagd voor de specificiteit van het beroep bij alle stakeholders binnen de gezondheidssector, maar worden ook steeds meer ontmoetingsplaatsen gecreëerd voor neuropsychologen en andere beroepscategorieën, in een geest van uitwisseling en reflectie over een rijk en gediversifieerd thema.

**Keywords: Neuropsychology, Association, Psychology, Belgium, ASBL**

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