



# **18<sup>th</sup> Annual PhD Meeting**

**Graduate School Neurosciences  
Amsterdam Rotterdam**

**24 and 25 November 2011**

**Woudschoten Conference Center, Zeist**

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Cover: Nouk Tanke

Dear PhD student,

Welcome to the 18th Annual Meeting of PhD-students of the Graduate School Neurosciences Amsterdam Rotterdam (ONWAR), in collaboration with the Rudolf Magnus Institute of Neuroscience in Utrecht, at Woudschoten Conference Center in Zeist.

This meeting is organized for and by PhD-students and offers the opportunity to present work in a friendly and informal atmosphere, to meet other PhD-students, and to get acquainted with each other's work. PhD-students in their 1<sup>st</sup> and 2<sup>nd</sup> year will present their work as a poster, PhD-students in their 3<sup>rd</sup> year will present a blitz-presentation in addition to a poster, and PhD-students in their 4<sup>th</sup> year will give an oral presentation.

The two-day program includes research topics on both fundamental and clinical neuroscience. For the first time in the history of the ONWAR Annual Meeting, parallel sessions are scheduled, on the first day of the program, to accommodate the high number of PhD-students with an oral presentation.

The meeting is also intended to learn how to present one's work to a wide audience. In order to improve your presentation skills, there will be a short plenary evaluation of the presentations after each oral session. In an attempt to get the best out of you, the best poster, the best blitz presentation and the best oral presentation will be awarded. The best posters of each poster session will be indicated by you. The 'poster committee', chaired by the last year's poster award winner Marie Orre, will then decide which poster presenter will win the award. The best blitz presentation will be chosen by the audience, the best oral presentation will be chosen by a jury of senior scientists. Prizes will be awarded on Friday.

We are pleased that Christian Keysers will give the Swammerdam Lecture on Thursday afternoon. He is professor of the Social Brain Laboratory at the Netherlands Institute for Neuroscience (NIN), Amsterdam and the Department of Neuroscience, UMC, Groningen. Together with colleagues of the Social Brain Laboratory he is performing excellent work on the neurobiology of empathy. Prof. Keysers has devoted his career to investigating the function of how mirror neurons aid in our understanding of actions, sensations, and emotions. It is a great honour to have him as a speaker at the 2011 PhD-student meeting.

The organizers also would like to thank the senior scientists from Rotterdam, Utrecht and Amsterdam for chairing the oral sessions and giving didactic feedback to the presentations.

We hope that this PhD-student meeting in Woudschoten will give you a scientifically satisfactory exchange as well as a pleasant stay.

The organizing committee:

Bernard Bloem, Dept. of Integrative Neurophysiology, CNCR-VUA, NCA, Amsterdam

Sandra Cornelisse, Dept. of Neuroscience and Pharmacology, UMCU, Utrecht

Judith van der Harg, Dept. of Neurogenetics, AMC, Amsterdam

Bart Lubbers, Dept. of Molecular and Cellular Neurobiology, CNCR-VUA, NCA, Amsterdam

Eva Naninck, Center for NeuroScience, SILS, UvA, Amsterdam

Marie Orre, Netherlands Institute for Neuroscience, Amsterdam

Natasha Pasricha, Dept. of Neuroscience and Pharmacology, UMCU, Utrecht

Nouk Tanke, Dept. of Neuroscience, ErasmusMC, Rotterdam

Mark Verheijen, Dept. of Molecular and Cellular Neurobiology, CNCR, NCA, Amsterdam

Stella de Wit, Dept. of Psychiatry, VUmc, NCA, Amsterdam

Els Borghols ONWAR, Amsterdam

## Program Annual PhD Meeting - Thursday, 24 November 2011

09:00 – 09:30 Registration/ coffee and tea  
09:45 – 09:50 Words of welcome by day chair (Big Lecture Hall)

### Parallel Session Information: 2 locations!

#### Parallel Session A

Location: **Big Lecture Hall (Room 30)**  
Didactic comments: **Matt Self**  
Day Chair: **Natasha Pasricha**

#### Parallel Session B

Location: **Kapelzaal (Room 16)**  
Didactic comments: **Matthijs Verhage**  
Day Chair: **Bart Lubbers**

### Sessions:

10:00 – 11:15 **Session 1A: Addiction - Chair: Taco de Vries** (Big Lecture Hall)  
Nienke Broos  
*Impulsivity, a treatable risk factor of relapse to cocaine dependence?*  
Zsuzsika Sjoerds  
*Family history of alcohol dependence and the development of mood- and anxiety disorders: cognitive and emotional functions in the brain*  
Janna Cousijn  
*Neural predictors of problem severity in cannabis users*  
Ping Gao  
*Immediate early genes in cocaine self-administration*

10:00 – 11:15 **Session 1B: Gene Regulation - Chair: Koen Bossers** (Kapelzaal)  
Eva Verbeek  
*Fine mapping seven genes from a GWAS for Major Depressive Disorder*  
Rubén Saavedra Pascual  
*Microarray technology to unravel new regeneration-related characteristics of Olfactory Ensheathing Cells*  
Mattia Maroso  
*Activation of Toll-like receptor 4 (TLR4) and Receptor for Advanced Glycation End Product (RAGE) signaling contributes to ictogenesis and epileptogenesis*  
Laura van Berge  
*Leukoencephalopathy with brain stem and spinal cord involvement and lactate elevation is associated with cell type-dependent splicing of mtAspRS mRNA*

11:15 – 11:30 Coffee and tea

11:30 – 13:15 **Session 2A: Pharmacology - Chair: Matt Self** (Big Lecture Hall)  
Elodie Girault  
*Involvement of the hypothalamic Orexin system in Olanzapine induced metabolic side-effects*  
Anne Klomp  
*Lasting effects of chronic fluoxetine treatment on the late developing rat brain: Age-dependent changes in the serotonergic neurotransmitter system*  
Albertine Scheltema Beduin  
*Obsessive-Compulsive symptoms in patients with schizophrenia comparing treatment with Clozapine, Olanzapine, Risperidone and no antipsychotics: a naturalistic cross-sectional study of 543 patients*  
Lianne Schmaal  
*Effect of modafinil on neural correlates of response inhibition during stop signal task in alcohol dependent men*  
Susanne Koot  
*Stress and decision-making in rats: effects of corticosterone*

- 11:30 – 13:15 **Session 2B: Imaging - Chair: Klaus Linkenkaer-Hansen** (Kapelzaal)  
Joost Verbeek  
*Initial PET studies in rats with novel radiotracer [<sup>11</sup>C]D617 and comparison with (R)-[<sup>11</sup>C]verapamil*  
Saskia Woudstra  
*Piccolo/PCLO effect on neural correlates of emotional encoding*  
Giovanni Piantoni  
*Sleep deprivation and connectivity: why presenting on the first day of ONWAR is better than on the second*  
Jeroen Bos  
*Rat robot: spatial discrimination of multimodal, three-dimensional dynamic agents*  
Marloes Henckens  
*Corticosteroid regulation of the human amygdala*
- 13:15 – 14:15 Lunch
- 14:15 – 14:45 Blitz Session I (Big Lecture Hall)
- 14:45 – 15:15 Printer Workshop (Kapelzaal)
- 15:15 – 16:15 Poster Session I / Printers market
- 16:15 – 17:30 **Session 3A: Neurodegeneration - Chair: Wiep Scheper** (Big Lecture Hall)  
Anna Carrano  
*Cerebral amyloid angiopathy and blood-brain barrier alterations*  
Anke Dijkstra  
*Stage-dependent dopaminergic cell loss in the substantia nigra during Parkinson's Disease*  
Hyung Elfrink  
*Rab6 function during ER stress in Alzheimer's disease*  
Robin Verhaar  
*Increase in endoplasmic reticulum-associated tissue transglutaminase and enzymatic activation in a cellular model of Parkinson's disease*
- 16:15 – 17:30 **Session 3B: Diseases - Chair: Odile van den Heuvel** (Kapelzaal)  
Stella de Wit  
*Response inhibition in OCD patients and their unaffected siblings*  
Addy van Dijk  
*Deep brain stimulation in the nucleus accumbens core activates prefrontal monoamine release*  
Anouk den Braber  
*White matter differences in monozygotic twins discordant or concordant for obsessive-compulsive symptoms: A combined DTI-VBM study*  
Elsemarieke van de Giessen  
*Imaging the dopaminergic system in obesity*
- 17:30 – 17:45 Coffee and tea
- 17:45 – 18:45 **Swammerdam Lecture by Prof. Christian Keysers** (Big Lecture Hall)  
 Social Brain Laboratory at the Netherlands Institute for Neuroscience (NIN)  
 Amsterdam and Department of Neuroscience, UMC, Groningen  
*From mirror neurons to empathy*
- 19:00 – 20:30 Dinner
- 20:30 – 21:30 Evening Program **"Beauty and the Brain"**

## Program Annual PhD Meeting - Friday, 25 November 2011

08:00 – 09:00 Breakfast

*Location:* **Big Lecture Hall**  
*Didactic comments:* **Peter Burbach**  
*Day Chair:* **Stella de Wit**

09:00 – 10:30 **Session 4: Synaptic Transmission – Chair: Casper Hoogenraad**  
Tony Cijssouw  
*Synaptic recruitment of the release machinery component Munc18-1*  
Arthur de Jong  
*Target cell dependency of presynaptic properties in hippocampal neurons*  
Marieke Meijer  
*Dissecting the molecular interactions of Munc18-1 within the synaptic vesicle secretion machinery*  
Danielle van Versendaal  
*Inhibitory synapse turnover in adult ocular dominance plasticity*  
Marlene Vegh  
*The aging synapse: synapse proteomic alterations in mouse models of aging*

10:30 – 10:45 Coffee and tea

10:45 – 12:15 **Session 5: Electrophysiology - Chair: Christiaan de Kock**  
Simon-Shlomo Poil  
*Novel biomarker of memory: from model to cognition*  
Timo van Kerkoerle  
*Laminar profile of cortical waves*  
Laurens Witter  
*Processing in the cerebellar nuclei*  
Fleur Zeldenrust  
*The reliability of coding in thalamocortical relay cells*  
Natasha Pasricha  
*Glucocorticoid pulsatility*

12:15 – 13:15 Lunch

13:15 – 13:45 Blitz Session II

13:45 – 15:00 Poster Session II / Coffee and tea

15:00 – 16:15 **Session 6: Receptor Signalling - Chair: Rhiannon Meredith**  
Marlies Oostland  
*Transient expression and function of serotonin 5-HT<sub>3</sub> receptors on glutamatergic granule cells in the early postnatal mouse cerebellum*  
Rogier Poorthuis  
*Nicotinic receptor modulation of the prefrontal cortex and attention behaviour*  
Roxana Kooijmans  
*Calretinin and parvalbumin positive interneurons express opposite patterns of AMPA receptor subunits in macaque V1*  
Femke den Boon  
*Intracellular type-2 cannabinoid receptor activation evokes Ca<sup>2+</sup>-activated Cl<sup>-</sup> currents in prefrontal cortical pyramidal neurons*

16:15 – 16:30 Poster, blitz and oral presentation awards

16:30 – 16:45 Closing remarks

**Blitz Session I****24 November 2011, 14:15 – 14:45**

Danai Riga  
Alexander Diaz  
Marianne Klanker  
Martina Moeton  
Sarah Burke  
Marieke Schouw  
Emanuele Zurolo  
Julia Meuwese  
Celso Henrique Freitas Alves  
Lieke Geerts  
Regina Kanski  
Vasil Mecollari  
Caroline Bruinsma  
Elizabeth Moloney  
Julia Dawitz  
Adrian Negrean  
Jan-Peter van Wieringen  
Pieter Klein  
Torben Hager  
Annelinde Vandenbroucke

**Blitz Session II****25 November 2011, 13:15 – 13:45**

Gregoire Maroteaux  
Marie Orre  
Sasja Heetveld  
Esther Lips  
Sarah Janssen  
Carlyn Mamber  
Katelijne van Oortmerssen  
Remi Soleman  
Zimbo Boudewijns  
Nouk Tanke  
Ning Chen  
Rocío Díez Arazola  
Andrea Goudriaan  
Catherine van Engen  
Ilse Verweij  
Bernard Bloem  
Nitish Fagoe  
Matthijs Verhoog  
Stefan Hoyng

**Group A: Addiction**

1. **Danai Riga**  
The deeper the blues, the higher the booze?
2. **Rolinka Schippers**  
Does deep brain stimulation of the nucleus accumbens shell decrease heroin taking and seeking?
3. **Marcia Spoelder**  
Development of a model for alcohol addiction in rats

**Group C: Cognition 1**

7. **Alexander Diaz**  
Factor analysis of Resting-State Questionnaire data reveal five modes of cognition during rest
8. **Carly Sweegers**  
Recoding with regularities
9. **Marianne Klanker**  
Effects of deep brain stimulation in corticostriatal circuitry on dopamine release in the nucleus accumbens
10. **Joost Uilenreef**  
Effects of focus and expectancy on processing of nociceptive information in the rat using somatosensory evoked potentials

**Group E: Degeneration 1**

14. **Judith van der Harg**  
The UPR and disturbed glucose metabolism: early events in Alzheimer's disease
15. **Martina Moeton**  
Studying the role of Glial Fibrillary Acidic Protein isoforms in astrocytes
16. **Ashutosh Dhingra**  
Comparison of induced pluripotent stem cell derived dopaminergic neurons to transdifferentiated dopaminergic neurons
17. **Marta del Campo Milàn**  
Transmembrane protein X in early stages of Alzheimer's disease

**Group G: Psychiatric Disorders 1**

22. **Iván Chavarría-Siles**  
Schizophrenia is associated with reduction of the cortical thickness in the Anterior Cingulate Cortex
23. **Sarah Burke**  
Otoacoustic emissions: effects of sex hormones on the inner ear
24. **Moran Cohn**  
Persistence of childhood antisocial behavior: a functional MRI study

**Group I: Receptors**

29. **Anna Karataeva**  
Identification of interactors of the Shisa family of potential AMPA receptor modulatory proteins
30. **Marieke Schouw**  
Pharmacological Magnetic Resonance Imaging (phMRI) in healthy subjects using an i.v. challenge with d-amphetamine
31. **Emanuele Zurolo**  
The inflammatory molecules IL-1 $\alpha$  and HMGB1 can rapidly enhance focal seizure generation in rat entorhinal cortex

**Group K: Sensory Systems 1**

35. **Jesse Veenvliet**  
Retinoic acid-dependent and -independent gene-regulatory pathways of Pitx3 in meso-diencephalic dopaminergic neurons

- 36. Julia Meuwese**  
Does perceptual learning require consciousness or attention?
- 37. Celso Henrique Freitas Alves**  
CRB2 in retinal development.

**Group M: Signaling 1**

- 38. Lieke Geerts**  
Functional analysis of Tomosyn in the mammalian brain
- 39. Regina Kanski**  
Modification of GFAP-isoform expression in neurogenic astrocytes
- 40. Vasil Mecollari**  
Neutralization of Semaphorin signaling in the injured spinal cord

**Group O: Motor Systems**

- 48. Caroline Bruinsma**  
Cerebellar function in Angelman syndrome
- 49. Elizabeth Moloney**  
AAV6-mediated delivery of neuropilin-1 receptor-bodies to skeletal muscle: a gene therapy strategy to neutralise semaphorin 3A in the G93A-hSOD1 mouse model for ALS
- 50. Floor Buma**  
The role of the cerebellum in chronic stroke patienten with upper limb paresis
- 51. Sjirk-Jan Zijlstra**  
Alpha-synuclein and its role in Parkinson disease

**Group Q: Networks 1**

- 56. Marta Ruipérez Alonso**  
Shisa9 in hippocampal brain oscillations.
- 57. Julia Dawitz**  
Activity dependent network development in mental retardation
- 58. Adrian Negrean**  
Single-shot action potential detection using two-photon excited membrane potential sensitive dyes
- 59. Borbála Duray**  
Neuronal oscillatory changes in an animal model of Parkinson's disease

**Group S: Pharmacology**

- 63. Luuk van der Velden**  
Network structure of the ventral tegmental area
- 64. Jan-Peter van Wieringen**  
Characterization of 123I- and 18F-labeled pharmaceuticals for imaging dopamine D2 receptors in high-affinity state
- 65. Pieter Klein**  
Synthesis and evaluation of [11C]GMOM analogs for PET imaging of the NMDA receptor

**Group U: Behavior**

- 70. Emmeke Aarts**  
Extracting gene-behaviour relations using longitudinal data obtained through automated home cage testing
- 71. Torben Hager**  
The dynamics of conditioned fear behavior in an Automated Home Cage (DualCage) Environment
- 72. Danique Jeurissen**  
Object recognition and image parsing

**Group W: (f)MRI 1**

- 76. Sanne Menning**  
Structural, biochemical and functional indices of chemotherapy-induced cognitive deficits in breast cancer patients

**77. Annelinde Vandenbroucke**

Seeing without knowing: neural signatures of a visual illusion in the absence of report

**78. Rebecca Steketeer**

fMRI as a diagnostic tool for early dementia

**Group Y: Glial Cells**

**82. Karen Carney**

Towards identification of astrocyte-expressed genes involved in synaptic plasticity

**83. Stephanie Dooves**

Transgenic mice showing a Vanishing White Matter phenotype

**84. Karlijn Doorn**

Region-specific microglia phenotypes in Parkinson's Disease

**Poster Session II**

**25 November 2011, 13:45 – 15:00**

**Group B: Cognition 2**

**4. Gregoire Maroteaux**

Identification of genotypes and genes that influence avoidance learning in mice

**5. Roy Cox**

Reactivation of episodic memory traces during sleep and the distribution of sleep spindles

**6. Sarah Moens**

Defining subtypes of insomnia

**Group D: Degeneration 2**

**11. Sigrid Tolboom**

Identification of neurodegenerative and neuroprotective mechanisms in a MPTP marmoset model for idiopathic Parkinson's disease

**12. Marie Orre**

Isolation and gene expression analysis of adult astrocytes and microglia from an AD mouse model

**13. Sasja Heetveld**

Frontotemporal dementia and Parkinsonism linked to chromosome 17: from disease mechanism to new therapeutic strategy

**Group F: Genetics**

**18. Esther Lips**

JAG: a tool to analyse the Joint Action of Genes within genotypic data

**19. Veerle Eggens**

The role of RNA processing genes in pontocerebellar hypoplasia

**20. Sarah Janssen**

Gene expression profile and functional annotation of the human ciliary body epithelia

**21. Carlyn Mamber**

GFAP-delta: A neural stem cell marker in mouse?

**Group H: Psychiatric Disorders 2**

**25. Judy Luigjes**

Deep brain stimulation normalizes frontostriatal connectivity in obsessive-compulsive disorder

**26. Katelijne van Oortmerssen**

Prevalence of ADHD in substance use disorder patients: a prevalence study at the Jellinek Addiction Treatment Centre.

**27. Remi Soleman**

The effects of sex steroids on functional brain activity during emotional picture processing

**Group J: Sensory Systems 2**

**32. Zimbo Boudewijns**

Sensory processing in rat medial prefrontal cortex

**33. Nouk Tanke**

Impact of single-cell stimulation in rat barrel somatosensory cortex on the detection of whisker movements

**34. Bruno Dagnino**

The perception of phosphenes: On the interaction between feedforward and feedback connections

**Group L: Signaling 2**

**41. Oscar Stassen**

The role of the intermediate filament GFAP in mechanotransduction in reactive astrocytes

**42. Julia Kurps**

Munc18-1 regulates cortical F-Actin in chromaffin cells

**43. Ning Chen**

Pre-synaptic protein complexes identified by Interaction Proteomics

**Group N: Synapse**

**44. Nikhil Pandya**

Structural and functional characterization of novel AMPA receptor interacting protein – Pancortin.

**45. Johan Winnubst**

Activity-dependent clustering of functional synaptic inputs on developing hippocampal dendrites

**46. Rocío Díez Arazola**

C-2 domain proteins: a competition model

**47. Margherita Farina**

Secretory vesicles in neurons: capture and fusion

**Group P: White Matter**

**52. Andrea Goudriaan**

Differences in brain myelination in mouse recombinant inbred strains.

**53. Catherine van Engen**

Omega-oxidation of very long-chain fatty acids: a therapeutic option for X-linked adrenoleukodystrophy?

**55. Shailender Singh**

Preactive multiple sclerosis lesions reveal innate immune activation and immune-regulation

**Group R: Networks 2**

**60. Ilse Verweij**

Sleep deprivation leads to a loss of functional connectivity in frontal brain regions

**61. Bernard Bloem**

Studying the causal role of cholinergic, GABAergic and glutamatergic corticopetal projections from the basal forebrain in neocortical activation.

**62. Ioannis Kramvis**

The state of phasic and tonic GABAergic inhibition in the CA1 hippocampal region of the Fragile-X mouse model for neurodevelopmental disorders

**Group T: Regeneration**

**66. Loek van der Kallen**

Regulation of neuronal plasticity by basic leucine zipper transcription factors

**68. Nitish Fagoe**

Optimization of AAV expression vector for overexpression of transcription factors in DRG neurons targeting axonal regeneration

**69. Arie van Vliet**

Wnt5a in peripheral neuroregeneration

**Group V: (f)MRI 2**

**73. Myrle Kemperman**

CONNECT: Cognition and the neural network: effects of chemotherapy

**74. Niels Kloosterman**

Auditory Decision-Making depends on a prefrontal sensory comparator mechanism

**75. Niels Gerrits**

The neural substrate of set-shifting deficits in Parkinson patients

**Group X: Modeling**

**79. Richard Hardstone**

Critical-state dynamics of avalanches and oscillations jointly emerge from balanced excitation/inhibition in neural networks

**80. Bas Schotten**

Modeling responses to hypertonic sucrose solutions in Hippocampal autapses

**81. Joachim Kutzera**

Modelling protein protein interaction based on protein abundance data in neurons

**Group Z: Plasticity**

**85. Roeland Struik**

Glutamate receptors and spontaneous recovery after cue-extinction of nicotine seeking

**86. Matthijs Verhoog**

Rules and mechanisms of spike-time dependent plasticity at adult human neocortical synapses

**87. Cillian King**

Post-translational modifications of synaptic proteins

**88. Stefan Hoyng**

Developing a potentially immunologically-inert reverse tetracycline controlled lentiviral vector for gene therapy in the peripheral nerve system

## SWAMMERDAM LECTURE

### TITLE

**From mirror neurons to empathy**

### AUTHOR

Christian Keysers

### DEPARTMENT/INSTITUTE

Department Head, Social Brain Lab, Netherlands Institute for Neuroscience, KNAW, Amsterdam, and full professor UMC Groningen, Groningen

### ABSTRACT

Humans are social animals. I will summarize research from my lab over the last decade that shows that our brain makes us share the actions, sensations and emotions of others. Whenever we see others act, we activate our own premotor and parietal cortices as if programming our own actions. Whenever we see others be touched, we activate our own somatosensory cortices as if we had been touched ourselves. Whenever we see the emotions of others, we activate our insula as if we had felt similar emotions. Through these systems, we might acquire an intuitive sense of ethics: hurting others hurts vicariously, and helping others makes us rejoice. I will support this idea by showing that psychopathic criminals that seem to lack this intuitive sense of ethics also show abnormally low vicarious activations to the states of others.

Further Readings: "The Empathic Brain" see [facebook.com/theempathicbrain](https://facebook.com/theempathicbrain)

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**TITLE**

**Extracting gene-behaviour relations using longitudinal data obtained through automated home cage testing**

**AUTHORS**

Emmeke Aarts, Matthijs Verhage and Sophie van der Sluis

**DEPARTMENT/INSTITUTE**

Dept. of Functional Genomics, CNCR, VU University Amsterdam, Amsterdam

**ABSTRACT**

Automated home cage testing of different strains of mice offers excellent opportunities to study the genetics of complex behavior in a neutral setting.

Before being able to compare phenotypes of different genetic strains to induce the function of manipulated genes, the phenotypes have to be extracted from the automatically obtained data. The extremely longitudinal nature of these data provides a wealth of information, but at the same time poses various challenges. Behavioral phenotypes have often been described in terms of frequency and mean duration of various behaviors. These longitudinal data, however, provide the opportunity to quantify behavioral phenotypes also in terms of patterns and sequences of behaviors, and to determine transitions between behaviors, or clusters of behaviors. These transitions, or transition probabilities, are most likely not fixed over time: distinguishable behavioral phases are expected over prolonged periods of measurement. We therefore further develop and extend Markovian techniques to model behavioral patterns and transitions over time and to accommodate the specific requirements of data obtained in automated home cage settings.

Next, the patterns and sequences of the behaviors of different genetic strains are compared to induce the function of manipulated genes. To identify the aspects of the patterns and transitions that are under genetic influence, the Markovian model is put in a Bayesian framework. This Bayesian framework allows the modeling of the genetically different groups in a hierarchical fashion, thus extracting the systematic differences in the quantified behavioral phenotypes between the genetic strains.

This new information on behavioral phenotypes defined in terms of time-organized patterns and structured sequences of behavior is expected to uncover behavioral differences between genetic strains which classical behavioral tests, and classical statistical methods and analyses cannot detect.

**KEY WORDS:** Behavioural phenotypes, Markov chain model, automated home cage testing

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**TITLE**

**Leukoencephalopathy with brain stem and spinal cord involvement and lactate elevation is associated with cell type-dependent splicing of mtAspRS mRNA**

**AUTHORS**

Laura van Berge, Stephanie Dooves, Carola G.M. van Berkel, Emiel Polder, Marjo S. van der Knaap, Gert C. Scheper

**DEPARTMENT/INSTITUTE**

Dept. of Child Neurology, VU University medical center, Amsterdam

**ABSTRACT**

Leukoencephalopathy with brain stem and spinal cord involvement and lactate elevation (LBSL) is an autosomal recessive white matter disorder with slowly progressive cerebellar ataxia, spasticity and dorsal column dysfunction. This disorder was identified in 2003 on the basis of magnetic resonance imaging showing characteristic abnormalities in the cerebral white matter and specific brain stem and spinal cord tracts. LBSL is caused by mutations in the gene *DARS2*, which encodes mitochondrial aspartyl-tRNA synthetase (mtAspRS). Considering the ubiquitous expression of this protein, the selective involvement of specific white matter tracts in LBSL is striking. The fact that long tracts are affected in LBSL patients suggests that the disease is due to a neuronal / axonal defect.

Almost all LBSL patients have one mutation in intron 2 of *DARS2*, affecting the splicing of the third exon. Using splicing reporter constructs, we find cell type-specific differences in the sensitivity to these mutations. The mutations have a larger effect on exon 3 exclusion in neural cell lines, especially neuronal cell lines, than in non-neural cell lines. Furthermore, correct inclusion of exon 3 in the normal mtAspRS mRNA occurs less efficiently in neural cells than in other cell types and this effect is again most pronounced in neuronal cells. The combined result of these two effects may explain the selective vulnerability of specific white matter tracts in LBSL patients. By using a bifunctional antisense oligonucleotide we can increase the splicing efficiency of exon 3 in the reporter constructs in neuronal cells.

**KEY WORDS:** Aminoacyl-tRNA synthetase, mRNA splicing, white matter disease

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**TITLE**

**Studying the causal role of cholinergic, GABAergic and glutamatergic corticopetal projections from the basal forebrain in neocortical activation**

**AUTHORS**

Bernard Bloem and Huibert Mansvelder

**DEPARTMENT/INSTITUTE**

Dept. of Integrative Neurophysiology, CNCR, VU University Amsterdam, Amsterdam

**ABSTRACT**

The basal forebrain is a collection of subcortical cholinergic nuclei with projections throughout the cortical mantle and hippocampus. Through these projections the basal forebrain modulates cortical function, increasing arousal and attention and affecting plasticity and memory.

Whereas most of its effects on cortical processing are attributed to cholinergic signalling, anatomical studies using retrograde tracers in combination with immunocytochemistry have shown that only a minority of the projections from the basal forebrain to the cortex is actually cholinergic; the majority of the projections are GABAergic or glutamatergic. This suggests that part of the effects of the basal forebrain could be through non-cholinergic mechanisms.

In this study, the causal role of different subpopulations of basal forebrain neurons in the modulation of cortical activity will be probed in mice. This will be done using a combination of selective optogenetic stimulation of ChAT, parvalbumin and CaMKII corticopetal axons with 1) in vitro recordings of postsynaptic currents in neocortical neurons and 2) in vivo local field potential recordings.

We hypothesize that in addition to acetylcholine, also GABA and glutamate released by the basal forebrain are involved in neocortical activation. Mechanisms through which this could occur could be direct excitation, modulation of neuronal excitability, disinhibition and regulation of release of other neurotransmitters. In vivo this could be evident as a desynchronization of field potentials in the anaesthetized animal.

**KEY WORDS:** Basal forebrain, optogenetics, neurophysiology

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**TITLE**

**Intracellular type-2 cannabinoid receptor activation evokes  $Ca^{2+}$ -activated  $Cl^-$  currents in prefrontal cortical pyramidal neurons**

**AUTHORS**

Femke S. den Boon<sup>1</sup>, Qiluan Schaafsma-Zhao<sup>1</sup>, Willem van Aken<sup>1</sup>, Monica Bari<sup>2,3</sup>, Chris G. Kruse<sup>1</sup>, Mauro Maccarrone<sup>3,4</sup>, Wytse J. Wadman<sup>1</sup>, Pascal Chameau<sup>1</sup> and Taco R. Werkman<sup>1</sup>

**DEPARTMENT/INSTITUTE**

Dept. of Cellular and Molecular Neurobiology, SILS-CNS, University of Amsterdam, Amsterdam

**ABSTRACT**

The endocannabinoid (eCB) system is widely expressed throughout the central nervous system, however, there is little evidence for functional type-2 cannabinoid receptors (CB<sub>2</sub>R) in neurons. We show that, in rodent layer II/III pyramidal cells of the prelimbic cortex, CB<sub>2</sub>R are located intracellularly and that their activation results in IP<sub>3</sub>R-dependent opening of Ca<sup>2+</sup>-activated Cl<sup>-</sup> channels. This novel pathway might be determinant in controlling neuronal excitability through modulation of the membrane potential.

**KEY WORDS:** Endocannabinoids, CB<sub>2</sub> receptors, neuronal excitability

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**TITLE**

**Rat Robot: spatial discrimination of multimodal, three-dimensional dynamic agents**

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**ABSTRACT**

In order to efficiently function while working in a group it is important to not only have a notion of oneself, but also to have a representation of other agents around you, it being a friend or perhaps a competitor.

Up till now little is known about how we represent other agents around us and how these other agents influence the representations we have of ourselves.

In this study we will look into the capacity of rats to represent dynamic objects in space. Mapping the spatial representation of other dynamic agents does not have to require intricate highly cognitive processes. Rats make use of so called place cells in their hippocampi to create a representation of where they are themselves in space. We hypothesise that rats are able to use these same place cells not only for representing themselves in space, but also to represent other dynamic objects. Not unlike mirror neurons, who respond both to observed and performed actions or visual neurons which activate both during viewing and imagining of the same scene. This type of multi-use of the same areas for similar processes seems common in the brain.

For this purpose we designed a new task. An E-Puck (Guger Technologies) robot is semi-automatically controlled to drive around on a maze. Rats were positioned in a spatially confined compartment separate from the robot's trajectories, which allowed them to track the spatio-temporal behavior of the e-puck without directly making contact with the robot. The position of the robot could not only be detected by means of visual information, but also by e.g. auditory and olfactory information. In the first training stages, the rat learned to associate the presence of the robot with reward (15% sucrose solution), and the absence of the robot with absence of reward. Subsequently, rats learned to perform actions, namely approaching one of two reward wells, in association with the spatial behavior of the robot. Each trial, the robot moved to one out of four possible end-positions, with a unique spatial trajectory. During recordings this task was flanked by two pallet chasing sessions in which the rat walked around collecting food pellets (45mg Bio Serv) in the same area of the maze where the robot would drive around during the robot part of the task.

Seven rats were trained and recorded on the task. The rats were implanted with a hyperdrive holding 14 TTs aimed at CA1 of the hippocampus. Both single cells and local field potentials were recorded while the rat performed the task.

These recordings yield a first insight in how external dynamic agents are tracked and represented in rats.

**KEY WORDS:** Tetrode, hippocampus, social

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**TITLE****Sensory processing in rat medial prefrontal cortex****AUTHORS**

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**ABSTRACT**

Cortical representation of sensory stimuli has been widely studied at the level of (primary) sensory cortex. However, much less is known about representation of sensory stimuli in higher order cortical areas, where sensory information almost certainly used in decision-making processes. Here we made electrophysiological recordings of individual neurons in layers 2, 3 and 5 of the anaesthetised rat medial prefrontal cortex (mPFC) and subsequently labelled recorded neurons to allow reconstruction of dendritic morphology. Spontaneous activity was generally low and did not differ significantly between layers. When a multisensory stimulus was presented (combined auditory and somatosensory stimulus), a large fraction (~90%) of neurons responded reliably with 1 or more spikes. Three types of responses could be dissociated: 1) neurons that respond to both somatosensory and auditory stimulation 2) neurons that respond to either somatosensory or auditory stimulation alone 3) neurons that did not show a response to either stimulus. All three response types could be found in layers 2, 3 and 5. Latencies of responses were generally 150-250 ms, with considerable variation between trials and between individual neurons. Analysis of the local field potential responses showed that sensory stimuli were represented very reliably at the network level, even in cases where spiking was absent. Together, the data indicate that sensory stimuli of different modalities are reliably represented in the mPFC.

**KEY WORDS:** Prefrontal cortex, sensory responses, identified neurons

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## TITLE

**White matter differences in monozygotic twins discordant or concordant for obsessive-compulsive symptoms: a combined VBM-DTI study**

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## ABSTRACT

### Background

Neuroimaging studies of obsessive-compulsive disorder (OCD) patients point to deficits in cortico-striato-thalamo-cortical circuits that might include changes in white matter. The contribution of environmental and genetic factors to the various OCD-related changes in brain structures remains to be established.

### Methods

White matter structures were analyzed in 140 subjects with both diffusion tensor imaging and voxel-based morphometry. We studied 20 monozygotic twin pairs discordant for obsessive-compulsive symptoms (OCS) to detect the effects of environmental risk factors for obsessive-compulsive (OC) symptomatology. Furthermore, we compared 28 monozygotic twin pairs concordant for low OCS scores with 23 twin pairs concordant for high OCS scores to detect the effects of genetic risk factors for OC symptomatology.

### Results

Discordant pair analysis showed that the environmental risk was associated with an increase in dorsolateral-prefrontal white matter. Analysis of concordant pairs showed that the genetic risk was associated with a decrease in inferior frontal white matter. Various white matter tracts showed opposite effects of environmental and genetic risk factors (e.g., right medial frontal, left parietal, and right middle temporal), illustrating the need for designs that separate these classes of risk factors.

### Conclusions

Different white matter regions were affected by environmental and genetic risk factors for OC symptomatology, but both classes of risk factors might, in aggregate, create an imbalance between the indirect loop of the cortico-striato-thalamo-cortical network (to the dorsolateral-prefrontal region)—important for inhibition and switching between behaviors—and the direct loop (involving the inferior frontal region) that contributes to the initiation and continuation of behaviors.

**KEY WORDS:** Diffusion Tensor Imaging, Voxel-Based Morphometry, Obsessive Compulsive behavior

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**TITLE****Impulsivity, a treatable risk factor to relapse of cocaine dependence****AUTHORS**Nienke Broos, Anton N.M. Schoffelmeer, Taco J. de Vries\* and Tommy Pattij\*

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**ABSTRACT**Introduction

Impulsivity and drug addiction are closely related, but the direction of this relationship is still under investigation. Using animal models, this can be studied prospectively and distinct dimensions of impulsivity can be related to distinct stages of drug taking and seeking. Furthermore, the treatment potential of impulsivity as risk factor for addiction is still questionable. Therefore, this study explored the bidirectional relationship between impulsivity and cocaine taking and seeking.

Methods

Rats were trained in either the five-choice serial reaction time task (impulsive action) or the delayed reward task (impulsive choice). Upper and lower quartiles were selected and subsequently trained in a cocaine self administration paradigm measuring onset, motivation, sensitivity, extinction and context-induced reinstatement of cocaine seeking. Importantly, throughout cocaine taking and seeking, impulsivity levels were weekly monitored. In addition, transient (pharmacological) manipulations of impulsivity and context-induced reinstatement were performed.

Results

Impulsive action did not predict cocaine taking or seeking. However, cocaine intake induced a transient increase of impulsive action and omissions. Pre-existing levels of impulsive choice predicted extinction resistance and sensitivity to context-induced relapse. Measures of impulsive choice remained stable throughout cocaine taking and seeking. Finally, (pharmacologically) provoked and transient changes in impulsive choice or action were not related to changes in context-induced relapse to cocaine seeking.

Discussion

Our data suggest that heightened levels of impulsive action might be a consequence of cocaine intake, whereas trait levels of impulsive choice are predictive of persistent cocaine seeking. Since transient changes in impulsivity did not correspond to changes in relapse sensitivity, the treatment potential of impulsivity as risk-factor for drug dependence warrants further investigation.

**KEY WORDS:** Impulsivity, addiction, cocaine**TELEPHONE NUMBER:** 020-4445677**EMAIL ADDRESS:** n.broos@vumc.nl

**TITLE****Cerebellar function in Angelman syndrome****AUTHORS**Caroline F. Bruinsma, M. Schonewille, G. van Woerden, C.I. de Zeeuw and Y. Elgersma**DEPARTMENT/INSTITUTE**

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**ABSTRACT**

Angelman syndrome (AS) is a genetic disorder caused by a mutation or deletion of the UBE3a gene. It is characterized by epilepsy, mental retardation, absence of speech and excess laughter, but also by movement and balance problems. The latter symptoms may indicate the presence of cerebellar deficits in this disorder.

Using immunohistochemistry we indeed found that the E6AP protein, the product of the Ube3a gene, is abundantly expressed in Purkinje cells. To examine cerebellar function we studied motor performance and motor learning by recording compensatory eye movements in Angelman (AS) and wild type (WT) mice. We observed no differences in the optokinetic reflex (OKR) and the vestibulo-ocular reflex in the dark (VOR) and in the light (VVOR), indicating that motor performance was not affected by the mutation. Furthermore, short-term learning, tested by mismatched optokinetic and vestibular stimulation aimed at decreasing the VOR gain, was normal. However, AS mice were not able to reverse the phase of their VOR following a multiple day training paradigm. To test whether this very specific phenotype was caused by impaired Purkinje cell function and plasticity, we measured parallel-fiber-Purkinje cell plasticity in vitro and in vivo spontaneous firing electrophysiological recordings. In both experiments no deficits were seen in Purkinje cell physiology, suggesting that this specific behavioral defect did not originate from Purkinje cells. This was further supported by the observation that expression of  $\alpha$ -CaMK2 T305/6VA mutant protein in Purkinje cells could not rescue the motor learning phenotype, while the same mutation in the hippocampus could rescue both the electrophysiological and the behavioral phenotype of AS mice.

At present, our results suggest normal cerebellar cortex function in AS mice and thus do not explain the observed deficit in phase reversal training. Hence, further research is needed to find the neuronal origin of the motor learning problem.

**KEY WORDS:** Cerebellum, Angelman syndroom, motor performance**TELEPHONE NUMBER:** 06-23879313**E-MAIL-ADDRESS:** c.bruinsma@erasmusmc.nl

**TITLE**

**Cerebellar involvement in the chronic ischemic brain; an fMRI, and DTI study**

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**ABSTRACT**

Little is known about the involvement of the cerebellum in motor recovery after cerebral stroke. We attempted to investigate differences seen in the cerebellar function after cerebral stroke in well recovered patients compared to healthy controls. We used fMRI and DTI to study both the functional and structural difference in cerebellar involvement in recovery after stroke. We recruited 7 patients with chronic ischemic stroke and 7 age-matched control subjects. fMRI scans were acquired on a 3T MR scanner. Whole brain tractography was done using TBSS. The three cerebellar peduncles, the superior (SCP), middle (MCP) and inferior (ICP) peduncles were evaluated with fiber tracking using *ExploreDTI*. We were able to see structural and functional differences between healthy controls and stroke patients in the cerebellum, even though patients were well recovered and had lesions in the capsula interna. There were no significant findings in cerebral motor areas in task related activity in a flexion-extension task. This could indicate that automatization of motor skills might still be in issue in chronic stroke patients even though their motor skills are comparable to those of healthy controls.

**KEY WORDS:** fMRI, DTI, stroke

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**TITLE**

**Otoacoustic emissions: effects of sex hormones on the inner ear**

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**ABSTRACT**

Click-evoked otoacoustic emissions (CEOAEs) are echo-like sounds that are produced by the inner ear in response to click-stimuli. CEOAEs generally have a higher amplitude in women than in men and already neonates show a sex difference in CEOAE amplitudes. It is proposed that this sex difference might develop due to differential amounts of androgen, especially testosterone, male and female fetuses are exposed to prenatally. Presumably, testosterone affects emission strength by inhibiting the development of the inner ear. Therefore, males have lower CEOAEs, since they are exposed to higher amounts of testosterone during early development. Determining CEOAEs might thus give an indication of the prenatal androgen environment. Puberty is a second phase in development when sex hormone levels rise, possibly affecting CEOAEs as well. Individuals diagnosed with Gender Identity Disorder (GID) are characterized by a strong identification with the other gender and discomfort about their natal sex. GID patients are hypothesized to be exposed to aberrant sex hormone levels during a critical period of prenatal sexual differentiation of the brain.

The first aim of the present study was to examine CEOAEs in children, diagnosed with GID and compare these to control boys and girls. Furthermore, a second aim was to investigate changes in CEOAEs during puberty and whether the sex difference in CEOAEs already present pre-pubertally, would be consolidated by the surge of sex hormones during puberty.

Boys and girls, aged 8-18 years, diagnosed with and without GID are currently tested. Data collection and analyses are currently ongoing and results will be presented at the conference.

**KEY WORDS:** Otoacoustic emissions; sex difference; puberty

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## TITLE

### Transmembrane protein X in early stages of Alzheimer's disease

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## ABSTRACT

### Introduction

Alzheimer's disease (AD) is diagnosed in advanced stages of the disease. The current analysis of amyloid beta, tau and phosphorylated tau in cerebrospinal fluid (CSF) has limited value for early diagnosis. Using a proteomics approach, we found a significant increase of Transmembrane protein X (pX) levels in CSF from patients with AD and mild cognitive impairment (MCI) who later progressed to AD compared to controls and stable MCI patients. Therefore, we hypothesize that pX is a promising biomarker for early diagnosis of AD. The aim of this study is to confirm the initial findings of increased pX levels in AD patients compared to controls in CSF and human brain homogenates using immunological analysis. The relationship between pX, Phospho-TAU and amyloid peptide will be also analyzed.

### Methods

Polyclonal antibodies against a specific pX peptide were produced by Biogenes (Germany). Pools of CSF and post-mortem human brain homogenates (HBH) from AD (CSF n = 10; HBH n = 3) patients and controls (CSF n=9; HBH n=3) were analyzed by western blot. Immunohistochemistry analysis (n= 4) was performed on paraffin sections from AD patients and age-matched controls.

### Results

Specific pX bands of various molecular weights were observed by Western blot. In pools of CSF samples the intensity of pX-positive bands appeared to be decreased in AD patients. Inversely, preliminary results show higher pX levels in pools of AD human brain homogenate samples compared to controls. Evaluation of brain sections showed an increased intracellular and extracellular immunoreactivity in AD compared to Non AD brain tissue. Moreover pX is partly co-localized with neurofibrillary tangles and amyloid plaques.

### Conclusions

Our data suggest that pX levels in both brain homogenates and CSF are modified in AD patients, supporting the potential utility of pX for diagnosis of AD. The different bands found indicate that pX might be processed and attached to other proteins. Furthermore, pX is partly co-localized with the main pathological structures of AD, suggesting a central role in the disease process. Confirmation of our hypothesis will not just contribute to the development of new assays for early diagnosis and prognosis of AD but also might open new insights in AD pathophysiology.

**KEY WORDS:** Alzheimer's disease, early biomarkers, CSF

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**TITLE**

**Towards identification of astrocyte-expressed genes involved in synaptic plasticity**

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**ABSTRACT**

The contributions of glial cells to the modulation of adult synaptic plasticity are becoming increasingly more appreciated. Astrocytes in the supra optic nucleus (SON) of the hypothalamus demonstrate prominent temporary structural changes during transient physiological events such as dehydration, hyperosmosis, and lactation. The retraction of astrocytes processes from the synapses induces well characterized changes in the electrophysiological properties of the synapse, due in part to reduced availability of the gliotransmitter D-serine, the primary NMDA receptor co-agonist in this region. Astrocyte derived D-serine and other gliotransmitters also play critical roles for the modulation of long term potentiation (LTP) in hippocampal CA1 neurons. Although astrocytes in both the SON and hippocampus are crucial contributors to synaptic structural and functional plasticity, little is known about the astrocyte genes involved in these changes.

In this project we aim to establish astrocyte gene and protein expression profiles for synaptic changes in the SON during lactation and and salt water- induced hyperosmolarity. For this, SON from virgin, lactating, post-lactation, and hyperosmotic rats were collected from acute hypothalamic slices. The homogenized tissue samples were enriched for glial and synaptic membrane vesicles. Samples were analyzed by mass spectrometry to identify proteins differentially regulated by the changes in synaptic structure induced by lactation and hyperosmolarity. These mass spectrometry results will be combined with analysis results from additional techniques to obtain pure astrocyte samples from these models. Magnetic-activated cell sorting (MACS) and isolation of astrocytic membranes directly apposed to synapses are being tailored for the separation of astrocytes from *in vivo* tissues. In addition, astrocytes from co-cultures with neurons are purified using our recently developed Cold-jet method. With these astrocyte purification techniques, samples will be analyzed by microarray to examine gene expression, and by mass spectrometry to investigate protein expression. The results of these analyses from the *in vivo* and *in vitro* systems will be used to select promising target genes for *in vivo* targeting approaches, and as such determine the specific roles of selected astrocyte-expressed genes in synaptic plasticity.

**KEY WORDS:** Astrocytes, synaptic plasticity, neuron-glia interaction

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**TITLE**

**Amyloid beta induces oxidative stress mediated blood-brain barrier changes in capillary amyloid angiopathy**

**AUTHORS**

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**ABSTRACT**

Cerebral amyloid angiopathy (CAA) is frequently observed in Alzheimer's disease (AD) and is characterized by deposition of amyloid beta (A $\beta$ ) in leptomeningeal and cortical brain vasculature. In 40% of AD cases A $\beta$  mainly accumulates in cortical capillaries, a phenomenon referred to as capillary CAA (capCAA). The aim of this study was to investigate blood-brain barrier (BBB) alterations in CAA-affected capillaries with the emphasis on tight junction (TJ) changes. First, capCAA brain tissue was analyzed for the distribution of TJs. Here, we show for the first time a dramatic loss of occludin, claudin-5 and ZO-1 in A $\beta$ -laden capillaries surrounded by NADPH oxidase-2 (NOX-2)-positive activated microglia. Importantly, we observed abundant vascular expression of the A $\beta$  transporter receptor for advanced glycation endproducts (RAGE). To unravel underlying mechanism, a human brain endothelial cell line was stimulated with A $\beta$ 1-42 to analyze the effects of A $\beta$ . We observed a dose-dependent cytotoxicity and increased ROS generation, which interestingly was reversed by administration of exogenous antioxidants, NOX-2 inhibitors and blocking RAGE. Taken together, our data evidently show that A $\beta$  is toxic to brain endothelial cells via binding to RAGE and induction of ROS production, which ultimately leads to disruption of TJs and loss of BBB integrity.

**KEY WORDS:** Alzheimer's disease, blood-brain barrier, oxidative stress

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**TITLE**

**Schizophrenia is associated with reduction of the cortical thickness in the Anterior Cingulate Cortex**

**AUTHORS**

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**ABSTRACT**Background

Schizophrenia is a chronic psychiatric disorder that affects 1% of the population. It is characterized by hallucinations, delusions and disorganized thinking and speech. Recently, cognitive dysfunction was recognized as a fundamental feature of schizophrenia and has been shown repeatedly to have a negative association with functional outcome. The main domains of cognition that are disrupted significantly in schizophrenia include attention, executive function, working memory, learning and memory. We previously found that a group of genes associated with cognitive ability is related to differences in the grey matter volume of the Anterior Cingulate Cortex (ACC). In the current study we sought to investigate the association of ACC cortical thickness and schizophrenia and its effect on cognitive ability.

Methods

Cortical thickness of the ACC and general cognitive abilities (IQ) were measured in 157 schizophrenia subjects and 158 matched healthy controls of the Mind Clinical Imaging Consortium MRI study of Schizophrenia (MCIC).

Results

We found that the cortical thickness of the ACC was significantly reduced in schizophrenic subjects ( $p=0.001$ ) compared to controls after controlling for total brain volume, sex and age. We also found that the IQ scores were significantly lower in the subjects with schizophrenia compared to controls ( $p < 0.0001$ ). We then examined the correlation between IQ and ACC cortical thickness and found a significant positive correlation between IQ and ACC cortical thickness ( $r^2=0.05$ ,  $p=0.001$ ) in schizophrenic subjects, whereas in the control subjects no correlation was observed. In the schizophrenic subjects we additionally found a significant negative correlation between duration of illness and ACC cortical thickness ( $r^2=0.15$ ,  $p < 0.001$ ) and IQ scores ( $r^2=0.05$ ,  $p=0.001$ ).

Conclusions

Our results show that the ACC cortical thickness and IQ scores are significantly reduced in schizophrenic subjects compared to healthy controls. The IQ scores were highly correlated with the ACC cortical thickness only in the schizophrenic group; this finding suggests that the ACC is important for normal cognitive functioning in schizophrenia. Based on the negative correlation of duration of illness with ACC cortical thickness and IQ in schizophrenic subjects we hypothesize that the progression of the disease produces a reduction in the ACC cortical thickness that consequently might lead to a decline in cognitive functioning.

**KEY WORDS:** Schizophrenia, Anterior Cingulate Cortex, cognitive ability

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**TITLE****Pre-synaptic protein complexes identified by Interaction Proteomics****AUTHORS**Ning Chen<sup>1</sup>, P. Klemmer<sup>1</sup>, R.C. van der Schors<sup>1</sup>, R. Toonen<sup>2</sup>, M. Verhage<sup>2</sup>, A.B. Smit<sup>1</sup>, K.W. Li<sup>1</sup>**DEPARTMENT/INSTITUTE**<sup>1</sup>Dept. of Molecular & Cellular Neurobiology, <sup>2</sup>Dept. of Functional Genomics, CNCR, VU University Amsterdam, Amsterdam**ABSTRACT**

Pre-synaptic terminals are specialized structures responsible for the stimulus-dependent quantal release of synaptic neurotransmitters. Exocytosis and endocytosis of synaptic vesicles (SVs) involves coordinated dynamic molecular events in networks of proteins in the pre-synaptic active zone. While a limited number of synaptic proteins have been examined extensively, the global synaptic protein interactome and their dynamics that underlie SVs release and replenishment remain largely unknown.

In this study we perform large-scale interaction proteomics to characterize the pre-synaptic protein interactome. From this we will construct a model to reveal the molecular basis of SVs release and recycling.

Presently, we have characterized 40 pre-synaptic protein complexes including those involved in docking, priming, vesicle fusion, endocytosis and vesicle recycling. In a typical experiment approximately 150 proteins were characterized; semi-quantitative data was generated based on spectral-counting of the sequenced peptides. We confirmed most of previously reported protein-protein interactions, and overlapping sets of protein interactors among distinct pre-synaptic protein complexes. Importantly, novel interacting proteins and new functional groups were detected. A draft of elements of this pre-synaptic protein network in SVs docking will be presented as an example, demonstrating the complexity of the pre-synaptic protein interactome and the power of this type of analysis.

**KEY WORDS:** Synaptic interactome, proteomics, exocytosis**TELEPHONE NUMBER:** 020-5989898**E-MAIL-ADDRESS:** ning.chen@falw.vu.nl

**TITLE****Synaptic recruitment of the release machinery component Munc18-1****AUTHORS**Tony Cijssouw, Tim Kroon, Matthijs Verhage, Ruud F. Toonen**DEPARTMENT/INSTITUTE**

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**ABSTRACT**

Munc18-1 is a crucial component of the release machinery of synaptic vesicles<sup>1</sup> in neurons and dense-core vesicles in chromaffin cells<sup>2</sup>. Varying expression levels of Munc18-1 controls synaptic recovery<sup>3</sup> and regulation of synaptic Munc18-1 levels can therefore be an important regulatory mechanism of neurotransmitter release. But how Munc18-1 is temporally and spatially controlled at the cellular and molecular level in the dynamic environment of the cell is not known. Therefore, we generated knock-in mice expressing Munc18-1-Venus, a fusion protein of Munc18-1 and Venus (an enhanced Yellow Fluorescent Protein), from the endogenous Munc18-1 locus. This allows for live cell imaging of endogenous Munc18 in neurons and adrenal chromaffin cells. Here we find that Munc18-1 is synaptically enriched and can be divided into two populations, a highly dynamic population exchanging fast with the axon and a highly immobile one. We investigate the role of Munc18 binding partners Syntaxin, Liprins and Mint but also the effect of neuronal activity on both the exchange between the axon and the synapse and the synaptic immobilization of Munc18.

**KEY WORDS:** Munc18-Venus, Synapse, FRAP**TELEPHONE NUMBER:** 020-5986929**E-MAIL-ADDRESS:** tony.cijssouw@cncr.vu.nl

**TITLE****Persistence of childhood antisocial behavior: a functional MRI study****AUTHOR**Moran D. Cohn**DEPARTMENT/INSTITUTE**

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**ABSTRACT**

Children with early-onset Disruptive Behavior Disorders (DBD) are at risk of developing a persistent pattern of severe antisocial behavior and numerous adult psychosocial problems (1). Studies on persistence within groups of children with early-onset DBD are scarce, while studies relating persistence to neurobiological characteristics do not exist.

This project aims to investigate longitudinal patterns of early-onset DBD with respect to two etiological theories on neurobiological processes and their interaction with contextual psychosocial characteristics. The first theory proposes that conscience development in individuals with DBD is hampered by deficient fear conditioning, a basic emotional learning process in which a previously neutral stimulus becomes aversive, due to its repeated coupling with an unconditioned aversive stimulus. Deficient fear conditioning has been found in adult antisocial populations, and is associated with differences in limbic and prefrontal brain function (2). The second theory proposes that antisocial individuals choose short-term rewarding antisocial strategies, because of higher sensitivity to reward, and lower sensitivity to punishment than normal controls (3).

Participants for this project will be recruited from a cohort of 308 adolescents (mean age 17 years) who have been arrested before the age of twelve and of whom 80 were previously diagnosed with DBD (4). Participants with a previous diagnosis of DBD will be psychiatrically re-investigated and divided in two subgroups (persistent versus desistent, n=25-30 for each group). These subgroups will be compared with each other and with 25 matched healthy controls from the same cohort both psychosocially and by structural, functional and DTI-MRI. The functional MRI-protocol will consist of a differential fear conditioning paradigm (2) and a reward/punishment-sensitivity paradigm (5).

As the study is ongoing, preliminary results of our fear conditioning experiment will be presented.

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**KEY WORDS:** fMRI, antisocial, adolescence**TELEPHONE NUMBER:** 020-8901545**E-MAIL-ADDRESS:** m.cohn@debascul.com

**TITLE****Neural predictors of problem severity in cannabis users****AUTHORS**Janna Cousijn<sup>1,2</sup>, R.W. Wiers<sup>1</sup>, K.R. Ridderinkhof<sup>3</sup>, W. van den Brink<sup>2</sup>, D.J. Veltman<sup>2</sup>, A.E. Goudriaan<sup>2</sup>**DEPARTMENT/INSTITUTE**<sup>1</sup>ADAPT-lab, Dept. of Psychology, University of Amsterdam, Amsterdam, <sup>2</sup> Amsterdam Institute for Addiction Research, Dept. of Psychiatry, Academic Medical Centre, University of Amsterdam, Amsterdam, <sup>3</sup>Amsterdam Center for the Study of Adaptive Control in Brain and Behavior, Dept. of Psychology, University of Amsterdam, Amsterdam**ABSTRACT**

Cannabis is the most commonly used illegal drug in most countries and treatment demands have strongly increased over the last decades. A key question on the transition from occasional to compulsive cannabis use is: Why do some heavy-using individuals develop abuse and dependence, while others do not, even after prolonged drug use? A potential predictor of the course of drug use is the approach-bias, i.e. the relatively automatic tendencies to approach rather than avoid drug-related stimuli. Here we investigated the neural mechanisms of cannabis approach and avoid responses with the Stimulus Response Compatibility task (SRC) in heavy cannabis users (n = 33) and non-using controls (n = 36). Moreover we assessed the predictive relationship between neural approach responses and cannabis problem severity after six months. Compared to controls, heavy cannabis users showed widespread deactivations during the SRC-task vs. active baseline in reward, motivation, cognitive control, and motor circuits. On top of behavioural measures of baseline problem severity and cue-induced craving, cannabis specific approach responses in the dorsolateral prefrontal cortex and anterior cingulate cortex significantly predicted increased problem severity after six months. These findings suggest that heavy cannabis users with lack of control over cannabis specific approach responses are more likely to increase cannabis related problems. Also, cannabis specific approach responses in the dorsolateral prefrontal cortex and anterior cingulate cortex may be used as predictors of the course of cannabis use to identify individuals especially at-risk for developing a cannabis abuse disorder.

**KEY WORDS:** cannabis, approach-bias, fMRI**TELEPHONE NUMBER:** 020-5256729**E-MAIL-ADDRESS:** j.cousijn@gmail.com

**TITLE**

**Reactivation of episodic memory traces during sleep and the distribution of sleep spindles**

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**ABSTRACT**

The active role of sleep in episodic memory consolidation has been firmly established by now. Converging lines of evidence suggest that neural patterns present during initial encoding are being replayed during sleep, and that this reactivation is key to the formation of stronger, more durable memory traces. In addition, a large body of work indicates that consolidation involves a gradual shift in memory dependence from hippocampal regions to neocortical areas, and this process too might be subserved by reactivation during sleep. At the electrophysiological level, sleep spindles make for interesting candidates in this relocating of memory traces, as these oscillatory events occur over most of cortex and correlate with post-sleep memory performance.

We aim to establish a more direct link between memory reactivation and sleep spindles. Reactivation can be triggered by providing cues present at the time of learning to sleeping participants. Hence, we employed a memory task in which different blocks of stimuli were shown in the presence of distinct (and different) odors. This enabled us to selectively reactivate one 'block' of memory traces during subsequent sleep by administering the associated odor. By manipulating certain aspects of the stimuli over blocks, we have a tool to assess the cortical distribution of sleep spindle parameters in relation to reactivated stimulus properties. We will present preliminary findings.

**KEY WORDS:** Sleep, memory, EEG

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**TITLE**

**The perception of phosphenes: on the interaction between feedforward and feedback connections**

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**ABSTRACT**

How visual awareness emerges in our brain, is probably one of the major unanswered questions in neuroscience. During the past years a major effort has been done in order to elucidate the neural mechanisms involved in this process. One of the most researched venues is based on the hypothesis that feedback connections from higher areas play a fundamental role in the access of information to consciousness. This proposal has been supported by several human studies including a paper with a double TMS technique which suggested that feedback projections are necessary for visual awareness (Pascual-Leone & Walsh 2001). As the TMS pulses activate large regions of cortex, we here sought to replicate the interaction between areas of the visual cortex using the much more precise microstimulation technique, probing two areas of the visual cortex.

We used microstimulation (MS) in area V1 and area V4 in a task where monkeys had to detect the presence of a phosphene evoked by the stimulation of an electrode in area V1. Will electrical microstimulation in area V4 modulate the perception of phosphenes evoked by this V1 stimulation? Monkeys were trained to detect suprathreshold microstimulation of a small group of V1 neurons and to report if they see a phosphene by making a delayed saccade to its spatial location. We paired the V1 microstimulation with a subthreshold V4 microstimulation, at various stimulus-onset asynchronies, while ensuring overlap between the receptive fields of the stimulated neurons in V1 and V4. We found that there was no difference in the V1 phosphene detection threshold between the different stimulus-onset asynchronies tested. We were surprised by this result. In line with what has been reported previously with TMS experiments, we were expecting to observe a decrease in the V1 detection threshold if the MS in V4 precedes the MS in V1. In opposition to this prediction, we did not find modulation in the phosphene threshold for any of the tested delays. We conclude that the intriguing interactions between areas in the visual cortex that have been observed with double-pulse TMS do not occur with the spatially more precise microstimulation techniques.

**KEY WORDS:** Visual perception, electrical stimulation, feedback connections

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**TITLE**

**Activity dependent network development in mental retardation**

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**ABSTRACT**

Wiring of neuronal networks during development is strictly regulated by several activity-dependent and independent (genetic) processes. A hallmark pattern of activity-dependent network wiring is spontaneous, synchronous network activity (SSNA) that is consistently described in many different developing neuronal systems of retina, spinal cord, hippocampus and neocortex. Disturbance of those processes e.g. caused by a genetic aberration in the regulating system, can lead to incorrect wiring in the brain and cause mental retardation and other learning impairments.

We investigated the effects of the *Fmr1* gene, a regulator of mRNA translation in neurons, upon SSNA during early brain development. The *Fmr1* gene is impaired in Fragile X syndrome, the most common monogenic cause of mental retardation and closely associated with autism. A phenotype commonly seen in patients and the mouse model are learning impairments of spatial orientation and navigation memory. The superficial entorhinal cortex (EC) encodes the highly-structured grid cell activity thought to be crucial for spatial orientation and memory. Therefore, using calcium imaging of bulk-loaded EC slices, we tested whether SSNA was altered in *Fmr1*-KO mice compared to controls during the first two weeks of postnatal brain development. We find that EC activity is developmentally-regulated during this period and that the time course of SSNA differs for *Fmr1*-KO mice compared to healthy controls.

Even though SSNA is a common feature of the development of many neuronal networks the underlying mechanisms on how the SSNA arises and is regulated are diverse. We show that in EC SSNA is action-potential dependent, being abolished in the presence of TTX. Network activity is modulated by both fast AMPA-mediated and GABA-A-mediated transmission. Understanding the mechanisms of SSNA in healthy EC will help to identify new targets for treatment of incorrect network wiring caused by a disturbed SSNA pattern during brain development.

**KEY WORDS:** Spontaneous network activity, development, mental retardation

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**TITLE**

**Comparison of induced pluripotent stem cell derived dopaminergic neurons to transdifferentiated dopaminergic neurons**

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**ABSTRACT**

Nuclear transfer reprogramming and induced pluripotent stem (iPS) cells have provided evidence that cell fate is not forever. Furthermore, recent work has shown the direct conversion of one mature cell type to another cell type without the intermediary step of ES cell formation, called transdifferentiation. At present it is not clear which method gives a better representative population of the desired cell type.

We would like to compare dopaminergic neurons derived from the directed differentiation of iPS cells to those obtained from transdifferentiation of fibroblasts. To aid in the identification of dopaminergic neurons, we will use a reporter mouse embryonic fibroblast which expresses GFP under tyrosine hydroxylase promoter. Four transcription factors Oct3/4, Sox2, Klf4, c-Myc are used for making iPS cells and Mash1, Nurr1, and Lmx1a for transdifferentiation.

Cap analysis gene expression (CAGE) will be performed on TH-positive neurons to gain a better insight into the transcriptional profile of neurons obtained from the different methods. A better understanding of the transcriptional profile of dopaminergic neurons may refine current differentiation protocol for dopaminergic neurons which in turn, might have significant implications *in vitro* disease modeling and cell replacement therapies.

**KEY WORDS:** Induced pluripotent stem cells, transdifferentiation, dopaminergic neurons

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## TITLE

**Factor analysis of Resting-State Questionnaire data reveal five modes of cognition during rest**

## AUTHORS

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## ABSTRACT

Over the course of a day, individuals frequently engage in activities such as mind wandering [1] and introspection [2, 3]. To allow for systematic measurement and characterization of resting-state cognition, our group developed the Resting-State Questionnaire (RSQ), a 50-item survey rated on a five-point Likert-scale depending on the level of agreement [4]. Here, we describe a factor model of RSQ data obtained as part of an online test battery implemented in the Netherlands Sleep Registry (NSR).

Participants ( $n = 605$  [375 female], mean age 53+13 years) were instructed to create a quiet environment around their computer, and follow the procedures presented on-screen. After five minutes eyes-closed rest participants were notified through headphones and instructed to fill out the online RSQ.

The data set was preprocessed in SPSS to screen for outliers, e.g., extremely high or low (sum-) scores on the RSQ. Additionally, participants were asked to indicate whether they were disturbed during the experiment, in which case they were removed from further analysis. Subsequently, exploratory (Promax rotation,  $\kappa = 4$ ) and confirmatory factor analyses were performed on the screened data set ( $n = 536$ ) in Mplus 5.1. Criteria for the EFA factor selection were 1) eigenvalues of the R-matrix  $>1$  and explaining  $\geq 3\%$  of the total variance, 2) factor loadings  $>.32$  and 3) an item should not load on more than 2 factors. The thus obtained factors were then used as a template to build a confirmatory factor model.

Based on the RSQ items associated with the five-factor solution, we labeled the factors: self-projection, self-reflection, somatic attention, discontinuity of mind, and feelings. In future research, this model will be used to relate neurophysiological measures of the resting state, e.g., EEG and fMRI, to cognition. In addition, the model may be used to investigate the impact of brain-disorders on mind wandering and other aspects of resting-state cognition.

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**KEY WORDS:** Cognition, factor analysis, resting-state

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**TITLE****C2-domain proteins: a competition model****AUTHORS**Rocío Díez Arazola, A.J.A. Groffen, L.N. Cornelisse, M. Verhage**DEPARTMENT/INSTITUTE**

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**ABSTRACT**

By coupling  $\text{Ca}^{2+}$  signals to synaptic vesicle fusion events, synaptotagmin-1 is essential for evoked but not spontaneous neurotransmission. Here we show that DOC2B mediates spontaneous fusion. Hippocampal cultured neurons of DOC2A/B double knock-out mice display normal evoked release, but their spontaneous release frequency is significantly reduced. Acute DOC2B expression in DOC2A/B null neurons restored the spontaneous release frequency to WT levels. Previous studies showed a calcium dependent translocation of DOC2, which has synaptotagmin-like calcium binding C2 domains but lacks a transmembrane domain. Calcium dependence of spontaneous release mediated by DOC2 was tested by bath application of BAPTA-AM which significantly reduced mini frequency in both WT and null neurons to the same extent after incubation for 15 minutes. In addition, we show that DOC2 proteins act analogously to synaptotagmin-1 with a  $\text{Ca}^{2+}$ -sensitivity in the sub- $\mu\text{M}$  range, an order of magnitude higher than that of synaptotagmin-1. DOC2 proteins target to SNARE complexes where they compete with synaptotagmin-1 for SNARE binding. Thus, different classes of multiple C2-domain-containing molecules trigger synchronous versus spontaneous fusion. We conclude that DOC2B is an important, although not exclusive, high affinity calcium sensor that mediates spontaneous neurotransmitter release in hippocampal neurons. In addition, we use triple KO mice (Doc2A/B-Syt1 TKO) primary cultures, on which we overexpress different mutants, to study the interplay between different C2-domain proteins at the synapse.

**KEY WORDS:** Synaptotagmin, Doc2, release**TELEPHONE NUMBER:** 06-16395240**E-MAIL-ADDRESS:** rocio.diezarazola@gmail.com

**TITLE**

**Deep brain stimulation in the nucleus accumbens core activates prefrontal monoamine release**

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**ABSTRACT**

Recent publications have shown promising results of deep brain stimulation (DBS) in the nucleus accumbens (NAc) for patients with obsessive compulsive disorder and major depressive disorder.<sup>1-3</sup> Though DBS appears to be effective in treatment resistant cases, the precise mechanism of action of DBS is still unknown. One of the possible effects of DBS might be the modulation of phasic or tonic monoamine release near the electrode site or at a distant, connected area.

The aim of the current study is to evaluate how DBS affects the extracellular concentration of monoaminergic neurotransmitters in the target region the NAc core and in the orbital and the medial prefrontal cortex, respectively. These areas have either direct anatomical connections to the stimulation target or have been shown to be affected by accumbens stimulation.<sup>4</sup> Male Wistar rats were divided over 3 groups. Group 1 had one unilateral bipolar electrode implanted in conjunction with a custom-made concentric microdialysis probe into the NAc core to measure the effect of DBS on local monoamine release in the NAc core. Group 2 and 3 had two bilateral bipolar electrodes into the NAc core and one microdialysis probe in either the orbital (OFC) or the medial prefrontal cortex (mPFC). The bipolar electrode consisted of two twisted 60 µm platinum/ iridium wires with 2 poles at a distance of 500 µm. Testing started after a recovery period of 7 days. Freely moving animals were stimulated with 300 µA (120Hz, pulse width 80 µs) in the NAc core for 2 hours with a bipolar, biphasic pulse. We measured extracellular concentrations of dopamine, serotonin, their metabolites and noradrenaline using in vivo microdialysis one hour before and during stimulation. We report rapid increases in the release of dopamine and serotonin in the mPFC and dopamine and noradrenaline in the OFC after onset of stimulation in the NAc core but no effect in the NAc core itself.

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**KEY WORDS:** OCD, major depressive disorder; deep brain stimulation, microdialysis

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**TITLE**

**Stage-dependent dopaminergic cell loss in the substantia nigra during Parkinson's Disease**

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**ABSTRACT**

Alpha-synuclein pathology may spread in a predictive manner throughout the brain in six stages as defined by Braak and colleagues. Aged individuals with alpha-synuclein pathology in the brainstem (Braak stage 1-3) may represent the premotor stage of Parkinson's disease. The present study aims to investigate dopaminergic cell loss in the substantia nigra (SN) in Braak stage 0-6 and correlate this with disease duration and alpha-synuclein pathology load in the SN.

Lewy bodies and Lewy neurites were assessed in twelve brain regions in elderly without any clinical reference of neurological disorders and Parkinson patients. In total 56 donors were included of which the frozen or paraffin-embedded SN was available for histological processing. Dopaminergic cell density was determined in eight donors per Braak stage by counting neuromelanin-containing neurons in the entire SN using design-based stereology.

No significant decrease in the dopaminergic nigral density was observed between the premotor stages. A 35% decrease in dopaminergic density was observed in Braak stage 4 compared to stage 3 ( $p=0.02$ ), and 60% overall decrease between controls and Braak 6 ( $p=0.01$ ). No significant correlation between dopaminergic cell density and disease duration was found, but the alpha-synuclein load in the SN does correlate with dopaminergic cell density ( $r=-0.43$ ,  $p=0.02$ ).

Our results suggest profound loss in dopaminergic nigral density in Braak stage 4, but not in the premotor stages of PD. The alpha-synuclein pathology load is correlated to dopaminergic loss in the SN.

**KEY WORDS:** Parkinson's Disease, neurodegeneration, substantia nigra

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**TITLE**

**Region-specific microglia phenotypes in Parkinson's Disease  
Acute microglia isolation from fresh human and rat brain tissue**

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**ABSTRACT**

Recent data indicates that the characterization of Parkinson's Disease (PD) as an isolated disorder of the dopaminergic system is an oversimplification of the complex pathology of the disease (Braak and Braak, 2000). Besides the selective dopaminergic cell and axonal loss in the nigrostriatal system, various other brain areas are affected in PD, showing  $\alpha$ -synuclein accumulation mainly in neurons, a major hallmark of PD pathology (Braak *et al.*, 2003). In addition to the classical motor symptoms, also non-motor clinical features, such as neuropsychiatric symptoms, autonomic dysfunction, sensory and cognitive impairment, pain and sleep disturbances are common in PD and may antedate motor dysfunction (Dickson *et al.*, 2009; Grinberg *et al.*, 2010; Reichmann *et al.*, 2009).

Furthermore, in recent years, neuroinflammation has been found to be characteristic for various neurodegenerative diseases associated with aberrant protein accumulations, including PD. Activation of microglial cells, the key innate immune regulators of the central nervous system (CNS), occurs in various brain regions, including the substantia nigra and olfactory bulb of PD patients which have been widely implicated in disease progression (Glass *et al.*, 2010; Lull and Block, 2010; McGeer and McGeer, 2008; Qian *et al.*, 2010; Vroon *et al.*, 2007; Zhang *et al.*, 2005). Interestingly, there is overt neuronal loss within the substantia nigra but lacking in the olfactory bulb of PD patients. This suggests that local factors e.g. microglial cells can contribute to the brain-region specific outcome of a neuropathological process. Recent data suggest that microglia do not represent a single, uniform cell population but rather encompass a family of cells with diverse phenotypes that occur brain region specific (de Haas *et al.*, 2008). It has subsequently been pursued that activated microglia due to their local phenotype can have beneficial effects next to its known detrimental action (Graeber and Streit, 2010; Schwartz *et al.*, 2006; Streit *et al.*, 2009).

In my PhD project we hypothesize that region-specific microglia phenotypes determine the local neuropathological outcome of PD by their distinct inflammatory profile. To this end, we will use various approaches to test our hypothesis. One of which is determining the presence of different microglia phenotypes in neuropathological differently affected brain regions of PD patients or relevant rat models (e.g. brain region-specific alpha-synuclein overexpression). We therefore acutely isolate microglial cells from fresh human and rat post-mortem brain tissue which enables us to discover region-specific microglia phenotypes by FACS analysis. We will show initial FACS data of microglial cells isolated from human and rat grey matter representative of a technical development essential within my project.

Understanding how microglia can be protective in one brain region, while promoting neurotoxicity in another, will ultimately not only increase our knowledge about specific microglial phenotypes, but also in their role in neurodegenerative processes. As such, it could open new avenues for the development of local PD therapy.

**KEY WORDS:** Parkinson, microglia, FACS

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**TITLE**

**Transgenic mice showing a Vanishing White Matter phenotype**

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**ABSTRACT**

Vanishing white matter (VWM) disease is a severe leukoencephalopathy, classically starting in childhood. The disease is characterized by neurological symptoms like ataxia which are slowly progressive. Episodes of rapid deterioration are triggered by stress and can lead to coma and eventually death. The disease is caused by mutations in any of the five genes encoding the subunits of the eukaryotic translation initiation factor 2B (eIF2B). Although this protein has an important function in all cells, mainly the cells in the brain white matter (astrocytes and oligodendrocytes) are effected by these mutations. Pathologically the brain shows cystic degeneration without gliosis, dysmorphic astrocytes, myelin damage and an increased number of oligodendrocytes. Two mouse models for VWM are developed to elucidate the disease pathology further and to test possible treatments. The 2B4 mouse line has mutations in the *Eif2b4* gene, encoding the  $\delta$  subunit, and the 2B5 mouse line has mutations in the *Eif2b5* gene, encoding the  $\epsilon$  subunit. Both of these mutations have been described in human patients before. Preliminary data show that these mice have neurological symptoms like ataxia and epilepsy. Pathologically, the myelin is effected and the astrocytes are dysmorphic. Although more research is necessary, it seems that these transgenic mice will be a good model for VWM.

**KEY WORDS:** Vanishing white matter, eIF2B, glial cells

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**TITLE**

**Neuronal oscillatory changes in an animal model of Parkinson's disease**

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**ABSTRACT**Introduction

According to clinical studies significant alterations can be detected in synchronous neuronal oscillatory activity in Parkinson's disease (PD) patients. These aberrant synchronous patterns alter interregional communication in the brain and might underlie the cognitive and motor deficits apparent in PD. In our study we aim to investigate interregional connectivity in the cortex and in deep structures in a hemiparkinsonian rat model.

Methods

Six naïve rats were implanted with an electrode array consisting of two frontal (left and righthemisphere) and two depth electrodes targeting the subthalamic nucleus. After one week baseline recordings were executed. One week later we injected 6-OHDA in the medial forebrain bundle to induce the hemiparkinsonian state. New measurements followed the injection (during and after the development of dopaminergic cell loss in the substantia nigra).

During baseline and experimental recordings several behavioural patterns (sit, walk, etc..) were identified and neuronal activity was analysed during these phases. Plexon recording and analysis tools were used. For further analysis we used custom-made Matlab programs and BrainWave (a program assessing coupling measures in human clinical data).

Results and conclusion

In the first set of experiments electrophysiological data was acquired from the four recording sites. Four animals showed spontaneous turning behaviour after the 6-OHDA injection, which supports dopaminergic cell loss. We were able to record animals up to 63 days after the operation; 4-49 days after the 6-OHDA injection. We just started data analysis and we encountered the following phenomena: The local field potentials show alterations after the injection. We observed different patterns occurring in depth, and surface electrodes. Synchronicity measures showed changes in between regions (comparing pre-, and post-injectional data) and in between measure points of one region. Coupling between cortical and subcortical structures also shows differences after 6-OHDA injection.

Our preliminary data seems to underlie the clinical findings: the oscillatory activity of deep brain structures alter by dopaminergic cell loss.

**KEY WORDS:** Parkinsons disease, neurophysiology, synchronisation

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**TITLE**

**The role of RNA processing genes in pontocerebellar hypoplasia**

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**ABSTRACT**

Pontocerebellar hypoplasias (PCH) represent a group of autosomal recessive neurodegenerative disorders with prenatal onset. Thus far, seven subtypes of PCH have been distinguished (PCH 1-7), all showing hypoplasia of cerebellum and pons as well as poor mental and motor development. Up to now, genes encoding for the tRNA splicing endonuclease (TSEN) complex, mitochondrial arginyl-tRNA synthetase (RARS2) and vaccinia-related kinase 1 (VRK1) have been associated with PCH. The TSEN complex consists of four subunits, TSEN54, -34, -15 and -2. The RNA kinase cleavage and polyadenylation factor I subunit (Clp1) associates with the TSEN complex and is involved in tRNA, mRNA and siRNA processing.

Based on in situ hybridizations, we made a 3-dimensional model of TSEN54 mRNA expression in the human embryonic brain at 8 weeks gestational age. This 3D model shows high expression of TSEN54 in the developing cerebellum and telencephalon.

The current understanding suggests a role for tRNA processing in the development of PCH, but the exact disease mechanism remains unclear. In order to investigate the role of (t)RNA processing on the developing brain, we have developed a zebrafish model. Fish harbouring a homozygous nonsense mutation in *tSEN54* or *tSEN34* die within nine days post fertilization. We hypothesize that these mutants survive the early stages of development on maternal tRNAs.

Zebrafish harbouring a homozygous nonsense mutation in *Clp1* show abnormal development including an S-curved body and tail and reduced head and eye size. At 4 days post fertilization, *Clp1* homozygous mutants have lost all reaction upon physical touching. In situ hybridization shows abnormal expression of the developmental brain marker *otx2* in these mutants, demonstrating the importance of *Clp1* during brain morphogenesis.

Our findings in zebrafish *Clp1* and TSEN mutants point towards an important role for tRNA splicing and RNA processing in brain development and the development of PCH.

**KEY WORDS:** RNA processing genes, pontocerebellar hypoplasia, brain development

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**TITLE****Rab6 function during ER stress in Alzheimer's disease****AUTHORS**Hyung L. Elfrink<sup>1</sup>, R. Zwart<sup>1</sup>, M. Lopèz-Cavanillas<sup>1</sup>, F. Baas<sup>1,2</sup> and W. Scheper<sup>1,2</sup>**DEPARTMENT/INSTITUTE**<sup>1</sup>Dept. of Genome Analysis, Academic Medical Center, Amsterdam, the Netherlands<sup>2</sup>Dept. of Neurology, Academic Medical Center, Amsterdam, the Netherlands**ABSTRACT**

Alzheimer's disease (AD) is a neurodegenerative disorder, characterized clinically by progressive cognitive decline and memory loss and pathologically by deposits of aggregated proteins, hyperphosphorylated tau in so called neurofibrillary tangles and amyloid  $\beta$  in senile plaques. We have previously implicated the unfolded protein response (UPR) of the endoplasmic reticulum (ER) in the early stages of AD. The UPR is a stress response that activates after insults, such as protein accumulation, that challenge ER homeostasis. Furthermore, we have shown that the small GTPase Rab6, involved in retrograde Golgi to ER- and intra-Golgi transport, is also increased in the early stages of AD. Both observations show a strong positive correlation.

In this study we investigate the function of Rab6 during ER stress in Alzheimer's disease in more depth.

We employed Rab6 overexpression or knockdown in cell models and investigated the effect of Rab6 on the UPR. Our results show that Rab6 overexpression reduces the UPR and that Rab6 knockdown has the opposite effect. Interestingly, Rab6 function or dysfunction has no effect on UPR signalling. However, we found a profound effect of Rab6 on the recovery from ER stress.

Our results show that Rab6 function modulates the UPR. The increased levels of Rab6 in AD brain might therefore be indicative of a failed attempt to recover from prolonged ER stress. We are currently investigating the mechanism of Rab6 modulation in the UPR.

**KEY WORDS:** Alzheimer's disease, unfolded protein response, Rab6**TELEPHONE NUMBER:** 020-5665889**E-MAIL-ADDRESS:** h.l.elfrink@amc.uva.nl

**TITLE**

**Omega-oxidation of very long-chain fatty acids: a therapeutic option for X-linked adrenoleukodystrophy?**

**AUTHORS**

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**ABSTRACT**Introduction

X-linked adrenoleukodystrophy (X-ALD) is characterized biochemically by increased levels of very long-chain fatty acids (VLCFA) in plasma and tissues. The variation in phenotypic expression is striking and unpredictable as there is no genotype-phenotype correlation. Phenotypes include the fatal, rapidly progressive cerebral demyelination observed in childhood cerebral ALD (CCALD) but also milder phenotypes such as adrenomyeloneuropathy (AMN) and primary adrenocortical insufficiency. All patients have mutations in the ABCD1 gene encoding the peroxisomal membrane protein ALDP. ALDP deficiency strongly increases VLCFA levels by two mechanisms <sup>(1)</sup>: 1) an increase in cytosolic levels of very long chain fatty acyl-CoA, which are then further elongated to VLCFA and 2) accumulation of VLCFA due to impaired peroxisomal beta-oxidation. Interestingly, VLCFA can be degraded via an alternative pathway called omega-oxidation. Key enzymes in this pathway are CYP4F2 and CYP4F3B <sup>(2)</sup>. We hypothesize that induction of these enzymes could up-regulate omega-oxidation activity and thus reduce VLCFA levels in X-ALD patients.

Methods/Results/Conclusions

We have created a cell model allowing us to investigate the effects of CYP4F2 over-expression on both omega-oxidation activity and VLCFA levels. This model enables us to take into account a possible effect of CYP4F2 polymorphisms on our results. Preliminary results will be presented at the meeting in November.

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**KEY WORDS:** X-linked adrenoleukodystrophy, omega-oxidation, very long-chain fatty acids

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**TITLE**

**Optimization of AAV expression vector for overexpression of transcription factors in DRG neurons targeting axonal regeneration**

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**ABSTRACT**

Limited or no regeneration takes place after injury in the mammalian central nervous system (CNS), while injured neurons in the peripheral nervous system (PNS) do regenerate. One reason why regeneration in the PNS is more successful than in the CNS is that injured neurons in the PNS switch on a large number of regeneration-associated genes upon injury, while CNS neurons do not. This response is crucial for successful regeneration and it would be of great interest to be able to manipulate CNS neurons to induce such a regeneration program after injury.

The sensory neurons of the PNS are commonly used as a model to study the intrinsic response of neurons after injury. These reside outside the spinal cord in the dorsal root ganglia (DRG). DRG neurons have one axonal branch in the peripheral nerve and another that enters the spinal cord. Spontaneous regeneration takes place after injury to peripheral axons, while regeneration of central axons from the same cell body is limited after injury. Interestingly, a lesion of the peripheral branch also boosts regeneration of the central axon after subsequent damage. This is caused by the upregulation of a wide variety of regeneration-associated genes in the DRG cell body as response to the peripheral injury.

A number of transcription factors (TFs) are also known to be switched on after axotomy and these are likely to regulate the regeneration-associated gene expression program. Our aim is to artificially induce a regeneration program in a dorsal root lesion model by overexpression of TF(s) in DRG neurons using adeno-associated viral (AAV) vectors, by direct injection or intrathecal administration to the CSF.

Our vectors express a TF and green fluorescent protein (GFP) to label axons of transduced neurons. The most common way to express two proteins is to use a single promoter construct with an internal ribosome entry site (IRES). However, using a construct with IRES, the second protein is expressed very inefficiently. Therefore, we developed a dual promoter construct, which expresses both the TF under study and GFP. Here, we show that our optimized AAV dual promoter construct is able to both express the TF under study and label axons of transduced DRG neurons with GFP efficiently in the dorsal root injury model.

**KEY WORDS:** Regeneration, AAV, transcription factors

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**TITLE**

**Secretory vesicles in neurons: capture and fusion**

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**ABSTRACT**

Synaptic transmission is modified by external modulation via a variety of chemical signals, such as neuropeptides, hormones and growth factors. These signals are secreted from large dense core vesicles (DCVs) in neurons, by mechanisms that differ at least in some respect from the mechanisms by which synaptic vesicles release classical neurotransmitters.

Neuropeptides play a key role in many of the most important brain function such as memory, learning, reward but also mood, pleasure and pain perception. Defects in neuropeptide release have been linked to the onset of psychiatric disease.

Despite the importance of DCV mediated signalling, our knowledge on DCV transport, capture and release is still limited.

In order to follow the vesicles trafficking, neuropeptide Y (NPY), a DCVs marker, has been fused to a red fluorescent reporter (RFP) and to a pH-sensitive variant of GFP (pHluorin) to detect vesicle fusion. Two color imaging allows the tracking of DCVs from the cell soma where they are formed until they fuse with the plasma membrane releasing their content.

**KEY WORDS:** Neuropeptide, DCVs, secretion

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**TITLE****CRB2 in retinal development****AUTHORS**

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**ABSTRACT**

In Leber congenital amaurosis vision is lost within the first year of life. In about 10% of the cases this is caused by mutations in the Crumbs homolog 1 or *CRB1* gene. Mice lacking CRB1 show retinal degeneration in up to one quadrant of the retina, which might be due to overlapping functions with CRB2 and/or CRB3 in the other quadrants.

To answer to that question we generated *Crb2* conditional knockout (cKO) mice. The *Crb2* cKO mice were generated and crossed with *Chx10-Cre* transgenic mice. Animals from embryonic day 16 till 1.5 year of age were analyzed. In order to investigate the possible ectopic localization of proteins and cells we performed immunohistochemistry (IHC) on the retinas of these animals. Optical coherence tomography (OCT), scanning laser ophthalmoscopy (SLO) and electroretinography (ERG) were performed in adult animals. Moreover, a morphological analysis was performed using toluidine blue staining in Technovit sections. Retinal disorganization starts at embryonic day 18 at the periphery. At post-natal days 6 and 10, progressive disorganization was detected in all four quadrants of the retina, characterized by gaps in the outer limiting membrane (OLM) and ectopic cell nuclei in the subretinal space. At a later stage, retinal degeneration of the outer and inner retina was detected. IHC experiments performed on adult animals, showed loss of localization of members of the Crumbs complex at regions with disrupted OLM. We detected unusual localization of MPP4 and PSD95 in the outer nuclear layer suggesting ectopic synapses. We also observed increased levels of GFAP and CD11b in these animals suggesting gliosis and activated microglia. In *Crb2* cKO retinas, we observed smaller cone photoreceptor segments at P15 and P21, whereas the total number of cones is not affected at these time points, suggesting that CRB2 plays a role in determining the length of photoreceptor segments. ERG demonstrated a progressive reduction of the electrical retinal activity in the *Crb2<sup>F/F</sup>;Chx10-Cre<sup>Tg/+</sup>* animals from one month of age.

Loss of CRB2 results in a more severe retinal degeneration than loss of CRB1. CRB2 seems be necessary for proper retinal development, especially at a late time point. The lack of the protein leads to early retinal disorganization and at a later stage to retinal degeneration.

**KEY WORDS:** Crumbs homologue 2 (CRB2), conditional knockout, Leber congenital amaurosis (LCA)

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**TITLE****Altered function of cortico-striatal systems after cocaine self-administration****AUTHORS**

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**ABSTRACT**

In cocaine addiction, the descent from casual, recreational drug use into the compulsive patterns of cocaine taking that characterize addiction is thought to result from altered function of limbic corticostriatal systems underlying drug reward, motivation and habit formation. To investigate the neural underpinnings of cocaine addiction, we examined the expression of 20 immediate early genes (IEGs) using quantitative PCR in dorsal and ventral corticostriatal systems after short-term (10 days) and long-term (60 days) cocaine self-administration (SA) in comparison to SA of sucrose or control conditions. We next selected the two most strongly regulated IEGs for in situ hybridization (ISH) to determine changes in cell numbers and/or cellular expression levels of IEG mRNA. QPCR showed that short-term cocaine SA significantly ( $p < 0.05$ ) upregulated *c-fos*, *MKP-1*, *Egr2* and *FosB* mRNA in medial prefrontal cortex (mPFC), and dorsal (DS) and ventral striatum (VS). *Arc* mRNA increased in mPFC and DS only. Long-term cocaine SA showed the same response, but *Egr4*, *Homer1a*, *TrkB*, *BDNF*, *c-Jun*, *Sgk* and *Dclk* were significantly changed as well, after cocaine or sucrose SA, mainly in mPFC. ISH results from the two selected IEGs - *c-fos* and *MKP-1* - after short-term cocaine SA, confirmed the qPCR data. Moreover, subregional analysis showed response gradients of *c-fos* and *MKP-1* expression in mPFC and orbitofrontal cortex (OFC), with anterior cingulate having stronger upregulation than prelimbic and infralimbic cortex, and medial orbital displaying bigger changes than ventral lateral and lateral orbital cortex. Compared to the sucrose SA or control animals, *c-fos* or *MKP-1* cell numbers were significantly increased in DS, VS, mPFC, OFC and ventral agranular insular cortex but not in dorsal agranular and dysgranular insular cortex in the animals self-administering cocaine. In striatum, compared to the control animals, stronger increases in cell numbers were found in DS (303%) than in VS (177%), with the strongest regulation in the medial part of DS (332%). In summary, our short-term cocaine SA data suggest a prominent involvement of the dorsal mPFC, medial OFC and medial DS in this behavior. These findings point to a major role of the dorsal striatum - possibly as part of the prelimbic – medial dorsal striatum projection system – in cocaine reinforcement in animals with limited cocaine SA experience. Whether the neural substrates of cocaine SA change after long-term cocaine SA experience is currently being investigated.

**KEY WORDS:** Cortico-striatal system, cocaine, immediate early gene

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**TITLE****Functional analysis of Tomosyn-2 in the mammalian brain****AUTHOR(S)**Lieke (C.J.) Geerts, K.B. Janssen, M. Verhage and A.J.A. Groffen**DEPARTMENT/INSTITUTE**

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**ABSTRACT**

Synapses in the brain are specialized in the secretion of signalling molecules. Secretory events depend on the gradual assembly of the SNARE complex. SNARE assembly is regulated by various accessory proteins, among which is tomosyn. The mammalian tomosyn family comprises two isoforms encoded by different genes, tomosyn-1 and -2. Tomosyn is thought to compete with the vesicle-bound SNARE protein synaptobrevin for SNARE complex binding. Since tomosyn is not associated with vesicles, this has been suggested to inhibit secretion. Thus far, most studies have focused on tomosyn-1 while tomosyn-2 is the predominantly expressed isoform during postnatal and adult development of the nervous system. The aim of this project is to identify the function of tomosyn-2 in the mammalian nervous system. We generated a mouse line with a conditional null ('floxed') allele of the tomosyn-2 gene. Here we describe the generation of a tomosyn-2 null mice by mating the floxed allele with a Cre deleter strain. These mice are found to have an early postnatal sublethal phenotype. No defects in neurite formation, outgrowth and synaptogenesis were observed in primary cultures of dissociated neurons. Additionally, postnatal development assessed by body weight was unaffected in tomosyn-2 null mice. Also, autapse electrophysiology measurements were performed to investigate effects on synaptic strength and plasticity. Synaptic transmission was found to be enhanced in tomosyn-2 null mice, indicating an inhibitory role for tomosyn-2 in secretion of synaptic vesicles.

**KEY WORDS:** Tomosyn, SNAREs, synaptic transmission**TELEPHONE NUMBER:** 020-5986931**E-MAIL-ADDRESS:** lieke.geerts@cncr.vu.nl

**TITLE****The neural substrate of set-shifting deficits in Parkinson patients****AUTHORS**Niels J.H.M. Gerrits, Y.D. van der Werf and O.A. van den Heuvel**DEPARTMENT/INSTITUTE**

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**ABSTRACT**Introduction

Parkinson's disease (PD) patients often suffer from cognitive deficits, such as a failure to flexibly switch between response options, so-called set-shifting. Previous research investigated the neural substrate of this deficit and found that PD patients made more errors and had a reduced activation in the caudate nucleus and the prefrontal cortex. Often, however, the medication effects and test paradigm, using other cognitive functions beside set-shifting, represent potentially confounding factors. We therefore designed a new paradigm and used medication-free patients.

Methods

Healthy controls (N=22) and PD patients (N=11) categorized an arrow according to its location (left or right) or to the direction in which it pointed (up or down). The correct response depended on which stimulus dimension was relevant. After a correct response, positive feedback was given, after an incorrect response, negative feedback. Then, without notice, the relevant stimulus dimension changed and participants had to set-shift. The neural activity during the task was recorded using functional MRI and analysed using first- and second-level analyses in SPM8.

Discussion

We found that healthy controls made fewer errors and showed more activation in the right dorsolateral prefrontal cortex and left striatum than PD patients, in accord with previous findings.

**KEY WORDS:** Parkinson's disease, cognitive deficits, functional MRI**TELEPHONE-NUMBER:** 020-4449635**E-MAIL-ADDRESS:** n.gerrits@vumc.nl

**TITLE****Imaging the dopaminergic system in obesity****AUTHORS**Elsmarieke van de Giessen<sup>1,2</sup>, Barbara de Weijer<sup>3</sup>, Swen Hesse<sup>4</sup>, Wim van den Brink<sup>5</sup>, Jan Booij<sup>1</sup>**DEPARTMENT/INSTITUTE**<sup>1</sup> Dept. of Nuclear Medicine, Academic Medical Center, Amsterdam, <sup>2</sup> Dept. of Neurogenetics, Academic Medical Center, Amsterdam, <sup>3</sup> Dept. of Endocrinology, Academic Medical Center, Amsterdam, <sup>4</sup> Dept. of Nuclear Medicine, University of Leipzig, Leipzig, Germany, <sup>5</sup> Dept. of Psychiatry, Academic Medical Center, Amsterdam**ABSTRACT**Introduction

Overeating of highly palatable and caloric foods is thought to play a major role in the pathophysiology of obesity. The dopaminergic brain reward system is involved in the reinforcing effects of food and therewith overeating. For example, it has been shown that food is able to induce a dopamine release in the striatum and that dopaminergic drugs such as methylphenidate and amphetamine are able to reduce appetite. Thus, it has been questioned whether the dopaminergic reward system in obese people might be affected. First evidence for this is the finding that obese people have decreased striatal dopamine D2 receptor (DRD2) availability, which is similar to the decrease found in addiction. The presented studies further assess the dopaminergic reward system in obesity to gain more insight the role of this system in the pathophysiology of obesity.

Methods

Three studies using single photon emission computed tomography (SPECT) imaging are performed to assess the striatal dopaminergic system in obesity. 1. In 15 obese and 15 control women, it is assessed whether obese subjects have a decreased reactivity of the dopamine system on an amphetamine challenge. 2. In 19 obese women, it is assessed whether acute weight loss after bariatric surgery leads to a change in striatal DRD2 availability. 3. In a European sample of 116 subjects, it is assessed whether a high body mass index (BMI) correlates with striatal dopamine transporter (DAT) availability.

Results and discussion

Preliminary results of study 1 indicate that obese women have lower striatal DRD2 availability than control women, which replicates the previous finding. Further results on the reactivity of the dopamine system will be shown at the meeting. Study 2 shows that there is no significant change in striatal DRD2 availability after acute weight loss by bariatric surgery. This suggests that weight change does not directly influence the dopaminergic reward system on short term. At last, it was shown in study 3 that there is no correlation between BMI and striatal DAT availability. This indicates that the pre-synaptic side of the dopaminergic system is not affected in obesity. In short, only the post-synaptic side of the dopaminergic reward system is affected in obesity, reflected by lower striatal DRD2 availability, and this lower availability does not change shortly after acute weight loss.

**KEY WORDS:** Dopamine, obesity, SPECT scan**TELEPHONE NUMBER:** 020-5668323**E-MAIL-ADDRESS:** e.m.vandegiessen@amc.uva.nl

**TITLE****Involvement of the hypothalamic Orexin system in Olanzapine induced metabolic side-effects****AUTHORS**Elodie M. Girault<sup>1</sup>, E. Foppen<sup>1,2</sup>, M.T. Ackermans<sup>3</sup>, E. Fliers<sup>2</sup>, A. Kalsbeek<sup>1,2</sup>**DEPARTMENT/INSTITUTE**<sup>1</sup>Netherlands Institute for Neuroscience, KNAW, Amsterdam, <sup>2</sup>Dept. of Endocrinology and Metabolism, and <sup>3</sup>Laboratory of Endocrinology, Dept. of Clinical Chemistry, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands**ABSTRACT**

Atypical antipsychotic drugs such as Olanzapine (Olan) induce weight gain and metabolic changes associated with the development of type 2 diabetes. The mechanisms underlying these undesired side-effects are currently unknown. Therefore, we investigated the effects of Olan on glucose metabolism in undisturbed, awake and freely moving male Wistar rats.

In the 1st experiment, we administered Olan continuously for 160 min via an intragastric (IG) cannula. Blood glucose levels in the Olan-infused rats showed an acute increase from  $\pm 5.5$  to  $\pm 8.5$  mmol/L, while blood glucose levels in Vehicle-treated rats showed no significant increase. Surprisingly, endogenous glucose production (EGP) only showed a minor change in Olan-treated rats. Therefore, the increased basal blood glucose levels upon the IG administration of Olan are most likely mainly due to decreased glucose uptake and utilization. In a 2nd set of experiments, we investigated the effects of acute Olan administration on insulin sensitivity by using the hyperinsulinemic-euglycemic clamp with stable isotope technique. In the Vehicle-treated rats elevating circulating plasma insulin levels 2-fold as compared to basal levels caused the expected decrease in EGP of  $\pm 40\%$ . The IG Olan-treated rats showed only a minimal decrease in EGP ( $\pm 10\%$ ). Similar results were found when circulating plasma insulin levels were elevated 6-fold: EGP decreased  $\pm 80\%$  in controls and only  $\pm 55\%$  in Olan rats. Obviously, acute IG administration of Olan induces severe hepatic and peripheral insulin resistance in rats.

It has been shown that peripheral injections of Olan induce Fos activation in neurons of the lateral hypothalamus/perifornical area and that a large part of these activated neurons are Orexin A-positive (Stephanidis et al., 2009). In ongoing experiments we aim to confirm the involvement of the central Orexin system in the metabolic side-effects of Olan by comparing the Olan infusions animals treated ICV with Orexin A antagonist or vehicle.

**KEY WORDS:** Olanzapine, Orexin, glucose metabolism**TELEPHONE NUMBER:** 020-5664524**E-MAIL-ADDRESS:** e.girault@nin.knaw.nl

**TITLE**

**Differences in brain myelinisation in mouse recombinant inbred strains**

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**ABSTRACT**

Proper functioning of the brain depends on the adequate integration of information of multiple brain regions. White matter tracts mediate the information flow between distant brain areas. Myelination of axons is essential for high-speed conductance and propagation of nerve impulses. Myelin may affect cognitive processes in the brain. Defects in myelin insulation in multiple sclerosis patients cause abnormal synchronization of brain areas and impair cognitive function. Vice versa, cognitive training of adults induces changes in white matter structure. However, an active plastic role for myelin in cognitive functions still has to be determined. Here we study the possible role of myelin in cognition, by analyzing myelination in inbred recombinant inbred strains of mice. We performed a clustering analysis on hippocampal gene expression of myelin genes in C57/BL6 (C57), DBA/2J (DBA) and their BXD offspring. We showed that all of the myelin genes were similarly regulated and that C57 and DBA mice were among the extreme ends of myelin expression across the inbred strains. Our Western blot analysis on brain, hippocampus and cerebellum homogenates from C57 and DBA mice confirmed strain differences at the protein level. Ongoing experiments involve electron microscopy to verify myelin differences at the protein level and visualize myelin differences in the brain. Moreover, conduction velocity measurements will be performed to relate myelin gene and protein expression to long tract information processing and behavior.

**KEY WORDS:** DBA, C57, myelin

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**TITLE**

**The dynamics of conditioned fear behavior in an automated Home Cage (DualCage) environment**

**AUTHORS**

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**ABSTRACT**

Fear conditioning is an important test in behavioral neuroscience, used to investigate the neural systems and molecular basis of various aspects of emotional learning across a wide range of species including rodents. Dysfunction of the fear circuits is implicated in mechanisms of affective disorders. Classical fear conditioning methods require frequent handling which confounds the measurement of fear-related parameters such as the behavioral response and autonomic responses (e.g. heart rate) of the animal. We therefore developed a novel fully automated home cage environment (DualCage; see *HomeCage<sup>Plus</sup>*, Biobserve) to explore the dynamics of fear responses during deliberate and/or motivated choice of mice. The DualCage consists of a home compartment (HC) attached to a shock compartment (SC) separated by an automatically operated sliding door. A separation of compartments is necessary to exploit the novelty-seeking behavior of mice at distinct times after training to investigate short- and long-term memory.

The behavior is monitored by two cameras using specific tracking software (Viewer©, Biobserve) with a software script controlling the actions of the hardware. This includes opening and closing of doors and shock delivery at specific times and/or depending on the position of the mouse in an operant fashion, e.g. after an instrumental response such as entering the SC. The behavior is monitored for several days during the light and dark phases and the video is stored digitally. Mice are not handled during the entire experiments to monitor spontaneous exploratory behavior during training and testing. Tests were performed with C57BL/6J mice exposed to either a single or no shock (US) during the first SC visit, and the subsequent progression of exploration was monitored.

The spontaneous behavior elicited by contextual fear memory is characterized by risk assessment when access to the SC is granted including peeking through the door in a stretch-attend posture before eventually revisiting the SC. Mice not subjected to a US revisited the shock compartment immediately after access was provided, whereas US-exposed mice showed a slow progression of various behavioral displays indicative of risk assessment and avoidance before fully entering the SC with considerable delay (median latency > 6 hr). Revisits of the SC eventually occurred despite the lack of e.g. rewarding reinforcement indicating the natural motivation of mice to explore their environment despite previous negative experiences.

This DualCage system exploits naturalistic-like behavior of mice that is characterized by trade-offs between avoidance and novelty-seeking (curiosity) in conflict situations with quantification on long time scales based on deliberate choice. The DualCage system with a separation of HC and attached compartment is equipped with versatile add-on components designed to integrate any commercially available hardware for multi-purpose use depending on the scientific questions and the behavior of interest. This approach will allow avoiding any unspecific interference by the experimenter and may increase the replicability of findings.

**KEY WORDS:** Automated in vivo phenotyping, fear conditioning, naturalistic behaviour

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## TITLE

**Critical-state dynamics of avalanches and oscillations jointly emerge from balanced excitation/inhibition in neuronal networks**

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## ABSTRACT

Variability in background neuronal activity has traditionally been viewed as random noise with little interest shown in its source or function. Recent empirical evidence has shown that this "noise" has a distinct character, suggesting that the brain is acting in a critical state poised between order and disorder<sup>1</sup>. In this state scale-free modulations occur, meaning that no spatial or temporal scale takes precedence over another. Research has characterized this critical behaviour into two separate and seemingly irreconcilable lines of research: neuronal avalanches<sup>2</sup> on short time scales and scale-free modulation of oscillations on long time scales<sup>3</sup>. Neuronal avalanches are defined as field potential propagation in local networks. Theoretical studies have shown optimum information processing<sup>4</sup> and reactivity<sup>5</sup> when a network produces scale-free neuronal avalanches, whereas pre-clinical studies have shown that scale-free modulation of oscillations is altered in epilepsy, schizophrenia and Alzheimer's disease<sup>6</sup>.

A major unresolved issue is whether neuronal networks are capable of simultaneously producing neuronal avalanches and oscillations with critical-state dynamics.

Here we study a widely accepted model of neuronal networks and show that critical-state dynamics on the level of neuronal avalanches on short time scales (< 120 ms), and that of neuronal oscillations on long time scales (> 2 s) can be unified through a common mechanism of balanced excitation/inhibition. We find that any deviation from balanced connectivity leads to a breakdown of scale-free behaviour in both neuronal avalanches and oscillations. Our model provides a framework within which the classic notion of homeostatic synaptic plasticity<sup>7</sup> can be reconciled with recent evidence of critical behaviour on different levels of neuronal organisation. This raises the possibility that modulations in oscillations may be used as a proxy to predict changes in the dynamics of neuronal avalanches—and vice versa.

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**KEY WORDS:** Oscillations, criticality, avalanches

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**TITLE**

**The UPR and disturbed glucose metabolism: early events in Alzheimer's Disease**

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**ABSTRACT**Background

Accumulation of misfolded proteins in the endoplasmic reticulum (ER) leads to activation of the unfolded protein response (UPR), a protein quality control mechanism that initially protects the cell against ER stress toxicity. We have previously shown that the UPR is activated in neurons in the AD brain very early in pathology. Decreased glucose metabolism is an established trigger to activate the UPR. Interestingly, multiple epidemiologic studies indicate that the metabolic syndrome is a strong risk factor for AD and that AD patients have disturbed glucose metabolism.

Aim

In this study we investigate if metabolic stress results in activation of the UPR to restore glucose homeostasis.

Methods

Differentiated SK-N-SH cells were treated with ER stressors and the levels of UPR markers and glucose transporters were analyzed using Western Blotting, qPCR and immuno-fluorescence. Uptake of glucose was analyzed using a hexokinase assay.

Results

We show that UPR activation affects the expression of the major neuronal glucose transporter GLUT3 via a post-transcriptional mechanism and consequently increase glucose uptake.

Conclusion

Our data indicate that activation of the UPR under conditions of metabolic stress actively enhances the capacity of the cell to restore the energy homeostasis. Failure to restore the homeostasis will result in prolonged activation of the UPR which can lead to tau phosphorylation. We propose that the UPR provides a mechanistic connection between metabolic disturbances and AD pathology.

**KEYWORDS:** Alzheimer's Disease, glucose metabolism and UPR

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**TITLE**

**Frontotemporal dementia and Parkinsonism linked to chromosome 17: from disease mechanism to new therapeutic strategy**

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**ABSTRACT**

Frontotemporal dementia and Parkinsonism linked to chromosome 17 (FTDP-17) is an early onset dementia that primarily affects frontal and temporal lobes. Despite extensive research efforts, the origins of this devastating disease remain poorly understood. Pathologically, FTDP-17 is characterized by focal atrophy, neuronal loss and accumulation of hyperphosphorylated microtubule associated protein tau (MAPT). Tau is important for promoting tubulin polymerization and is involved in axonal transport. In adult human brain, six tau isoforms are produced from a single gene by alternative mRNA splicing. Three of these isoforms contain 4 microtubule-binding repeats (4R); whereas the other three contain only 3 microtubule-binding repeats (3R). Until now, more than 40 mutations in MAPT are known to cause FTDP-17. The majority of these mutations affect alternative splicing of the repeat domain, encoded by exon 10. As a consequence, the ratio of 4R/3R shifts towards increased expression of 4R tau. Overexpression of 4R tau correlates with increased hyperphosphorylated 4R isoforms, 4R inclusions and neuronal loss.

In this study, we want to know all important splicing factors modifying inclusion of MAPT exon 10. Identifying these splicing factors might provide us with interesting target genes for FTDP-17 therapeutics. To examine alternative splicing regulation of MAPT exon 10, we will conduct a shRNA screen of ~330 components of the human spliceosome. Alterations in 4R and 3R transcript levels will be determined by quantitative PCR, which has been optimized to specifically detect the different isoforms.

A more directed therapeutic strategy would be to normalize the ratio between 3R and 4R tau. To explore this possibility we tested different shRNAs and were able to identify shRNAs that could specifically silence 4R MAPT expression in human neuroblastoma cell lines. We aim to extend these findings into primary cortical neurons of humanized MAPT mice and determine if normalization of the ratio can prevent disease related phenotypes. Combination of these studies will give more insight into the underlying gene network for MAPT exon 10 inclusion and determine if the use of shRNAs targeting 4R tau could represent a new strategy for therapeutic intervention.

**KEY WORDS:** FTDP-17, tau, alternative mRNA splicing

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**TITLE****Corticosteroid regulation of the human amygdala****AUTHOR**Marloes J.A.G. Henckens**DEPARTMENT/INSTITUTE**

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**ABSTRACT**

Adequate responding to acute threatening situations is of critical importance to survival. The amygdala is a key player in this process, regulating vigilance and heightening attention towards threat. Its activity is boosted upon threat exposure and its connectivity to other regions of the brain's vigilance-network strengthened. Obviously, this mechanism is beneficial for survival, but may at the same time become maladaptive and culminate in mental diseases such as post-traumatic stress disorder (PTSD) when not properly regulated. Corticosteroids, the end product of the hypothalamic-pituitary-adrenal (HPA) axis, are thought to be involved in this regulation by controlling brain- as well as HPA-activity by providing negative feedback to the brain. However, it is unknown how corticosteroids affect the human amygdala and the neural circuitry it is connected to. Implementing a randomized, double-blind, placebo-controlled design, we investigated the effects of 10 mg hydrocortisone on both amygdala responsivity and connectivity, using task-related and resting state functional MRI respectively. Results showed that hydrocortisone generally decreased responses of the amygdala to emotional input. Furthermore, hydrocortisone reduced *positive* functional coupling of the amygdala to brain regions involved in the initiation and maintenance of the stress-response, i.e. the locus coeruleus, hypothalamus, and hippocampus, while reducing *negative* functional coupling to brain regions involved in executive control; the middle frontal and temporal gyrus. These results suggest that corticosteroids avoid amygdala overshoot during stress and enable adequate recovery thereafter by reducing its impact on the rest of the brain. Thus, corticosteroids seem to critically contribute to the restoration of homeostasis by normalizing brain processing in the aftermath of stress.

**KEY WORDS:** Cortisol, amygdala, fMRI**TELEPHONE NUMBER:** 0634315715**E-MAIL-ADDRESS:** M.J.A.G.Henckens@umcutrecht.nl

**TITLE**

**Development of an immunologically-inert reverse tetracycline controlled lentiviral vector for gene therapy in the peripheral nervous system**

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**ABSTRACT**

The outcome of surgical intervention after severe traumatic injury to the peripheral nervous system (PNS) is often limited. Current interventions mainly consist of reconstructive techniques like grafting and nerve transfer. At present, these techniques have reached an almost perfect refinement. Novel therapeutic strategies are needed to further enhance regeneration.

A promising strategy is gene therapy. Over the past decade lentiviral vectors (LV) have emerged as potent gene delivery vehicles for Schwann cells in the PNS. We have shown that lentiviral vectors are effective in directing sustained and enhanced expression of neurotrophic factors in the injured peripheral nerve. The main setback, however, is that following transduction these neurotrophic factors are expressed in an uncontrollable way. High levels of neurotrophic factors have been shown to limit axonal outgrowth and cause systemic side effects. A possible solution to this problem would be the development of an LV system that can be switched on and off.

The focus of the current project is to develop an LV system that expresses a transgene of interest under a regulatable promoter. A candidate system that has proven its efficacy in-vitro and in -vivo is the tetracycline-dependant transcriptional regulatory system. This system however induces an immune response directed against the transactivator proteins in the immune-competent PNS. In order to circumvent the immune response against the transactivator producing Schwann cells, we developed an LV construct exploiting the evasion mechanism of the Epstein-Barr virus Nuclear-Antigen 1 strategy. We present here the first in-vitro results comparing the classical system to our stealth variant.

**KEY WORDS:** Lentivirus; inducible promoter; peripheral nervous system

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**TITLE**

**Gene expression profile and functional annotation of the human ciliary body epithelia**

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**ABSTRACT**

The ciliary body (CB) of the eye is a neuroepithelium that consists of the non-pigmented (NPE) and pigmented (PE) epithelium. One of its multiple functions is the production of the aqueous humor (AH). AH is necessary to build up and maintain intra-ocular pressure (IOP). Increased IOP is a major risk factor for the development of primary open angle glaucoma. So far, little is known about the molecular machinery and functional properties of the NPE and PE.

To study the gene expression profile and functionalities of the CB, we isolated NPE and PE cells from 4 healthy human donor eyes using laser dissection microscopy. Next, RNA isolation, amplification, labeling and hybridization against 44k Agilent microarrays were carried out. We subdivided the expression data in different sub-datasets and analyzed these with bioinformatics (Ingenuity) to assign functional properties. For conformation, we performed immunohistochemistry on frozen ciliary body slides (8µm).

We found that the gene expression profiles of the NPE and PE resembled each other, with 3100 genes highly and commonly expressed in both cell layers. However, the NPE and PE also showed substantial differences. The comparison between NPE and PE revealed 265 genes that were statistically significant higher expressed in NPE compared to PE and 952 genes significantly higher expressed in PE compared to NPE (both after correction for multiple testing, p-value < 0.05). We found that the main functionalities of the NPE and PE related to immunological functions, embryonic stem cell properties, endocrine regulations and neural characteristics. Immunohistochemistry confirmed our data.

**KEY WORDS:** Ciliary body, microarray, gene expression profile

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**TITLE**

**Object recognition and image parsing**

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**ABSTRACT**

Based on low-level analysis of a visual scene, our visual system groups parts of an object together and separates different objects from the background and each other. A widely held view is that the grouping process occurs without attention and in parallel across the visual scene. We challenge this view and hypothesize that attention spreads from one point on the object towards the boundaries, thereby labeling the perceptual object as one entity in the visual cortex. In our psychophysics study, we investigated the time-course of image-parsing of natural images. Participants judged whether two cues are on the same or on two different objects. We found that image-parsing was serial, as participants were slower when the distance between cues was larger, and even slower when cues are on different parts of the object. Classification of images as animals or vehicles was fast and efficient. Moreover, when comparing up-right and reverted images, we find that object-familiarity facilitates image parsing. Our study suggests that object classification is a fast process which is based on the feedforward information of image features to higher visual areas. Subsequently, a serial image parsing process, facilitated by object familiarity, groups image features together to a single perceptual object.

**KEY WORDS:** Natural images, attention, perceptual grouping.

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**TITLE**

**Target cell dependency of presynaptic properties in hippocampal neurons**

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**ABSTRACT**

Presynaptic terminals of the central nervous system form a highly heterogeneous population. Even synapses originating from the same neuron may vary substantially in both structural and functional properties, including synaptic release probability (Pr) and the number of vesicles. Furthermore, Pr can change over time, depending on the pattern of neuronal activity (short-term plasticity, STP) and the activation of presynaptic receptors. Experiments performed in acute slices of the hippocampus and cortex have shown that Pr, STP and presynaptic receptor expression depend on the identity of the postsynaptic target cell. Molecular mechanisms of this target dependency are not well understood. In the current study we investigated target dependency of presynaptic properties in cultured hippocampal neurons; a system well suitable for mechanistic studies. Using immunocytochemical stainings, we found that the synaptic expression levels of many presynaptic proteins, including Munc18, Bassoon and VAMP, depends on the identity of the postsynaptic target. This was true for both GABAergic and glutamatergic synapses. Surprisingly, the total surface area of the presynaptic terminal did not depend on the target identity, suggesting that terminal content, but not terminal size depends on the postsynaptic target. Neuronal activity or synaptic transmission does not seem to play a role, since 1-7 day incubation with TTX or DNQX+AP5 had only very limited effect. Live imaging experiments using SypHy, a fluorescent marker for vesicle release, showed that Pr correlated very well with the target dependency of presynaptic protein levels. Thus, both structural and functional properties of presynaptic terminals depend on the identity of the postsynaptic target, but this is independent of neuronal activity. Current research focuses on the molecular mechanisms, using pharmacological and genetic tools.

**KEY WORDS:** Synaptic transmission, presynaptic strength, imaging

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**TITLE**

**Regulation of neuronal plasticity by basic leucine zipper transcription factors**

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**ABSTRACT**

Neuronal cells are plastic in their connectivity, both in healthy and injured states. Previous research showed that the basic leucine zipper (bZIP) transcription factors CREB, NFIL3 and C/EBP play important roles in regulating gene expression during neuronal repair. Further analysis showed that NFIL3 and CREB cooperate in a so-called incoherent feed-forward transcriptional regulatory loop: CREB induces NFIL3, and NFIL3 subsequently represses the transcription of CREB target genes. Transcriptional feed-forward loops are known to be important for determining temporal aspects of gene regulation. Additional findings showed that C/EBPs, which are downstream targets of CREB, share many target genes with NFIL3, suggesting that C/EBPs are part of the CREB-NFIL3 regulatory loop. Interestingly, CREB and C/EBPs are also essential transcriptional regulators of long-term synaptic plasticity and memory in the hippocampus. However, a regulatory role for NFIL3 herein has not been demonstrated yet. We show that activation of the cAMP-PKA-CREB pathway in cultured primary hippocampal cells induces NFIL3 expression. Therefore we hypothesize that, analogous to neuronal repair, hippocampal neurons require NFIL3 to fine-tune the expression of CREB and C/EBP target genes that are involved in synaptic plasticity. To test this hypothesis, we generated an NFIL3 conditional (floxed) knockout mouse, and we plan to use these animals to study the effects of hippocampal NFIL3 gene deletion at the molecular, cellular and behavioral levels.

**KEY WORDS:** Fear conditioning, transcription factors, NFIL3

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**TITLE**

**Interaction of the intermediate filament protein GFAP with Notch signalling**

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**ABSTRACT**

Adult neural stem cells (NSCs) express the intermediate filament protein glial fibrillary acidic protein (GFAP), typifying them as astrocytes. Newly discovered GFAP-isoforms facilitate distinction of subpopulations of GFAP-expressing cells. Our lab showed that in the human brain, the alternatively spliced isoform GFAP- $\delta$  is specifically expressed in NSCs of the subventricular zone, which is one of the two neurogenic niches in the adult brain.

We hypothesise that a change in intermediate filament composition, induced by alternative splicing of the GFAP gene, is functionally important for the stem cell characteristics of the neurogenic astrocytes. Therefore, we will modulate GFAP- $\delta$  expression in these cells to investigate, whether this isoform is not only a marker for NSCs, but can also influence the stem cell characteristics, such as self renewal(mitosis), differentiation and cell type specific signaling.

We aim to specifically silence GFAP- $\delta$  by targeting an alternative exon, which is expressed in the GFAP- $\delta$  isoform, but not in the canonical isoform GFAP- $\alpha$ . In addition, we target the 3'UTR of GFAP- $\alpha$  to downregulate the canonical transcripts, but not GFAP- $\delta$ , which is feasible because GFAP- $\delta$  encodes an alternative terminal sequence. For interference with GFAP expression, short hairpin RNAs (shRNA) are used, resulting in isoform-specific downregulation of endogenous GFAP. Moreover we designed an approach to skip the alternative exon of GFAP- $\delta$  using an U7 small nuclear RNA (snRNA) carrying antisense sequences targeting splicing enhancers. Moreover, proteasomal inhibition, which was shown to downregulate GFAP mRNA in general, can be used to change the isoform ratio of GFAP- $\delta$  versus GFAP- $\alpha$ .

Investigation of changes in cell signaling will be analysed, based on the fact that intermediate filaments are increasingly recognized as important signaling platforms in the cell. Our research focuses on Notch signaling, which is crucial for stem cell maintenance, since it has been shown that GFAP- $\delta$  interacts with presenilin, which is part of the proteolytic complex that cleaves Notch.

Ultimately, understanding the role of alternative splicing in neurogenic astrocytes may help to comprehend the identity of neural stem cells in the human adult brain.

**KEY WORDS:** Alternative splicing, neurogenic astrocytes, Notch signaling

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**TITLE**

**Identification of interactors of the Shisa family of potential AMPA receptor modulatory proteins.**

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**ABSTRACT**

The AMPA-type glutamate receptor is widely expressed in the brain. Its tight regulation of function is prerequisite to long term synaptic plasticity and synaptic efficacy and is crucially involved in memory formation and storage.

Identification of AMPA receptor interacting proteins is currently under intense study, and several new auxiliary subunits for the AMPA receptor were found over the last years. TARPs (the transmembrane AMPA receptor regulatory proteins) control AMPA receptor subcellular localization and channel kinetics. Similarly, Cornichons CHIN 2 and 3 – a family of small transmembrane proteins - modulate AMPAR function. Recently, we discovered another novel AMPA receptor interacting protein, Shisa 9, or CKAMP44 (cystine-knot AMPAR modulating protein). Shisa 9 is a type I transmembrane protein of 44 kDa expressed in the brain, most abundantly in hippocampus. In contrast to TARPs and cornichons, Shisa 9 does not affect subcellular localization of AMPA receptors, but instead increases receptor desensitization. Shisa 9 belongs to the protein family characterized by the single transmembrane domain and a Cys-knot structure in the N-terminus. Two other members of this family Shisa 6 and Shisa 7 are also expressed in the brain what makes them attractive candidates of possible AMPA receptor interactors.

Here, I focus on the biochemical characterization of Shisa 9. By screening the library of total brain cDNA in a yeast two hybrid system we identified a number of novel putative interactors of Shisa 9. The next step was validation of identified interactors by GST pulldown and co-immunoprecipitation of 'Shisa9-interactor' complexes in a heterologous system (HEK293 cells). In total, we confirmed that 5 interactors represent true partners of Shisa 9. We also came to the conclusion that interaction between Shisa 9 and its partners occurs via PDZ-domain of the partner and PDZ-binding domain (Glu-Val-Thr-Val) in Shisa 9. The further step is to identify conditions under which these interactions occur.

**KEY WORDS:** Shisa9, AMPA receptor, excitatory synapse

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**TITLE****CONNECT: Cognition and the neural network: effects of chemotherapy****AUTHORS**Myrle M. Kemperman, S.B. Schagen, S.B. de Ruiter, L. Reneman, W. Boogerd, L.S. Laméris**DEPARTMENT/INSTITUTE**

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**ABSTRACT**Introduction

A substantial group of patients that has received chemotherapy report cognitive complaints. These problems are corroborated by neuropsychological studies that show impairments in some patients. The exact nature and causes of these impairments, however, need further elucidation. Therefore, it is important to gain more insight in the influence of chemotherapy on the brain with state-of-the-art imaging techniques combined with neuropsychological performance.

Objective

To investigate the late effects of chemotherapy on neuropsychological performance, and MRI brain function and structure in patients that received standard-dose chemotherapy for breast cancer or compared to disease specific and healthy controls.

Methods

We will select 25 previously diagnosed and treated breast cancer patients (on average 10 yrs ago) who had received a standard-dose cytotoxic regimen consisting of 5 cycles of Fluorouracil (5FU), Epirubicin and Cyclophosphamide (FEC) at the NKI-AVL, VUMC, LUMC or DdHK. Another group of 15 previously diagnosed and treated breast cancer patients who did not require chemotherapy will be selected from the sample of a previously conducted pilot study (De Ruiter et al., 2010). This pilot study from our group also included 19 patients that received high dose chemotherapy (4 x FEC + one high-dose CTC (cyclophosphamide, thiotepa, carboplatin) and autologous peripheral blood hematopoietic progenitor-cell transplantation. These 3 groups will be compared to each other, as well as to a group of 25 healthy female controls, matched for age.

Results

To date, 10 FEC patients and 20 healthy female controls participated in this study. All MRI data and neuropsychological data will be analysed in close collaboration with statistical experts of the NKI-AVL and AMC.

Discussion

This study adds to previous studies by using state of the art imaging techniques in a large group of patients to characterize aspects of white and grey matter injury underlying cognitive dysfunction in breast cancer survivors. The relevance of this project lies in increasing knowledge about the late effects of chemotherapy, and their underlying mechanisms.

**KEY WORDS:** MRI, chemotherapy, cognition**TELEPHONE NUMBER:** 020-5668324**E-MAIL-ADDRESS:** m.kemperman@nki.nl

**TITLE****Laminar profile of cortical waves****AUTHOR**Timo van Kerkoerle**DEPARTMENT/INSTITUTE**

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**ABSTRACT**

A visual stimulus evokes local high frequency activity in the visual cortex (30-100Hz; gamma band), and simultaneously suppresses the more global low frequency fluctuations (in particular 8-12Hz; alpha band). Selective attention has been shown to amplify this transition. The high frequencies have recently been suggested to be involved in feed-forward processing, while low frequencies have been suggested to be involved in recurrent interactions. This hypothesis can be tested in a straight forward way by analyzing in which cortical layers the different frequencies are generated, as feed-forward and feedback connections target different cortical layers.

We recorded from the primary visual cortex (V1) of macaque monkeys performing a figure-ground segmentation task. The monkeys are rewarded for detecting a figure as defined by oriented texture elements. A laminar probe allowed us to simultaneously measure the neural activity of the different cortical layers. The appearance of the visual stimulus evokes high frequency activity and suppresses low frequencies. Also, the high frequencies are boosted at the figure location, while low frequencies are boosted at the ground location.

By averaging oscillations in different frequency bands, we find that low and high frequency waves move in opposite directions through the different cortical layers. High frequencies start out in layer 4 and gradually move to deep and superficial layers, while low frequencies are initiated in the deep and superficial layers and subsequently shift to layer 4. This suggests that high frequencies are involved in the feed-forward processing of information while low frequencies are involved in recurrent interactions. Whether the low frequencies are generated by feedback from higher visual areas, by lateral interactions, or even by input from the pulvinar is difficult to distinguish based on the laminar profile that we find because all of these inputs target deep and superficial layers while avoiding layer 4.

**KEY WORDS:** Visual cortex (V1), monkey, electrophysiology**TELEPHONE NUMBER:** 020-5664841**EMAIL ADDRESS:** t.van.kerkoerle@nin.knaw.nl

**TITLE**

**Molecular regulation of synaptic output**  
*A role for the Ubiquitin Proteasome System*

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**ABSTRACT**

Homeostatic synaptic scaling is a form of neuronal plasticity in which a neuron has the ability to scale up or down its synaptic strengths according to the perturbations it faces. Although the phenomenon is well studied and described, molecular mechanisms that implement and sustain these scaling events are not well understood. Specifically, how is presynaptic strength tuned according to the activity-state of a neuron?

ERK1 is an activity-induced kinase that targets several substrates in the presynaptic terminal. We have recently discovered that one of these substrates is Munc18-1, a protein of which its presence is crucial for exocytosis and its abundance is strongly correlated with synaptic efficacy. Here, we hypothesize that sustained neuronal activity leads to a homeostatic scaling event in which Munc18 levels are controlled through ERK1 phosphorylation and subsequent degradation via the Ubiquitin Proteasome System. Preliminary evidence for a candidate E3-ligase that may mediate this activity-dependent ubiquitination of Munc18-1 is also discussed. This class of E3-ligases called F-Box proteins may regulate multiple plasticity related proteins and will therefore be the focus of future proteomics and genetic studies.

**KEY WORDS:** Plasticity, synapse, homeostatic scaling

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**TITLE**

**Effects of neuromodulation in the cortico-striatal circuit on striatal dopamine release and motivated behaviour**

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**ABSTRACT**

Deep Brain Stimulation (DBS) in cortico-striato-thalamo-cortical (CSTC) circuits has proven to be an effective treatment in otherwise treatment-resistant patients with obsessive compulsive disorder (OCD) and major depressive disorder (MDD). Within the CSTC circuits, stimulation of several target areas (e.g. ventral striatum, anterior cingulate, subthalamic nucleus) has resulted in reduction of clinical symptoms. Despite successful use of DBS in the clinic, the mechanism by which DBS reduces symptoms remains elusive. In addition, it is not clear which target area can best be stimulated to result in optimal clinical effects. Dysfunction in CSTC circuits and altered DA transmission are thought to be a hallmark of both OCD and MDD pathology. Under normal circumstances, CSTC circuits are important for motivated and goal-directed (e.g. reward-related) behaviour. These circuits are involved in learning and adapting associations between stimulus, response and outcome and for that depend on dopamine (DA) neurotransmission. One of the effects of DBS may therefore be that it stabilizes dysfunctional activity in the CSTC circuit, resulting in normalization of DA release and behaviour. We are interested in the effects of DBS in the CSTC circuit on motivated behaviour and DA release in the striatum. Specifically, we will combine DBS in cortical (ventromedial PFC) and/or subcortical (lateral habenula) areas with measurements of phasic dopamine release in the striatum using fast-scan cyclic voltammetry (FSCV).

**KEY WORDS:** Deep brain stimulation, dopamine, fast-scan cyclic voltammetry

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## TITLE

Synthesis and evaluation of [ $^{11}\text{C}$ ]GMOM analogs for PET imaging of the NMDA receptor

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## ABSTRACT

**Objectives:** The N-methyl-D-aspartate receptor (NMDAr) complex belongs to the ionotropic glutamate receptor family and is involved in many physiological processes. Imaging the NMDAr complex in living animal or human brain by PET provides useful information on the role of the NMDAr complex in various neurological disorders such as Alzheimer's disease and other neurodegenerative disorders. Until now [ $^{123}\text{I}$ ]CNS 1261 is the only radiotracer that has been used on patients affected by schizophrenia. Unfortunately one had to conclude that small changes in NMDAr distribution could not be quantified. [ $^{11}\text{C}$ ]CNS 5161, [ $^{18}\text{F}$ ]fluoromemantine, [ $^{123}\text{I}$ ]iodo-MK-801 and [ $^{11}\text{C}$ ]ketamine showed promising in vitro properties, although in humans results were not encouraging. [1] The 1-(2-chloro-5-(methylthio)phenyl)-3-phenylguanidine structure of GMOM [2] is used as a template for a new series of substituted N,N-diarylguanidines. Analogs showing high affinity for the NMDAr ion channel site will be radiolabelled with either carbon-11 or fluorine-18.

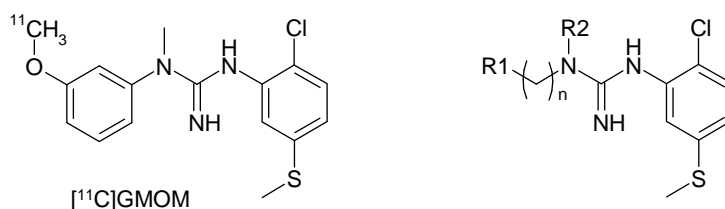


Figure 1. Chemical structures of [ $^{11}\text{C}$ ]GMOM and analogs.

**Methods:** A series of 18 analogs of GMOM was synthesized according to methods described in literature [3]. The competition binding assays on NMDAr were performed against [ $^3\text{H}$ ]MK-801 with rat fore-brain membranes.

**Results:** A set of 18 substituted N,N-diarylguanidines was synthesized in yields comparable to the literature [3]. The  $K_i$  values are shown in Table 1 (nM).

R1								
R2	H Me	H Me	Me	Me	Me	Me	Me	Me
n=0	857 262	207 20.7	442	9.0	14.3	4.4 $\mu\text{M}$	>10 mM	220
n=1	>10 >10 mM mM	1.56 2.7 $\mu\text{M}$ $\mu\text{M}$						
n=3	5.9 >10 $\mu\text{M}$ mM	>10 262 mM mM						

Table 1.  $K_i$  of substituted N,N-diarylguanidines for the NMDAr ion channel site (nM).

**Conclusions:** Introducing one or more methylene groups between R1 and the guanidine moiety, or pyridinyl in R1 is not tolerated as the binding affinity decreases dramatically. A methyl group at R2 increases the binding affinity. Introducing Fluorine in the methoxy moiety increases the binding affinity two-fold with respect to GMOM. Replacing the methyl at the methoxy moiety with 1-fluoropropane decreases the binding affinity 10 fold, apparently only small substituents are allowed at this position. Radiolabelling of the compounds with the highest affinity is in progress.

**Research Support:** This work was supported by CTMM LeARN, work package 02N-101-01.

**References:** [1] F. Sobrio et al., (2010), Mini-Reviews in Medicinal Chemistry, 10, 870-886, [2] F. Dumont et al., (2002), Bioorganic & Medicinal Chemistry Letters, 12, 1583-1586, [3] N. Reddy et al., (1994), Journal of Medical Chemistry, 37, 260-267.

**KEY WORDS:** PET, NMDA, GMOM

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## TITLE

**Lasting effects of chronic fluoxetine treatment on the late developing rat brain: age-dependent changes in the serotonergic neurotransmitter system**

## AUTHORS

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## ABSTRACT

### Rationale

With the growing prevalence of psychotropic drug prescriptions among children and adolescents, the need for studies on lasting effects of drug exposure on the developing brain rises. Fluoxetine is the only selective serotonin reuptake inhibitor (SSRI) officially registered to treat major depressive and anxiety disorders in children. Although various (pre)clinical studies have assessed the (long-term) effects of fluoxetine exposure in the perinatal period and in adulthood, limited data is available on its effects on the developing brain later in life, i.e. during adolescence.

### Objective

We aimed at investigating the effects of age following chronic SSRI treatment on the central serotonin (5-HT) system. To this end, the serotonergic system of chronic fluoxetine-treated (5 mg/kg, oral gavage for 3 weeks) juvenile (PND25) and adult rats (PND65) was assessed after a one-week washout period, using several different imaging and biochemical techniques. These included 1) pharmacological MRI with an acute fluoxetine challenge (5 mg/kg, i.v.), 2) autoradiography using storage phosphor imaging to determine SERT binding ratio with [<sup>123</sup>I]β-CIT, 3) quantitative real-time PCR (RT qPCR) to examine gene expression of a number of 5-HT related genes, and 4) immunohistochemical (IHC) staining of several 5-HT related targets.

### Results

With 5-HT phMRI, we observed a diminished brain response to the acute challenge in adult-treated animals when compared to control animals, whereas this response was increased in juvenile-treated rats. As a result, significant age by treatment interaction effects were seen in several (subcortical) 5-HT related brain regions. Storage phosphor imaging with [<sup>123</sup>I]β-CIT did not reveal any changes in SERT availability after chronic treatment, either in adult- or juvenile-treated animals. Preliminary results of the qPCR and IHC studies will be shown at the meeting.

### Conclusion

Using phMRI, an opposite response to the acute 5-HT challenge was seen after chronic fluoxetine treatment in the developing brain compared to that in the matured brain. These findings are indicative of age-dependent effects of chronic fluoxetine on serotonergic function and most likely reflect neuronal imprinting effects of juvenile SSRI exposure. In order to unravel the underlying neurobiological processes, a number of biochemical techniques were used to characterize the serotonergic system of juvenile-treated compared to adult-treated rats.

**KEY WORDS:** Juvenile SSRI exposure, 5-HT system, pharmacological MRI

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**TITLE****Auditory decision-making relies on a prefrontal sensory comparator mechanism****AUTHORS**

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**ABSTRACT**

It has been suggested that decision variables are computed in prefrontal cortex using a difference-based comparator mechanism that weighs the sensory evidence associated with different alternatives (Heekeren et al., 2004, 2008). Since this view has been developed based on mostly visual studies, we asked whether a similar mechanism might also account for auditory decisions. Consistent with these studies, we hypothesized that a prefrontal area involved in decision making should fulfill two conditions. First, its activity should be coupled with the difference in activation between pools of neurons representing task-relevant stimulus characteristics. Second, it should show the greatest activity on trials in which the evidence for a given perceptual category is greatest. To address these issues we employed two discrimination tasks on the same stimuli in our fMRI-experiment. 14 subjects listened to piano tone ranges (2 s) of either up- or downward pitch, played louder from either left or right. In each trial, we assigned one of two discrimination tasks. In the first task subjects had to decide whether the range was located left or right. In the other task subjects decided whether the range's pitch went up or down. We created trials with high (easy, 95% correct choices) and low (hard, ~80% correct) levels of sensory evidence by simultaneously playing stochastically pitched tones at various intensities. We did not find stimulus-selective BOLD responses for the two pitch directions. In contrast, for the two locations we indeed found stimulus-selective regions, from which we extracted the timeseries and computed the absolute difference ( $|\text{Left}(t) - \text{Right}(t)|$ ). We used a psychophysiological interaction analysis to determine which voxels correlated more strongly with  $|\text{Left}(t) - \text{Right}(t)|$  during the location task than the pitch task. A network of brain regions showed this pattern of activity, including bilateral inferior frontal and precentral gyri, and right insula. One region, located in the left superior frontal gyrus, showed not only this task-dependent change in functional coupling but also a stronger activity during easy versus hard decisions. These findings suggest that the computation of a perceptual decision involves a network of regions, in which the prefrontal cortex plays a pivotal role. Importantly, we show that the strength of decision-related functional connectivity in the brain depends on task-relevance of sensory representations. In summary, our findings suggest that a similar mechanism might underlie auditory and visual decisions. This further supports the contemporary view on brain mechanisms that underlie perceptual decision making.

**KEY WORDS:** Perceptual decision making, sensory processing, fMRI

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**TITLE**

**Parvalbumin and calretinin positive interneurons express opposite patterns of AMPA receptor subunits in macaque V1**

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**ABSTRACT**

Inhibition plays an important role in signal processing in primary visual cortex (V1), impacting signal filtering and oscillation generation. Interneuronal activity can be mapped in sequence after excitatory input to the cortex, starting with layer 4 cells (excitatory), followed by parvalbumin (PV) positive cells (inhibitory) then pyramidal cells (excitatory), which in turn are followed by calretinin (CR) positive cells (inhibitory). For the PV and CR cells, this response can be completely blocked by CNQX, which suggests that input to both is mediated by AMPA receptors.

Inhibitory interneurons identified by their calcium binding protein (like PV and CR) display differing firing patterns. Several factors can influence firing patterns, including the expressed receptors.

In the present study, we compared the distribution of AMPA receptor subunits on parvalbumin versus calretinin positive interneurons in primate V1. We used markers for the GluR1, 2, 3, 4 subunits. We fluorescently tagged these proteins in slices of macaque area V1. Subsequently, we imaged the slices using a confocal microscope and calculated the co-localization probability of the various marker combinations.

We found systematic differences in the distribution of the various AMPA receptor subunits between cell types. Our data show that indeed PV and CR positive cells differentially express AMPA subunits, with the majority of PV cells expressing GluR2 and 3, while the majority of CR cells expresses GluR1 & 4.

**KEY WORDS:** AMPA, interneurons, macaque

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**TITLE****Stress and decision-making in rats: effects of corticosterone****AUTHORS**Susanne Koot<sup>1,2</sup>, A. Baars<sup>2</sup>, P. Hesselings<sup>2</sup>, R. van den Bos<sup>1,2</sup>, M. Joëls<sup>1</sup>**DEPARTMENT/INSTITUTE**<sup>1</sup>Rudolf Magnus Institute of Neuroscience, University Medical Centre Utrecht, Utrecht<sup>2</sup>Division Behavioural Neuroscience, Dept. of Animals in Science and Society, Faculty of Veterinary Medicine, Utrecht University, Utrecht**ABSTRACT**

In several domains of the society, such as in the military, financial business and health care, decisions have to be made under highly stressful conditions. Recently we showed that male subjects performed poorly under acute stress in the Iowa Gambling Task (IGT), which measures decision-making performance under uncertainty (Van den Bos et al., 2009, *Psychoneuroendocrinology* 34). This effect was dependent on stress-induced cortisol levels: high-responders performed poorly, while low-responders did not. Here we studied the effect of corticosterone (CORT) on decision-making performance of male rats in a rodent version of the IGT (rIGT; Van den Bos et al., 2006, *Behav. Res. Meth.* 38). Male Wistar rats, kept on a reversed day-night cycle under mild food-restriction (90-95% of free feeding weight), were tested in the rIGT (120 trials in total). CORT-injections (1mg/kg s.c., HBC-complex, Sigma) were given in the second half of the task (3 days, 20 trials/day), 30min prior to testing, 2h after dark onset. Compared to saline controls, CORT-injections led to strongly increased plasma CORT levels 30min after administration. Behavioural data showed that CORT-injections were followed by a poor rIGT performance: CORT-treated rats made more choices for the long-term disadvantageous option than saline-treated controls. Currently we are analysing c-Fos expression in prefrontal, striatal and amygdalar areas to unravel the underlying neural mechanisms. Thus, high levels of corticosterone may disrupt decision-making performance in male rats. The observed effect of CORT 30min after administration might be due to non-genomic effects, which is subject of further study.

**KEY WORDS:** Stress, decision-making, rats**TELEPHONE NUMBER:** 030-2534149**E-MAIL-ADDRESS:** s.koot@uu.nl

**TITLE**

**The state of phasic and tonic GABAergic inhibition in the CA1 hippocampal region of the Fragile-X mouse model for neurodevelopmental disorders**

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**ABSTRACT**

Fundamental to proper signal integration and processing within neuronal circuits is the finely tuned control of excitatory input by inhibitory synapses. Atypical GABAergic inhibitory control can lead to altered excitatory signal gains that interfere with the proper function of the underlying neurocircuit. In many cases aberrant inhibition can promote epileptogenic activity, impede cognitive processing, and is associated with many neurodevelopmental and neuropsychiatric disorders<sup>1,2</sup>.

Fragile X Syndrome (FXS), caused by silencing of the *Fmr1* gene, is the leading cause of inherited mental retardation and approximately a third of these patients also exhibit autistic spectrum disorders<sup>3</sup>. Research in patients and rodent models of FXS consistently revealed changes in GABA receptor composition and GABAergic cell density in several brain areas including the hippocampus, the learning and memory relay center of the brain<sup>4,5</sup>.

We investigated whether such changes in GABAergic receptor and cell density were reflected in changes of inhibitory signaling in the hippocampus of the FXS mouse model. GABA<sub>A</sub> receptor mediated signaling can be described as either phasic, due to transient synaptic receptor activation, or tonic, due to lasting extrasynaptic receptor activation. Using whole-cell electrophysiological recordings, we characterized the phasic and tonic GABAergic currents of CA1 hippocampal pyramidal neurons during the 2<sup>nd</sup> and 4<sup>th</sup> week of development, in the FXS mouse model.

GABA<sub>A</sub> mediated phasic inhibitory synaptic transmission is unaltered between WT and FXS mice at either 2 or 4 weeks of postnatal development. However, we observed a trend for altered tonic inhibition during the 4<sup>th</sup> postnatal week in FXS mice. To dissect this further, experiments will now test subunit-specific GABA<sub>A</sub> receptor function and tonic inhibition throughout the hippocampus in the FXS mouse model.

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**KEY WORDS:** GABAergic inhibition, neurodevelopment, electrophysiology

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**TITLE****Munc18-1 regulates cortical F-Actin in chromaffin cells****AUTHORS**Julia Kurps, Heidi de Wit**DEPARTMENT/INSTITUTE**

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**ABSTRACT**

Chromaffin cells are endocrine cells which release hormones and neurotransmitter from secretory granules. The secretory pathway in chromaffin cells closely resembles the pathway in neurons, including docking of granules to the plasma membrane, priming of granules and eventually fusion of the secretory granules with the plasma membrane in order to release their content.

Munc18-1 was shown to be an essential docking factor in chromaffin cells. Furthermore, the absence of Munc18-1 in chromaffin cells from Munc18-1 KO animals leads to a dramatic docking phenotype with an extremely reduced number of secretory granules docked to the plasma membrane. Besides its important role in docking, Munc18-1 was shown to be involved in the regulation of cortical F-actin in chromaffin cells. This dense network of actin filaments underneath the plasma membrane functions as a physical barrier for secretory granules. Upon stimulation, the cortical F-actin network is depolymerised and secretory granules can access their release sites at the plasma membrane. It was shown that this cortical network is thicker in the absence of Munc18-1.

The molecular mechanism underlying the Munc18-1 mediated regulation of cortical F-actin is still unclear. Therefore, we developed a new hypothesis, including Phospholipase D (PLD), Phosphatidylinositol 4,5-bisphosphate (PIP<sub>2</sub>) and several actin-regulating proteins in order to explain the described phenomenon.

**KEY WORDS:** Cytoskeleton, exocytosis, chromaffin cells**TELEPHONE NUMBER:** 06-47432866**E-MAIL-ADDRESS:** julia.kurps@cncr.vu.nl

**TITLE****Modelling protein protein interaction based on protein abundance data in neurons****AUTHORS**Joachim Kutzera, Huub Hoefsloot, Age Smilde, Ka Wan Li, Guus Smit**DEPARTMENTS / INSTITUTES**

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**ABSTRACT**

This project is about the reconstruction of protein-protein interaction networks based on abundance data from immunoprecipitation.

Much research is done on exploring PPI (protein protein interaction), concentrating on two or three specific proteins in cells. Communication pathways were discovered bit by bit through combining the work on different proteins. Until today, it is difficult to discover bigger protein communication networks in a cell, as it includes maybe thousands of proteins with different functions, and we cannot observe them interacting. The interaction ability itself can be detected for two proteins with tools like the Yeast-2-Hybrid system. However, this method cannot proof, whether two isolated proteins interact, it is only suitable to test on two gene sequences, whether the proteins resulting from the expression of the genes can connect to each other or not.

A widely used biochemical method for purifying specific proteins is IP (immunoprecipitation). This method uses antibodies that stick to a specific protein, allowing to isolate this protein together with all other proteins that are currently connected to it. After identification and quantification of the purified proteins, the result is a table with abundance values for this proteins in one single IP experiment. With the help of the abundance values, it is possible to get an insight into the protein-protein interaction network, especially, when a high number of IP experiments is done with different antibodies on the same cell sample to get different parts of the network.

The project is divided into two different parts. Part one concentrates on measuring protein abundance data (so called "wet work"), in part two we try to model the interaction networks from this data ("dry work"). My work is about the second task, as well as the poster.

In the real experiment, cell material extracted from mouse neurons is analyzed with IP. The purified proteins are split into peptides through trypsin digestion and the peptides are measured using LC/MS with tandem-MS. The identification of the peptides and the quantification of the resulting proteins is done with help of peptide spectra databases.

The determination of protein abundance values can be simulated in silico for a theoretical network.

This network is represented by a graph  $\{V, E\}$ , composed of nodes  $V$  for proteins and edges  $E$  between these nodes, that stand for the interactions.

Supposing that one pulls on a node  $v$  in the network and each edge has a certain probability to break. Then, the probability for each node  $v^*$  to be still connected directly or indirectly to  $v$  is below 1 and can be calculated. In the simulation, these connected nodes represent the proteins in one IP-experiment, that do not stick directly to the antibody, but get pulled out indirectly. This simulation is repeated for all nodes. A matrix containing the connectivity probability for all nodes to all other nodes is the result. This matrix is correlated partly to the data from real IP experiments. However, the simulated networks are much smaller than the networks in the real measurements. One can easily pull on an arbitrary node in the simulation but not in the real IP, as there is no antibody available for every protein. For this and other reasons, the simulation strongly simplifies the biological processes, but seems to be sufficient to explain the protein abundancies in IP results from a real protein network.

The poster shows, that an artificial communication network can be reconstructed roughly from the calculated connectivity matrix. The reconstruction concentrates on finding closely connected complexes and the Complex-Complex interactions, the so called CCI.

**KEY WORDS:** Protein Protein Interaction, neurons, immunoprecipitation**E-MAIL-ADDRESS:** j.kutzera@uva.nl

**TITLE****JAG: a tool to analyse the joint action of genes within GWA data****AUTHORS**Esther Lips<sup>1</sup>, Maarten Kooyman<sup>2,3</sup>, Josine Min<sup>1</sup>, Danielle Posthuma<sup>1</sup>**DEPARTMENT/INSTITUTE**<sup>1</sup>Dept. of Functional Genomics, CNCR, VU University Amsterdam, Amsterdam, <sup>2</sup>Netherlands Bioinformatics Centre, Nijmegen, <sup>3</sup>Delft Bioinformatics Lab, Delft University of Technology, Delft**ABSTRACT**

Despite the successes of Genome Wide Association Studies (GWAs), in which many statistically associated SNPs are identified for a range of complex traits, these SNP-by-SNP analyses have failed to identify a substantial proportion of the heritability. Furthermore, these associations do not necessarily reveal insight in the functional mechanisms that are involved in the trait. In addition, these studies have provided genetic evidence for a substantial polygenic component for (many) complex traits, in which a large number of SNPs of very small effect are involved in the etiology. It seems likely that these SNPs are not distributed randomly across the genome, but are distributed across genes that share a common biological function or pathway. Therefore, simultaneous examination of SNPs located within multiple biologically related genes may be an promising strategy to reveal a larger component of the heritability and to uncover molecular mechanisms involved for the trait under investigation.

We have developed an easy-to-use open source tool, Joint Action of Genes (JAG), applicable to individual genotypes from GWAs, which tests the combined effects of all SNPs in a predefined gene-set using self-contained and competitive testing approaches. Our tool assigns SNPs to a gene with an user-defined window and enables to analyse gene-sets that are grouped on the basis of gene ontology, molecular function or any other user-specific biological relation.

In the self-contained test, JAG analyses the joint action of the genes within the gene-set by calculating the product of the  $-\log^{10}$  converted P-values,  $\Sigma\text{-log}_{10}(P)$ , from the association signals from all the individual SNPs from GWAs. To allow unbiased interpretation of the test statistic, permutations are conducted which are implicitly conditional on linkage disequilibrium, sample size, gene size, the number of SNPs per gene and the number of genes per group, by permuting affection status over genotypes.

In the competitive test is tested whether random generated sets of SNPs, equal sized as the user-specified gene-set and matched for the number of genes or the number of (genic, non-genic, combination of genic/non-genic) independent SNPs, would provide an equally or more significant empirical P-value as compared to the empirical P-value from the original gene-set.

Our tool, which will soon be freely available at <http://ctglab.nl/software/jag>, has the potential to uncover novel associated genes, pathways and molecular mechanisms. We have shown this in a gene-set analysis in cognitive ability (self-contained test only) [Ruano *et al*, 2010] and schizophrenia [Lips *et al*, *in press*], where we identified several, distinct sets of genes that were grouped on the basis of involvement in synaptic processes and functions (e.g. G-proteins, cell adhesion molecules).

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**KEY WORDS:** GWAS, tool, gene-set analysis**TELEPHONE NUMBER:** 020-5982959**E-MAIL-ADDRESS:** e.s.lips@vu.nl

**TITLE**

**Deep Brain Stimulation normalizes frontostriatal connectivity in obsessive-compulsive disorder**

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**ABSTRACT**

Deep Brain Stimulation (DBS) is an adjustable, reversible, non-destructive neurosurgical intervention using implanted electrodes to deliver electrical pulses to areas in the brain. DBS of the nucleus accumbens (NAc) is currently investigated for the treatment of refractory obsessive-compulsive disorder (OCD) showing promising results: a recent study reports an average 46% symptom decrease after 8 months with nine responders out of 16 patients ( $\geq 35\%$  Y-BOCS score decrease). The precise mechanisms behind NAc DBS in OCD are still a matter of investigation. One hypothesis is that DBS normalizes the frontostriatal connectivity in OCD. In the present study we will examine the effects of DBS on functional connectivity between the NAc and the frontal cortex in patients suffering from OCD.

Ten OCD patients treated with DBS and ten matched controls underwent each two resting state functional MRI scan sessions with a week in between. [Patients: after a week of DBS (DBS on-phase) and after a week without DBS (DBS off-phase); Controls: T1 and T2]. The right and the left NAc were the seed regions for connectivity analysis and the frontal cortex the region of interest.

Results show significant interaction effects (scans session x group) of the functional connectivity between left/ right NAc and the inferior frontal gyrus (IFG) and superior frontal gyrus. A trend towards significance was observed between the left NAc and a cluster in the medial frontal cortex. Simple effects testing reveals a significant decrease of connectivity in patients between the left NAc and the IFG from DBS off to DBS on, in the DBS off phase there is an increased connectivity between these areas in patients compared to controls whereas no such difference is found in the DBS-on phase. The change in connectivity strength from DBS off to on between the left NAc and the IFG is positively correlated with the change in OCD symptom severity from DBS off to on.

This study supports the hypothesis that DBS normalized the frontostriatal connectivity in OCD. This normalization of connectivity is associated with a decrease in OCD symptom severity emphasizing the importance of this finding for understanding the therapeutic mechanisms behind DBS.

**KEY WORDS:** Deep brain stimulation, resting state fMRI, frontostriatal connectivity, obsessive-compulsive disorder

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**TITLE****GFAP $\delta$ : A neural stem cell marker in mouse?****AUTHORS**Carlyn Mamber, Nina L. Haring, Jori van der Raadt, Willem Kamphuis, Elly M. Hol**DEPARTMENT/INSTITUTE**

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**ABSTRACT**

A population of astrocytes residing in the subventricular zone has been shown to be adult neural stem cells. These astrocytes continue to produce neurons throughout a lifespan. In humans, these neurogenic astrocytes specifically express an isoform of the glial fibrillary acidic protein, GFAP $\delta$ . In addition, GFAP $\delta$  co-localizes with the stem cell marker nestin and the proliferation marker Ki67; indicating that GFAP $\delta$  is a specific marker for human neural stem cells. The question remains, however, if GFAP $\delta$  marks the same population of astrocytes in mice as it does in humans. Is GFAP $\delta$  also a specific stem cell marker in mice? This study aims at characterizing the expression pattern of GFAP $\delta$  throughout murine development and into adulthood. Immunohistochemistry was used to visualize all GFAP isoforms (pan-GFAP) and GFAP $\delta$  expression from embryonic day 12 (E12) until postnatal day 45 (P45). Furthermore, real time quantitative PCR was used to decipher gene expression levels of GFAP isoforms in various brain regions. This study illustrates that GFAP $\delta$  is first expressed at a later developmental time point than pan-GFAP. By adulthood, GFAP $\delta$  is expressed by all adult astrocytes - even those not residing in the subventricular zone. The levels of GFAP $\delta$ , however, are higher in neurogenic areas. These data indicate that GFAP $\delta$ , though expressed more highly in neurogenic regions, is probably not a specific stem cell marker in mice as it is in humans.

**KEY WORDS:** GFAP $\delta$ , astrocyte, adult neurogenesis**TELEPHONE NUMBER:** 020-5665508**E-MAIL-ADDRESS:** c.mamber@nin.knaw.nl

## TITLE

**Activation of Toll-like receptor 4 (TLR4) and Receptor for Advanced Glycation End Product (RAGE) signaling contributes to ictogenesis and epileptogenesis**

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## ABSTRACT

### Background

Brain inflammation contributes to the mechanisms of seizure precipitation and recurrence, but the impact of specific inflammatory mediators on the development of epilepsy is *incompletely understood*. Using models of acute and chronic seizures in mice, we discovered a novel proconvulsant pathway involving the release of a danger signal, High Mobility Group Box 1 (HMGB1), from neurons and glia and its interaction with TLR4, a key receptor of innate immunity<sup>1</sup>. Increased levels of HMGB1 and TLR4 in human epileptogenic and experimental brain tissue, suggest a role for the HMGB1-TLR4 axis in human epilepsy.

### Aim

We now explored the role of RAGE, another HMGB1 receptor, in ictogenesis and investigated the involvement of TLR4 and RAGE signaling in epileptogenesis.

### Methods

C57BL/6 adult male wild-type (WT) or TLR4 and RAGE knock-out (KO) mice were injected unilaterally in the hippocampus either with 200 ng kainic acid (KA) to induce status epilepticus (SE) followed by chronic seizures, or with 7 ng KA to induce acute recurrent seizures for about 90 min<sup>1</sup>. HMGB1 (10 µg) was intrahippocampally injected before KA in acute experiments. Seizure activity was monitored and quantified by EEG analysis in freely-moving mice.

### Results

RAGE KO mice had reduced acute seizures following KA injection, as shown by a 40% and 30% decrease ( $p < 0.05$ ) in the number of seizures and their total duration, respectively. Injection of 10 µg HMGB1 in WT mice 15 min before KA increased by 2- and 3-fold the number and duration of seizures, respectively ( $p < 0.01$ ) and accelerated seizure onset by 2-fold ( $p < 0.05$ ). The proconvulsant effects of HMGB1 were precluded in RAGE KO mice, except for its effect on seizures onset which remained unmodified.

TLR4 and RAGE KO developed SE after a high dose of KA; the time to onset of spontaneous epileptic activity (i.e. Hippocampal Paroxysmal Discharges, HPD) was accelerated in both strains as compared to wild-type mice (WT) (days, WT,  $10.3 \pm 0.3$  (10); TLR4 KO,  $7.8 \pm 0.4^*$  (8); RAGE KO,  $6.8 \pm 0.5^*$  (6),  $*p < 0.01$ ). The time spent in HPD and their number over 3 EEG recording days (from 9:00 am to 5:00 pm) was significantly decreased by 60% and 30% on average in TLR4 and RAGE KO, respectively vs WT mice ( $p < 0.01$ ).

### Conclusions

RAGE activated signaling in the hippocampus mediates the proconvulsant effects of HMGB1, as previously shown for TLR4<sup>1</sup>, thus highlighting receptor co-operation in the mechanisms of ictogenesis. Activation of both RAGE and TLR4 contributes to spontaneous epileptic activity developing after SE, denoting new molecular mechanisms involved in epileptogenesis.

### Reference

Maroso et al, Nature Med, 2011, 16, 413

**KEY WORDS:** Inflammation, HMGB1, epilepsy

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**TITLE**

**Identification of genotypes and genes that influence avoidance learning in mice**

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**ABSTRACT**

Recognizing and avoiding aversive situations are central aspects of mammalian cognition. These abilities are essential for health and survival and are expected to have a prominent genetic basis. We modelled these abilities in mice (selected strains covering >95% of natural variation and gene-trap mice) using an unsupervised, automated assay in an enriched environment containing a shelter with two entrances. Mice visit this shelter 35-1200 times/24h and 72 % of all mice (n=1966) develop a significant and often strong preference for one entrance within 4 days. Subsequently, an aversive stimulus, illumination of the shelter, was automatically delivered when mice used their preferred entrance, but not the other. Within 2 days, most mice responded by changing their preference, DBA/2J 80%; C57Bl/6J 76%; 129S1/Sv 56%, indicating that mice associated one specific entrance with the aversive stimulus. A few mice responded by radically reducing the total number of entries and started sleeping outside, 5% of C57Bl/6J, 12.5% of DBA/2J, indicating these mice associate the shelter in general with the aversive stimulus. In contrast, other mice showed no evidence for any association, but did show markedly shorter shelter residence times after illumination, indicating they did perceive illumination as aversive. Screening random gene-trap mutants yielded a novel gene, *specc1/cytospinB*, involved in avoidance learning. These data show that different genotypes express distinct associations with aversive stimuli and behavioural responses, and that *specc1/cytospinB* modulates this aspect of cognition.

**KEY WORDS:** Behavioral flexibility, high-throughput behavioral screening, SPECC1

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**TITLE****Neutralization of Semaphorin signaling in the injured spinal cord****AUTHORS**Vasil Mecollari, E. B. Moloney, E.M.E. Ehlert, R. Eggers and J. Verhaagen**DEPARTMENT/INSTITUTE**

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**ABSTRACT**

Spinal Cord Injury (SCI) leads to damage of the ascending and descending neural pathways and often results in permanent loss of sensory and motor function due to the limited capacity of adult CNS neurons to regenerate. The lack of regeneration after SCI has been attributed to an unfavorable balance between growth-enhancing neurotrophic factors and growth-inhibitory factors. Among one of the major inhibitory family of molecules are the class III Semaphorins that are secreted by the meningeal fibroblasts invading the core of the lesion after injury. To date there are very few studies that assess the contribution of class III Semaphorins in the regenerative failure following SCI. Therefore the main aim of this study was to develop an approach to specifically interfere with Semaphorin signaling. The receptor complex for the secreted Semaphorins is comprised of a Plexin-A signaling subunit (plexin A1-A4) and a Neuropilin binding subunit (Nrp1 or Nrp2). Truncation of the Nrp ectodomain and fusion to an Fc fragment for stabilization and detection, results in soluble Nrp receptor bodies that can abolish the repulsive effect of class III Semaphorins, acting like a scavenger. We have generated lentiviral vectors encoding the soluble neuropilin receptors (Fc-Nrp1 and Fc-Nrp2) which were shown to be functional, since they fully abolished the growth cone collapsing effects of Semaphorin3A in explants of rat primary embryonic Dorsal Root Ganglia (DRG) as demonstrated in a DRG growth cone collapse assay. Moreover, both types of receptor bodies interfered with the anti-proliferative effects of Semaphorin3B and Semaphorin3F in the A549 lung cancer cell line as demonstrated in an MTT cell proliferation assay. Employing the same neutralization strategy *in vivo* in a C<sub>4</sub> unilateral dorsal hemisection SCI model in rats (in order to specifically damage the rubrospinal tract), we found that Fc-Nrp2 receptors secreted from primary Schwann cells implanted in the lesion area have a significant effect in the outgrowth of rubrospinal tract fibers, compared to the Fc-Nrp1 or GFP expressing Schwann cells after 6 weeks (n=3). Furthermore in a second *in vivo* pilot experiment where GFP, Fc-Nrp1, Fc-Nrp2, Fc-Nrp1/2 and Fc-Nrp2/BDNF were secreted from primary fibroblasts implanted in the lesion area, Fc-Nrp2 treated animals demonstrated better functional recovery of their injured forelimb and hindlimb at 8 weeks post injury (n=5). These data suggest that secreted soluble neuropilin bodies might provide a new tool to improve the poor functional recovery of the acutely injured spinal cord of the adult rat and a higher prospective to assess the contribution of class III semaphorins in the failure of axonal regeneration after SCI. Future *in vivo* experiments will further elucidate the efficiency of this strategy and validate its potential.

**KEY WORDS:** Spinal cord injury (SCI), Semaphorins, neuropilins**TELEPHONE NUMBER:** 020-5665511**E-MAIL-ADDRESS:** v.mecollari@nin.knaw.nl

**TITLE**

**Dissecting the molecular interactions of Munc18-1 within the synaptic vesicle secretion machinery**

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**ABSTRACT**

Synaptic transmission depends critically on the SM-protein Munc18-1, but it is unclear precisely how Munc18-1 operates within the fusion machinery. It has been suggested that the main function of Munc18-1 is to promote fusion by binding the SNARE complex, while others believe that Munc18-1 mainly controls the accessibility of the SNARE protein Syntaxin1a. Both functions are suggested to depend on a short sequence at the N-terminus of Syntaxin1a, the N-peptide. The aim of this study is to examine the importance of the N-peptide interaction, for which we generated point mutations in the N-peptide binding pocket within Munc18-1. These mutations severely diminish the affinity for SNARE complexes and reduce the ability of Munc18-1 to inhibit SNARE complex formation in vitro.

Interestingly, expression of mutant Munc18-1 supports normal docking, priming and fusion of synaptic vesicles in cultured munc18-1 null neurons. These data support a prevailing role of Munc18-1 before/during SNARE-complex assembly, while its continued association to assembled SNARE-complexes is dispensable for synaptic transmission and might have become redundant in evolution.

**KEY WORDS:** SM-proteins, SNARE complex, exocytosis

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**TITLE**

**Structural, biochemical and functional indices of chemotherapy-induced cognitive deficits in breast cancer patients**

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**ABSTRACT**Background

Cancer patients who have been treated with systemic chemotherapy frequently complain of cognitive problems. Even five years after treatment, up to 50% of disease-free cancer survivors report these problems, negatively impacting their daily functioning. Neuropsychological studies have confirmed the existence of deficits on a behavioral level, reporting impaired performance on tests tapping specific cognitive functions in about 20-40% of cancer survivors treated with chemotherapy, up to ten years after completion of treatment. Up till now, the mechanisms underlying the cognitive deficits are poorly understood and have not been studied well.

Methods

60 newly diagnosed breast cancer patients who will be treated with chemotherapy (CT-group) will be compared to 60 women with breast cancer that will not be treated with adjuvant chemotherapy (BC-group) and with 60 healthy women (HC-group). Groups will be matched for age and level of education. Participants will be tested twice, once after surgery and before subsequent therapy and a second time, six months after completion of chemotherapy, or, in the case of controls, at equivalent time intervals. Each test assessment consists of a semi-structured interview, several questionnaires, several neuropsychological tests, and an MRI scanning session. With use of MRI, functional and structural measures of the brain will be obtained.

In addition, a small hair sample will be collected to measure cortisol. Genetic factors involved in cognition will be analyzed in saliva samples and blood will be collected to assess levels of sex hormones and levels of pro-inflammatory cytokines.

Results

To date, 19 CT patients, 38 BC patients and 16 HC subjects have been included in the study, all of which have completed the first measurements. Inclusion of patients will continue until May 2012.

Discussion

This study will result in longitudinal information about the effects of chemotherapy on the brain. By collecting neuropsychological data, different MRI sequences and possible mediating factors, insight into underlying mechanisms of chemotherapy-induced changes in cognition will be provided.

**KEY WORDS:** Chemotherapy, cognition, (f)MRI

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**TITLE**

**Does perceptual learning require consciousness or attention?**

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**ABSTRACT**

It has been proposed that visual attention and consciousness are separate (Koch and Tsuchiya, 2007) and possibly even orthogonal processes (Lamme, 2003). The two converge when conscious visual percepts are attended, and hence become available for conscious report. A lack of reportability can however have two causes: the absence of attention or the absence of a conscious percept.

This raises an important question in the field of perceptual learning. It is known that learning can occur in the absence of conscious reportability, but given the recent theoretical developments it is now suddenly unclear which of the two ingredients – consciousness or attention – is not necessary for learning.

We present textured figure-ground stimuli, and manipulate reportability either by masking (which only interferes with consciousness) or with an inattention paradigm (which only interferes with attention). During the second session (24 hours later) learning is assessed via differences in figure-ground ERPs and via a detection task.

Preliminary findings suggest that early learning effects are found for stimuli presented in the inattention paradigm, and not for masked stimuli. These results suggest that learning requires consciousness, and not attention, and further strengthen the idea that consciousness is separate from attention.

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**KEY WORDS:** Consciousness, attention, EEG

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**TITLE****Defining subtypes of insomnia****AUTHORS**

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**ABSTRACT**

Phenotypical variability in complex disorders, like insomnia, hampers progress in elucidating the mechanisms underlying this disorder and makes it difficult to identify possible genetic predispositions. To bridge the gap between genetics and phenotypes, identification of endophenotypes is crucial. Phenotypical dissection of psychological or behavioral characteristics is an important step towards identifying such endophenotypes.

In this study we aimed to evaluate whether phenotypical complexity can be reduced by deriving subtypes of insomnia, based on psychological assessment. The large cohort studies of NESDA (the Netherlands Study for Depression and Anxiety) and NEMESIS (the Netherlands Mental Health Survey and Incidence Study) both provide psychological measurements related to sleep as well as diagnostic tools to assess possible comorbidities, thus allowing us to evaluate whether distinct homogenous subgroups can be found.

Preliminary findings of a latent class analysis of the NESDA dataset identified three subgroups. One cluster described people without noteworthy sleep problems, while two other clusters were characterized by (group 1) early morning awakening in combination with sleep maintenance problems and short sleep, and (group 2) early morning awakening due to an early chronotype profile ('larks'), without any further sleep complaint. Thus, early morning awakening in itself represents a common symptom of two distinct groups. Although this analysis was restricted to a few variables it already reveals the potential of this approach towards identifying homogenous subgroups of insomnia.

**KEY WORDS:** Insomnia, subtypes, endophenotypes

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**TITLE**

**The role of GFAP isoforms in the intermediate filament network of neurogenic astrocytes**

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**ABSTRACT**

Neural stem cells, unlike fully differentiated neurons, still have the ability to self renew and mature into neurons, astrocytes and oligodendrocytes. The central nervous system was always thought to be deprived of stem cells but now it is well accepted that new neurons can be born after embryonal development. Adult neurogenesis in the brains of rodents and humans is confined to two niches, which are the dentate gyrus in the hippocampus and the subventricular zone (SVZ). In the SVZ, these neural stem cells are able to self renew and differentiate into new neurons. They express glial fibrillary acidic protein (GFAP), an intermediate filament (IF) and are therefore classified as neurogenic astrocytes. IFs are part of the cytoskeleton and comprise a family of more than 70 proteins which are expressed in a tissue and cell type specific way. IFs are mostly perceived as a cytoskeletal component that contribute to the strength of the cells to cope with mechanical stress, but IFs are also important signaling platforms which regulates e.g kinases and are thought to transduce mechanical forces from the extracellular environment into a molecular response in the cell's nucleus.

Neurogenic astrocytes have a specific intermediate filament network. Besides GFAP, they contain vimentin and nestin which is commonly found in neural stem cells. GFAP has different isoforms and GFAP- $\delta$  has been found to be specific for human neural stem cells in the adult SVZ.

New functions of intermediate filaments (IFs) are emerging such as their role in signaling pathways as well as in migration and adhesion of cells. We therefore hypothesize that the neurogenic potential of these astrocytes is determined by a specialized IF network. We will test this by looking at the functional consequences of the incorporation of GFAP- $\delta$  within the IF network. Functional assays, like motility and migration assays, will be performed as well as proliferation assays. We have found that GFAP- $\delta$  is able to cause a collapse of the whole IF network, including nestin, vimentin and synemin, in astrocytoma cells. This collapse is concentration dependent since low levels of GFAP- $\delta$  are able to incorporate into the IF network without disrupting it. On the other hand, we will investigate the assembly of the different GFAP isoforms in the IF network and measure the dynamics of GFAP isoforms using fluorescent recovery after photobleaching (FRAP).

By elucidating the functional differences between GFAP- $\delta$  and other IFs we aim to determine its role in neurogenic astrocytes.

**KEY WORDS:** GFAP, neurogenic astrocytes, intermediate filaments

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**TITLE**

**AAV6-mediated delivery of neuropilin-1 receptor-bodies to skeletal muscle: a gene therapy strategy to neutralise semaphorin 3A in the G93A-hSOD1 mouse model for ALS**

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**ABSTRACT**

Amyotrophic Lateral Sclerosis (ALS) is characterised by a progressive degeneration of peripheral motor neurons leading to atrophy of the skeletal muscle and subsequent muscle weakness. Paralysis ensues due to degeneration of the brain and spinal cord motor neurons and death occurs largely from neuromuscular respiratory failure. The majority of cases (>90%) are sporadic. Familial cases make up the remaining 10%; several genes have been found to predispose an individual to typical ALS and ALS-like disorders, with a majority of familial ALS (fALS) being traceable to missense mutations in the Cu/Zn-superoxide dismutase 1 (SOD1) gene. Recent findings indicate that denervation of the hindlimb muscle in adult mice leads to increased semaphorin 3A (sema3A) expression in terminal Schwann cells (TSC) at the neuromuscular junction (NMJ) of type IIb muscle fibres. Semaphorin 3A expression in TSCs was also observed at this muscle fibre subtype in G93A-hSOD1 mice (a model for familial ALS) upon onset of the disease. Interestingly, type IIb muscle fibres are thought to be the first fibres to degenerate in ALS.

Semaphorin 3A is a secreted chemorepulsive axon guidance molecule that signals via a neuropilin-1 (nrp1) and plexinA membrane-receptor complex. We propose that semaphorin 3A plays an important role in the pathogenesis of ALS by creating a growth inhibitory environment at the NMJ. As a result, motor-terminals of vulnerable neurons disconnect or retract from their target muscle fibres. By using a soluble version of nrp1 to act as a scavenger for semaphorin 3A we aim to neutralise the growth inhibitory functions of semaphorin 3A at the NMJ in G93A-hSOD1 mice by means of a gene therapy approach.

The soluble nrp1 receptor-body construct (s-nrp1Fc) was created by removing the transmembrane and cytosolic domains and, in their place, the Fc fragment of IgG was fused (for stability and detection). Results suggest that s-nrp1Fc functions as a semaphorin 3A scavenger by preventing semaphorin 3A-mediated dorsal root ganglion growth cone collapse *in vitro*. We have also validated the use of Adeno-associated virus (serotype 6; AAV6) for muscle mediated transgene expression *in vivo*; direct intramuscular injection of AAV6-GFP or AAV6-seAP has demonstrated efficient expression and secretion of the transgene respectively from the transduced skeletal muscle. After intramuscular injection of AAV6-s-nrp1Fc into WT mice we were also able to detect s-nrp1Fc expression in the injected muscle. Currently we are analyzing the effects of intramuscular AAV6-s-nrp1Fc administration in the G93A-hSOD1 mouse to determine the efficacy of semaphorin 3A neutralization in disease progression and/or severity with a variety of behavioural and histological analyses.

**KEY WORDS:** Adeno-associated virus; Amyotrophic Lateral Sclerosis; Semaphorin 3A

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**TITLE**

**Single-shot action potential detection using two-photon excited membrane potential sensitive dyes**

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**ABSTRACT**

The electrophysiological properties of dendrites have been traditionally investigated using patch-clamp-based methods. Electrodes, however, suffer from severe spatial limitations. With the development of two-photon microscopy and suitable calcium-sensitive dyes, measurement of calcium dynamics in dendrites is now also a wide-spread method, but the kinetics of electrical signalling and calcium signalling can differ by an order of magnitude or more. Membrane-potential sensitive dyes do not have these limitations.

Up to now, two-photon excitation of membrane-potential sensitive dyes has been suffering from low sensitivity to a change in the membrane potential, bleaching and phototoxicity. With improved dye chemistry and two-photon excitation using long wavelengths generated by an optical parametric oscillator we have recorded optically single action potentials in hippocampal cultured neurons as well as from dendrites of hippocampal CA1 pyramidal cells in acute brain slices. We expect the present technique to be applicable for deep-tissue membrane potential recordings from dendrites and spines in vivo.

**KEY WORDS:** Two-photon microscopy, voltage-sensitive dyes, action-potential detection

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**TITLE**

**Prevalence of ADHD in substance use disorder patients: a prevalence study at the Jellinek Addiction Treatment Centre**

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**ABSTRACT**Background

Substance use disorders (SUD) are a major healthcare problem<sup>1</sup>. Patients with SUD often have comorbid psychiatric diseases. In the literature, there is growing evidence of a high prevalence of Attention Deficit Hyperactivity Disorder (ADHD) in these patients. Studies to date are mostly performed in the United States, where a prevalence of 23.1%% is found<sup>2</sup>. The objective of the current study is to replicate these findings in The Netherlands. Also, information is needed on the comorbidity with other psychiatric diseases in this patient population.

Objective

To obtain information on the prevalence of ADHD in SUD patients, and on the comorbidity with other psychiatric diseases.

Methods

500 patients presenting on the Jellinek addiction treatment centre are screened for ADHD. 230 of them receive a full assessment, in which ADHD is assessed by the Caadid interview. Borderline Personality Disorder (BPS) and Antisocial Personality Disorder (ASP) are assessed by the SCID and Mini Plus respectively. Mood disorders are assessed by the Mini Plus as well.

Results

Results are presented for the first 177 screened patients, of whom 66 patients have had a full diagnostic assessment. The prevalence of ADHD in the assessed patients was 21.2%. In patients with SUD and ADHD, the prevalence of (lifetime) depressive disorder was 38.5%, (hypo)mania was diagnosed in 16.7% of patients, and the prevalence of BPS and ASP were 15.4% and 38.5% respectively. Prevalences of BPS and ASP in these patients were significantly higher than in the SUD patients without ADHD.

Conclusions

ADHD is an important comorbid disease in Substance Use Disorder patients: 1 in every 5 patients presenting at a treatment addiction centre in Amsterdam has comorbid ADHD. This group of patients is also at enhanced risk for other psychiatric diseases.

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**KEY WORDS:** ADHD, addiction, comorbidity

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**TITLE**

**Transient expression of functional serotonin 5-HT<sub>3</sub> receptors by glutamatergic granule cells in the early postnatal mouse cerebellum**

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**ABSTRACT**

The serotonin 5-HT<sub>3</sub> receptor is the only ligand-gated ion channel activated by serotonin and is expressed by GABAergic interneurons in many brain regions, including the cortex, amygdala and hippocampus. Furthermore, 5-HT<sub>3</sub> receptors are expressed by glutamatergic Cajal-Retzius cells in the cerebral cortex. We used 5-HT<sub>3A</sub>/Enhanced Green Fluorescent Protein (EGFP) transgenic mice to show that 5-HT<sub>3</sub> receptors are also ubiquitously expressed by glutamatergic granule cells in the cerebellum during the first three postnatal weeks. Using whole-cell patch clamp recordings, we show that local application of either serotonin or the selective 5-HT<sub>3</sub> receptor agonist SR57227A to granule cells results in a small inward current, demonstrating a postsynaptic localization of the 5-HT<sub>3</sub> receptors. Functional 5-HT<sub>3</sub> receptors were also observed presynaptically at the parallel fiber – Purkinje cell synapse. Pharmacological block using the selective 5-HT<sub>3</sub> receptor antagonist tropisetron induced a reduction in the frequency of miniature synaptic events recorded from Purkinje cells. Paired-pulse stimulation of parallel fibers on whole-cell voltage clamped Purkinje cells from one week old mice did not yet show synaptic plasticity. In the presence of tropisetron, the parallel fiber – Purkinje cell synapse showed paired-pulse depression. Furthermore, cerebellar 5-HT<sub>3</sub> receptors appear to be involved in the morphological development of Purkinje cells. Purkinje cells in 9-day old, but not six-week old 5-HT<sub>3</sub> receptor knockout mice have an increase in the complexity of their dendritic tree as compared to wildtype mice. Taken together, these results show that functional 5-HT<sub>3</sub> receptors are present during early postnatal development in the cerebellum, where they are part of the mechanism regulating the development of synaptic plasticity and morphology.

**KEY WORDS:** Serotonin 5-HT<sub>3</sub> receptor, postnatal development, cerebellum

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**TITLE**

**Isolation and gene expression analysis of adult astrocytes and microglia from an AD mouse model**

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**ABSTRACT**

Alzheimer's disease is the most common cause of dementia among elderly. One of the main hallmarks of Alzheimer's disease (AD) is the accumulation of Amyloid  $\beta$  proteins ( $A\beta$ ) forming extracellular plaques in the brain. Surrounding the plaques, reactive astrocytes characterized by morphological changes and increased expression of cytoskeletal proteins such as GFAP and vimentin are found together with activated microglia characterized by an amoeboid morphology. What is causing this activation of glial cells is not fully understood. Inflammation is known to be secondary to protein accumulation in neurodegenerative diseases; microglia are the main immune cells of the brain and therefore the main player in the inflammatory events. However, the contribution of astrocytes in this process is unclear. We have set out to investigate the effect of  $A\beta$  plaque pathology on glial cells in the cortex of the APP<sup>swE</sup>/PS1<sup>dE9</sup> AD mouse model. We used a novel method involving different cell-surface markers and Fluorescence-activated cell sorting (FACS) that allowed us for the first time to isolate viable astrocytes together with microglia cells from aged control and AD mice. We performed genome wide analyses together with FACS analysis on these separate cell populations which allowed us to investigate altered inflammatory and other cellular pathways. Our study shows that the astrocyte contribution to the inflammation is greater than commonly expected.

**KEY WORDS:** Alzheimer's disease, astrocytes, gene expression

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**TITLE**

**Structural and functional characterization of novel AMPA receptor interacting protein – Pancortin**

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**ABSTRACT**

In the brain, the AMPA-type glutamate receptor (AMPA) is the major ionotropic receptor in glutamatergic synapses mediating fast excitatory transmission. The modulation of the density and conductance of this receptor in the synapse underlies neuroplasticity, which is at the basis of learning and memory. Aberrant functioning of AMPAR-mediated transmission underlies many brain disorders including drug addiction, mental retardation, schizophrenia and depression.

In recent years, several AMPAR-associated proteins have been identified and shown to regulate AMPAR properties. In the present study, we use an interaction proteomics approach to characterize the AMPAR protein complexes from different brain regions. AMPAR complexes were immuno-isolated with antibodies against AMPAR subunit 2 from crude synaptic membranes of cortex, hippocampus and cerebellum. The samples were separated by SDS-PAGE, digested by trypsin and analyzed by mass spectrometry. Several novel AMPAR interacting proteins were identified. One of these is Pancortin. Pancortins belong to the olfactomedin family of proteins and known to play important roles during development. Several members of the olfactomedin family of proteins are known to be secreted. Here, we aim to verify the interaction of Pancortin and the AMPA receptor using several approaches, such as reversed-Immunoprecipitation, Blue-Native PAGE 2D gel electrophoresis, and co-expression in HEK cells. On the long run we aim to understand the full functional significance of the Pancortin-AMPA receptor interaction in the context of synaptic plasticity and its importance in learning and memory using a multidisciplinary approach.

**KEY WORDS:** Proteomics, AMPA receptor, Pancortin

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**TITLE****Pulsatile glucocorticoid action in brain****AUTHORS**Natasha Pasricha<sup>1</sup>, Lenka Mikasova<sup>2</sup>, Laurent Groc<sup>2</sup>, Marian Joels<sup>1</sup>, Henk Karst<sup>1</sup>**DEPARTMENT/INSTITUTE**<sup>1</sup>Dept. Neuroscience & Pharmacology, University Medical Center Utrecht, Utrecht<sup>2</sup>Interdisciplinary Institute for Neuroscience, CNRS - Université de Bordeaux, Bordeaux, France**ABSTRACT**

The stress hormone, corticosterone, activates two types of receptors, the glucocorticoid receptors (GR) and the mineralocorticoid receptors (MR). Corticosteroids are thought to be important for normalization of brain activity and consolidation of memory via a genomic pathway. Electrophysiological data from our group, however, showed that corticosterone also exerts rapid non-genomic effects on glutamate release in the hippocampal CA1 and DG region via mineralocorticoid membrane receptors (mMR). Using single nanoparticle tracking, it has been shown that corticosterone rapidly and reversibly increases GluA2-AMPA receptor lateral diffusion, via activation of mMRs. Corticosterone is released in a circadian rhythm, with hourly ultradian pulses. We hypothesized that the rapid non-genomic pathway --and not the genomic pathway-- is able to translate these pulses into physiological effects.

Indeed, when hippocampal slices are exposed to 4 repetitive 100nM cort pulses in vitro, rapid non-genomic effects via membrane MR can follow the pulses. During the pulses an increase in mEPSC frequency, representing enhanced glutamate release, was observed, which went back to baseline frequency at the nadir of the corticosterone pulses. To investigate the impact of corticosterone pulses on glutamate receptor dynamics, we also studied the influences of repetitive 100 nM cort pulses in hippocampal cultured neurons with the single nanoparticle (Quantum Dot) tracking approach. This technique allows to image the dynamics of GluA2-AMPA and GluN1-NMDA receptor surface trafficking. Interestingly, early and late corticosterone pulses appear to differentially influence GluA2-AMPA surface diffusion whereas GluN1-NMDAR diffusion overall remained unaffected. We conclude that mMRs are important for the registration of changes in brain corticosterone level.

**KEY WORDS:** Corticosterone, quantum-dot tracing, electrophysiology**TELEPHONE NUMBER:** 088-7568877**E-MAIL-ADDRESS:** N.Pasricha@umcutrecht.nl

**TITLE**

**Sleep deprivation and connectivity: why presenting on the first day of ONWAR is better than on the second**

**AUTHORS**

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**ABSTRACT**

Our failure to appropriately respond to external stimuli can result in dramatic accidents, such as traffic collisions and workplace injuries. The risk is compounded by sleepiness and sleep deprivation. Unfortunately, our understanding of how sleepiness augments the propensity for lapses of attention and which brain mechanisms are particularly affected by sleep deprivation is poor.

We propose that sleep deprivation affects brain connectivity, which is the ability of distant brain areas to communicate with each other. The failure to communicate between brain regions brought about by sleep deprivation will then result in less effective information transmission, leading to an inappropriate response.

The effect of sleep deprivation on brain connectivity was investigated in two experimental paradigms. Both experiments consisted of two days: one day after normal sleep and one day after sleep deprivation. Brain activity was recorded using high-density EEG. In the first experiment, participants followed a resting state protocol, with 2 minutes of eyes-open and 2 minutes of eyes-closed for 5 sessions over the course of the day. During the second experiment, another group of subjects participated in a GO/NOGO task: they were asked to respond quickly to a target image, unless preceded by a NOGO signal.

Connectivity was assessed using Granger Causality (G-causality) on high-density EEG. G-causality quantifies the directed influence of area A onto area B and the other way round. This technique allows us to investigate how information spreads through the cortex and how our experimental manipulations affect the propagation of information.

Our results show that brain connectivity is strongly affected by sleep deprivation, both during resting states and during the execution of a task. Information flow from the visual cortex to the prefrontal cortex was selectively impaired during resting state. This impairment in brain connectivity correlates with participants' ability to correctly respond to the target stimuli. These findings demonstrate that lapses and slow reaction times are the consequence of an impaired information transmission as assessed by G-causality.

Based on these findings, I will convince you to go to bed early tonight, so that you can respond "appropriately" during the second day at the ONWAR meeting.

**KEY WORDS:** Sleep, connectivity, EEG

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**TITLE****Novel biomarkers of memory: from model to cognition****AUTHORS**Simon-Shlomo Poil, Huibert D. Mansvelder, Klaus Linkenkaer-Hansen**DEPARTMENT/INSTITUTE**

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**ABSTRACT**Introduction

Many brain disorders has been linked to an imbalance in the balance between excitation and inhibition in neuronal networks (Yizhar et al., Nature, 2011). Empirically we observe so-called scale-free dynamics on multiple levels as, e.g., scale-free auto-correlations in EEG/MEG oscillations on long time-scales (Linkenkaer-Hansen et al, 2001, J neurosci.) and a scale-free size distribution of local field potential bursts on short time-scales (Beggs & Plenz, 2003, *J. Neurosci.*). We claim that the scale-free dynamics is coupled with the balance between excitation and inhibition, and that on the conceptual level we can describe the brain as self-organized critical system (Bak et al, 1987, Phys. Rev. Lett.). By developing novel biomarkers sensing deviations from criticality in the brain we hope to identify pathophysiology at an early-stage. We also hypothesise that the temporal scale-free dynamics observed in ongoing oscillations is couple to memory and attention.

Research questions

Is it possible for an oscillatory neuronal network to be critical?

Can we study scale-free dynamics in oscillations *in vitro*?

Is the scale-free dynamics in oscillations impaired in Alzheimer's disease?

Approach

Model to cognition. We use an integrative approach, using both models and empirically data together.

Results

We introduce a novel theory of multi-level criticality which allows scale-free dynamics on short and long time scales using simple neuronal network. We observe that the scale-free dynamics is directly coupled with a balance of excitation and inhibition. And that each oscillation cycle is formed by multiple scale-free neuronal avalanches. On longer time scales we observe the oscillation cycles form oscillation bursts, and scale free amplitude fluctuations.

Scale-free dynamics has been shown to appear on short-time scales *in vitro* (Beggs et al, 2003, J. Neurosci.), but it has not been studied on longer time-scales in oscillations. We, therefore, studied fast network oscillations in hippocampal slices. We observed scale-free dynamics on long times scales, and that this dynamics is sensitive to modulation by the acetylcholine-like drug Carbachol (Poil et al, 2008, *Hum. Brain Mapp.*).

After this we showed, that the temporal scale-free dynamics of alpha oscillations (MEG) is impaired in Alzheimer's disease, - suggesting a link to between scale-free dynamics and memory (Montez et al, 2009, PNAS).

We conclude that biomarkers of criticality in oscillations may be sensitive to memory, and the balance of excitation and inhibition in the underlying neuronal networks.

**KEY WORDS:** Oscillations, memory, biomarkers**TELEPHONE NUMBER:** 0205989408**E-MAIL-ADDRESS:** simonshlomo.poil@cncr.vu.nl

**TITLE****Nicotinic acetylcholine receptor modulation of the prefrontal cortex and attention****AUTHORS**Rogier B. Poorthuis, Bernard Bloem, Benita Schak, Christiaan P.J. de Kock, Huibert D. Mansvelder**DEPARTMENT/INSTITUTE**

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**ABSTRACT**

Acetylcholine signalling in the prefrontal cortex (PFC) is crucial for attention. We found that cholinergic signalling through  $\beta 2^*$  nicotinic acetylcholine receptors (nAChRs) in the mPFC are critical for optimal attention performance and we are currently investigating the role of  $\alpha 5^*$  nAChRs. In the PFC network nAChRs are known to enhance glutamatergic inputs to LV pyramidal and interneurons and induce postsynaptic currents in layer V interneurons and layer VI pyramidal neurons. What the relative impact of nAChR stimulation is on activity in the different layers is unknown. Here we investigate nAChR modulation of all PFC layers and find a marked layer specificity for pyramidal neurons: LII-III pyramidal neurons and glutamatergic inputs to these cells do not express nAChRs, layer V and LVI pyramidal neurons express respectively  $\alpha 7$  and  $\beta 2^*$  nAChRs. Interneurons in LII-III do express nAChRs, as well as LVI interneurons. We then test the hypothesis that nAChRs activate the prefrontal cortex in a layer specific manner using 2-photon population imaging. Network activity is dominated by  $\beta 2^*$  nAChRs. In layer II-III only interneurons are activated. In layer V and VI both interneurons and pyramidal neurons are activated, the latter most strongly in layer VI. Together these results suggest that in the PFC nAChR activation results in inhibition of layer II-III pyramidal neurons. In layer V and VI nAChR induced activation of inhibitory and excitatory neurons results in a net augmentation of output neuron activation.

**KEY WORDS:** Nicotinic Acetylcholine receptor, prefrontal cortex, attention**TELEPHONE NUMBER:** 020-5987099**E-MAIL-ADDRESS:** rogier.poorthuis@cncr.vu.nl

**TITLE**

**The deeper the blues, the higher the booze?**

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**ABSTRACT**

The risk of alcohol dependence in individuals that suffer from Major Depression is twice as high when compared to the general population, questioning a common underlying substrate. Therefore, our long-term aim is to identify molecular substrates that could target concurrent depression and alcohol dependence. Previously, we introduced a preclinical model that links the maintenance state of depressive-like symptomatology with increased alcohol seeking and alcohol taking behavior in Wistar rats. Depression-like behavior in rats, characterized by a lowered affected state and cognitive performance, affects alcohol consumption, motivation to obtain an alcohol reward as well as cue-induced reinstatement of alcohol-seeking behavior. Currently, we are investigating the effects of depression-susceptibility on subsequent vulnerability to alcohol-taking and -seeking behavior. Drug-naïve Wistar rats were socially defeated and subsequently scored on depressive-like symptomatology as measured in four different behavioral tasks, namely Social Interaction, Social Avoidance, Social Memory and Object Place Recognition. Social defeat and protracted individual housing resulted in two stable distinct phenotypes that differ in depression-susceptibility: a highly affected susceptible (SUS) and an unaffected resistant population (UNSUS). The two populations displayed distinct performance in all four behavioral tests over a period of 2 months, with SUS rats showing progressive deterioration and UNSUS rats showing gradual improvement in performance. Preliminary results of the alcohol self-administration paradigm point to increased alcohol consumption, as exhibited by the SUS rats when compared with undefeated controls. This effect is absent in the UNSUS rats, indicating that depression-susceptibility might predict subsequent vulnerability to alcohol dependence.

**KEY WORDS:** Major depression, susceptibility, alcohol abuse disorder

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**TITLE**

**Shisa9 in hippocampal brain oscillations. An in vitro MEA study**

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**ABSTRACT**

Brain oscillations reflect the synchronized activity of neural networks. Synchrony is created by precise tuning of the excitatory and inhibitory drives. Oscillations can be chemically induced via stimulation of excitatory pathways. In vitro oscillations can be induced with carbachol (CCh), DHPG and Kainate. CCh is a cholinergic agonist. DHPG and kainate act on glutamate receptors.

AMPA is one of the ionotropic glutamate receptors. AMPA receptors mediate fast excitatory synaptic transmission and are therefore required in the generation of brain oscillations. The activity of the AMPA receptors is modulated by many proteins like TARPs, Cornichons, and Shisas. The Shisa family have been newly identified. Shisa 6, 7 and 9 are expressed in the brain and therefore are our main focus of interest.

Shisa9, also known as AIP1 or CKAMP44, has been shown to increase the deactivation time of AMPA mediated currents, to increase desensitization and to recover slower from desensitization.

We would like to know if Shisa9 is able to interfere in the generation of in vitro brain oscillations and to what extent. In order to investigate this, we measure field potential oscillations with multi-electrode arrays (MEAs) in young mice hippocampal brain slices that have been pre-incubated with the Shisa9 protein and compare the results with slices from the same mouse which had not been treated with Shisa9.

**KEY WORDS:** Shisa, hippocampus, oscillations

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## TITLE

### **Microarray technology to unravel new regeneration-related characteristics of Olfactory Ensheathing Cells**

## AUTHORS

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## ABSTRACT

Nerve regeneration after spinal cord injury (SCI) is virtually impossible. The primary olfactory nervous system however, is able to regenerate after neuronal damage throughout adult life and is an exceptional model of neural repair. This is a process supported by Olfactory Ensheathing Cells (OEC), which engulf outgrowing axons all along the primary olfactory pathway.

To examine the molecular mechanisms by which OEC support axon growth we conducted a microarray study on the Olfactory Nerve Layer after a lesion in the Olfactory Epithelium. Interestingly, we found significant changes in the expression of a number of genes involved in phagocytosis, specifically in Fc-receptor clustering, phagosome formation and closure, phagosome-lysosome fusion and Complement. OEC can promote neuronal regeneration and improve functional outcome when transplanted into rodents after SCI. Since some of the components of myelin are known inhibitors of neuronal outgrowth, we wanted to determine if OEC can neutralize them by phagocytosis of myelin. Our results show that cultured OEC take up myelin in a time-dependent manner and that it is present in phagosomes and lysosomes. All these results together suggest that OEC phagocyte debris after damage to the olfactory system and we show unequivocally that OEC are capable of phagocytosis and processing of myelin in vitro. If confirmed in in vivo studies, phagocytosis of myelin could be an important element of the neuroregenerative properties of OEC after transplantation into a SCI.

In addition, a meta-analysis of all published microarray gene expression datasets of cultured early-passage-OB-OEC with other cell types allowed us to identify 7 genes with uniformly higher or lower expression in early-passage-OB-OEC in all five comparisons. Of these seven genes, ENTPD2 is of particular interest, as it is a membrane-associated enzyme that hydrolyses extracellular ATP. In the setting of a SCI, ATP release is increased in peritraumatic area. ATP has been implicated in acute and chronic neuropathic pain and inflammation, and it is known to play a major role in increasing secondary damage by killing motor neurons surrounding the injury site. Application of an antagonist for the ATP-sensitive P2X7 receptor after SCI has been shown to improve behavioral recovery, reduce gliosis and diminish the immune response. Preliminary results have shown that OEC transplanted in spinal cord injury site express high levels of ENTPD2 7 days after transplantation. It is therefore possible that OEC expression of ENTPD2 after SCI inhibits P2X7 receptor activation by hydrolysing free ATP released after the injury and therefore, diminishing secondary damage, favouring neural repair and alleviating pain.

**KEY WORDS:** Olfactory Ensheathing Cells, microarray, spinal cord injury

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**TITLE**

**Obsessive-Compulsive Symptoms in patients with schizophrenia comparing treatment with Clozapine, Olanzapine, Risperidone and no antipsychotics: a naturalistic cross-sectional study of 543 patients**

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**ABSTRACT**Objective

We aim to compare the prevalence of obsessive-compulsive symptoms (OCS) in a population of patients with schizophrenia using clozapine, olanzapine or risperidone or using no antipsychotic medication.

Methods

Baseline data of the Genetic Risk and Outcome of Psychosis study were collected between April 2005 and October 2008. We conducted a naturalistic cross-sectional study of 543 patients with schizophrenia and related disorders, meeting Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) (American Psychiatric Association 1994) criteria, using no antipsychotic medication, using clozapine, olanzapine or risperidone. OCS severity was measured with the Yale-Brown Obsessive Compulsive Scale. We compared patients to a sample of 575 healthy controls.

Results

Prevalence of OCS in patients was significantly higher than in the control sample, 24.9% versus 6.4% ( $X^2=72.8$ ,  $p<.001$ ). Patients using clozapine reported significantly more often OCS during the last week (38.9%), when compared to patients using olanzapine (21.6%,  $X^2= 8.28$ ,  $p=.004$ ), risperidone (25.2%,  $X^2= 4.45$ ,  $p=.035$ ) and patients not using antipsychotics (21.4%,  $X^2=6.59$ ,  $p=.010$ ). When patients used clozapine for more than 6 months they reported OCS significantly more often than patients using clozapine for less than 6 months, 47.3% versus 11.8% ( $X^2=6.89$ ,  $p=.009$ ).

Conclusion

Treatment with clozapine in patients with schizophrenia is associated with a higher prevalence of OCS, especially when patients have been using clozapine for more than 6 months. We can not rule out the possibility that this association is related to illness characteristics. Patients treated with risperidone, olanzapine or without treatment with antipsychotic medication had comparable prevalence of OCS, significantly higher than the control sample.

**KEY WORDS:** Clozapine, schizophrenia, OCS

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**TITLE**

**Does deep brain stimulation of the nucleus accumbens shell decrease heroin taking and seeking?**

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**ABSTRACT**

Drug addiction is a chronic relapsing brain disease, characterized by compulsive drug-taking and continuation of drug use despite severe negative consequences. Though a variety of treatments is currently available, not all patients respond favourably. Deep brain stimulation (DBS) is a possible intervention for treatment refractory patients. Here we studied the effect of DBS in the nucleus accumbens shell (NAshell) on heroin seeking and taking in a rat model.

Rats were bilaterally implanted with bipolar electrodes aimed at the NAshell. DBS was applied during heroin self-administration for high (100 ug/kg) and low (25 ug/kg) doses of heroin, at the end of the extinction phase and during cue- and drug-induced reinstatement. As a control for natural rewards, NAshell DBS was conducted in rats that were trained to self-administer grain-based pellets. Results are currently being analyzed and will be presented at the meeting.

**KEY WORDS:** Deep brain stimulation, addiction, nucleus accumbens

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**TITLE**

**Effect of modafinil on neural correlates of response inhibition during stop signal task in alcohol dependent men**

**AUTHORS**

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**ABSTRACT**

Diminished impulse inhibition is a key feature of substance use disorders, including alcohol dependence. Therefore, improving inhibitory control might be a promising new treatment for substance use disorders. The pharmacological agent modafinil proved to enhance cognitive control functions in both healthy subjects and in psychopathology. However, the neural correlates underlying improvement in inhibitory control by modafinil in alcohol dependent subjects have not yet been investigated. We conducted a randomized, double-blind, placebo-controlled, cross-over study, using functional magnetic resonance imaging to examine the effects of a single dose of modafinil on response inhibition and underlying neural correlates in abstinent alcohol dependent patients (AD), and healthy control subjects (HC). Modafinil (200 mg) and placebo were administered orally, 2 hours before each of the two testing sessions. HC and AD did not differ in performance (SSRT) during modafinil and placebo sessions. However, SSRT on placebo correlated with modafinil induced SSRT improvement, indicating that high impulsive subjects show more improvement in response inhibition under modafinil. AD showed greater activity in thalamus, putamen and cingulate gyrus during stop success (SS), as contrasted with stop errors (SE), during modafinil>placebo. HC showed greater activity in the superior frontal gyrus. For both groups modafinil induced activity changes in the thalamus were positively correlated with baseline impulsivity and with modafinil induced SSRT improvement. The current findings indicate improvement in response inhibition in alcohol dependent patients due to modafinil through its effect on the thalamus, especially in subjects with higher baseline impulsivity. Clearly, for a successful treatment with modafinil, baseline patient characteristics such as impulsivity should be taken into account.

**KEY WORDS:** Alcohol dependence, modafinil, impulsivity

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**TITLE****Modeling responses to hypertonic sucrose solutions in Hippocampal autapses****AUTHORS**Bas Schotten, M. Verhage, L.N. Cornelisse**DEPARTMENT/INSTITUTE**

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**ABSTRACT**

Application of hypertonic sucrose solution is used to determine the size of the so-called 'readily-releasable pool' (RRP) (1), which is an important part of the synaptic vesicle cycle. However, it is still not understood what synaptic parameters are affected by this protocol. Furthermore, there is no 'golden standard' for determining the RRP size from the responses. We have developed a (minimal) model to simulate responses to various concentrations of hypertonic sucrose solutions in Hippocampal autapses.

Our simple model - which consists of merely three different vesicle 'states', and three rate constants - describes the main features of a sucrose response very well. It can also account for an interesting relation between the amount of vesicles released and the rate at which they are released (2). We plan to merge this model with models for calcium triggered release, taken from the literature (e.g. (3)). This will be used to explain/predict results from (possibly complex) protocols involving stimulations with both calcium and sucrose.

On the long run, the aim is to link presynaptic proteins to parameters of a model for short-term plasticity (STP), by constraining the model with plasticity data from genetic perturbation studies in autaptic Hippocampal neurons. Creating a model for STP is a first step in this endeavour – the above-mentioned sucrose-calcium model will serve as a backbone model, which will then be refined via a Bayesian modeling approach.

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**KEY WORDS:** Mathematical model, hypertonic solution, autapses**TELEPHONE NUMBER:** 020-5986931**E-MAIL-ADDRESS:** s.schotten@vu.nl

## TITLE

**Pharmacological Magnetic Resonance Imaging (phMRI) in healthy subjects using an i.v. challenge with d-amphetamine**

## AUTHORS

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## ABSTRACT

### Introduction

The dopamine (DA) system is pivotal in the pathology of several neuropsychiatric diseases. Pharmacological MRI (phMRI) can investigate neurotransmitter function by measuring the hemodynamic response to a pharmacological challenge. D-amphetamine (dAMPH), a drug that increases extracellular DA, is frequently used as a challenge. Jenkins et al (2004) have demonstrated in non-human primates that a dAMPH challenge can adequately assess DA function, since the hemodynamic response to dAMPH was blunted following DA lesioning. In addition, D2 receptor agonism has been found to influence regional cerebral blood flow (rCBF) (Chen et al. 2010). The purpose of this current study is to investigate how drug-induced brain hemodynamic changes correlate with D2 receptor availability in the human brain.

### Materials & Methods

Twelve healthy male volunteers participated in this study (mean age = 21.0, S.D = 1.47).

A 3DT1 anatomical scan was acquired on a Philips 3.0T MRI scanner, followed by a pseudo-continuous ASL sequence: TE/TR: 13.85/4.0 s, matrix size= 80x80, FOV = 240x119x240 mm, slices = 17, slice thickness = 7 mm, labelling duration = 1650 ms, post-labelling delay = 1525 ms, number of dynamics = 300. After the first 75 dynamics (10 minutes) of baseline scanning, 0.3 mg/kg AMPH was administered intravenously over 2 minutes. SPECT imaging took place under continuous infusion of the validated D2-receptor tracer [<sup>123</sup>I]IBZM, using a 12 headed dedicated brain SPECT camera (SME 810).

ASL images were averaged over 25 dynamics into 12 timebins. They were registered to 3DT1 scans and standard space using DARTEL (Ashburner, 2007) and smoothed (6 mm). Based on the literature (Jenkins 2004, Udo de Haes 2007) we chose striatum, anterior cingulate cortex (ACC), prefrontal cortex (PFC) and thalamus as ROIs using the WFU Pick Atlas toolbox. To control for general cardiovascular effects, a specific to non-specific CBF ratio (rCBF) was calculated. Mean baseline and post challenge CBF values were computed and subsequently analyzed using repeated measure ANOVA with Bonferroni correction.

### Results

Significant effects of dAMPH administration were observed in both striatum ( $F(3.30, 36.34) = 2.96, p = 0.041$ ) and ACC ( $F(3.45, 36.8) = 3.63, p = 0.018$ ), not in the PFC or thalamus. Planned contrasts in striatum showed a significant increase in rCBF from baseline to time-bin 1 ( $p = 0.002$ ), time-bin 2, ( $p < 0.0001$ ), time-bin 3, ( $p = 0.001$ ) and time-bin 5 ( $p = 0.002$ ). In ACC rCBF significantly increased from baseline to time-bin 2, ( $p = 0.024$ ), time-bin 4 ( $p = 0.004$ ), time-bin 5 ( $p = 0.041$ ), and time-bin 8 ( $p = 0.028$ ). SPECT images remain to be analyzed.

### Discussion

We report a significant increase in rCBF in response to an i.v. dAMPH challenge in specific brain regions involved in the DA brain circuitry of healthy control subjects. AMPH-induced changes in phMRI signal have previously been shown to correlate strongly with AMPH-induced increases in extracellular DA in rats (Chen *et al.*, 2005). Based on the similarities between these findings and our own results and the fact that this effect was visible despite a conservative cardiovascular correction, the AMPH-induced hemodynamic response is likely to reflect DA functioning. Future analysis SPECT data will determine whether this response is correlated to D2 receptor availability.

### Conclusion

ASL based phMRI using i.v. dAMPH as a dopaminergic challenge seems to be a useful tool in imaging DA function.

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**KEY WORDS:** phMRI, dopamine, amphetamine

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## TITLE

**Preactive multiple sclerosis lesions reveal innate immune activation and immune-regulation**

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## ABSTRACT

### Introduction

In multiple sclerosis (MS) brains, even after many years of disease, clusters of activated HLA-DR-expressing microglia form preactive lesions in normal-appearing white matter. The microglia clusters may represent an early stage of MS lesion formation that once triggered further develop into active demyelinating MS plaques. While the typical active, chronic active and chronic inactive lesions have been the focus of extensive pathological studies remarkably little is known about preactive lesions. Here we determined the incidence and composition of preactive lesions in a cohort of 21 MS patients. We demonstrate that the microglia clusters were not associated with T cells, axonal alterations, activated astrocytes or blood vessels and further revealed expression of IL-10, TNF- $\alpha$ , MMP9 but not IL-4 and MMP2 indicating a balance of pro-inflammatory and immune-regulatory mediators.

### Methods

FFPE tissue blocks (n=213) from MS patients (n=21) were selected and were sectioned to be stained for PLP and HLA-DR (LN3). For double IHC stainings, cryosections from different MS brain samples were stained with specific primary and thereafter Alexa 488/594 labeled secondary antibodies to perform microscopical analysis.

### Results

Preactive lesions were observed in 21% of tissue blocks and 67% of MS patients. The frequency of these lesions was higher in, but not restricted to regions in the vicinity of active demyelination and inflammation i.e. active and chronic active lesions. Examination of the cellular composition revealed that preactive lesions were not found in close proximity to blood vessels nor associated with CD3-positive lymphocytes, axonal alterations or activated astrocytes. The expression of both anti-inflammatory (IL-10) as well as pro-inflammatory cytokines (TNF- $\alpha$ , MMP9) was observed on many activated microglia including those associated with preactive lesions in MS brain tissue.

### Conclusion

Together our data reveal that preactive lesions occur in MS irrespective of disease duration, gender or sub-type of disease. Preactive lesions are not associated with blood vessels suggests an innate trigger within the CNS rather than activation by extrinsic factors crossing the blood brain barrier. Knowledge of the innate conditions that activate microglia and the initial stages of MS lesion development will enable the search for mechanisms underlying microglia activation as well as novel targets for therapeutic strategies aimed at inhibiting early lesion formation.

**KEY WORDS:** Preactive lesions, immune regulatory, multiple sclerosis

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**TITLE**

**Family history of alcohol dependence and the development of mood- and anxiety disorders: cognitive and emotional functions in the brain**

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**ABSTRACT**Background

A family history (FH) of alcohol dependence (AD) is known to increase the risk for, and the severity of, alcohol dependence. FH of AD is also associated with the occurrence of mood and anxiety disorders. However, it is unknown how a FH of AD may affect the neural substrates associated with the vulnerability for a clinical manifestation of mood and anxiety disorders. In this study, therefore, we investigated the effects of an alcoholic FH on performance and regional brain activation during a cognitive and emotional fMRI-task in non-alcoholic persons with a mood and/or anxiety disorder.

Methods

Participants were drawn from the Netherlands Study of Depression and Anxiety (NESDA)-Neuroimaging study. In a sample of subjects with mood and/or anxiety disorders a group with a first-degree FH of AD (FH+; n=31) was compared with a group without such a FH (FH-; n=77) on performance and regional brain activation during a visuospatial planning task and an emotional word memory task.

Results

On both fMRI tasks, accuracy levels were similar in both groups, but response times were slower in the FH+ group during the more demanding trials of the planning task. Moreover, using task-related ROI's the FH+ group showed a stronger BOLD response during planning in the middle frontal gyrus and right parahippocampal gyrus, and during encoding of positive words in the right insula compared to the FH- group. Whole brain uncorrected, reactivity to negative words remained absent in FH+ compared to FH-.

Conclusions

This study suggests subtle cognitive impairments during a planning task in people with mood and/or anxiety disorders and a FH of AD, and stronger BOLD responses in the FH+ compared to the FH- group during the planning and positive word encoding, while activity during negative words, or mood congruent stimuli, typical for mood- and anxiety disorders, was not visible in FH+. These findings may indicate the presence of a subgroup of people with a mood/anxiety disorder, showing mild cognitive impairments with the absence of mood disorder typical reactivity to emotional stimuli. Therefore, broader profiling of familial psychopathology needs to be considered during diagnosis as well as treatment.

**KEY WORDS:** Family history, alcohol dependence, depression, anxiety, emotion, cognition, fMRI

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## **TITLE**

**The effects of sex steroids on functional brain activity during emotional picture processing**

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## **ABSTRACT**

### Background

Although emotional processes are thought to be influenced by sex hormones, the literature is currently inconclusive about the effects of sex steroids on emotional processing. Studies in women during the course of their menstrual cycle indicate a relation between estrogens and processing of negative stimuli. To the best of our knowledge, no study has looked at the effect of hormonal suppression on affective picture perception thus far. In this functional Magnetic Resonance Imaging (fMRI) study we compare emotional picture processing of female-to-male (FtM) transsexuals, who are hormonally suppressed with a GnRH analog for eight weeks with a healthy control group in the early follicular phase of the menstrual cycle.

### Methods

Ten FtM transsexuals and 10 healthy control women were recruited in the VU University Medical Center and underwent a fMRI scan during the rating of complex emotional pictures adapted from the International Affective Picture System (IAPS). The gonadal hormone production of the FtM group was suppressed for eight weeks; the healthy control group did not receive any hormonal treatment before the fMRI. Blood samples were collected to assess hormonal levels.

### Results

Estradiol levels differed significantly between control women and FtM transsexuals. Key structures for emotional processing showed increased activation in the healthy control women compared to the FtM transsexuals when emotional pictures were rated: areas in insula, amygdala, hippocampus, dorsolateral prefrontal cortex and the orbitofrontal cortex. FtM transsexuals under gonadal suppression only showed increased activation in the mid part of the temporal lobe compared with control women.

### Conclusion

Gonadal hormones, estradiol in particular, seem to influence emotional neuronal processes. In this study differences in activation patterns were found in key regions for emotions when control women were compared with gonadally suppressed FtM transsexuals. In contrast to previous studies that have found associations between negative emotions and estrogens, we have now found an additional association for positive emotional stimuli and estrogens. The findings of this study seem to confirm the relation between emotional picture processing and estrogens.

**KEY WORDS:** Emotion, Sex steroids, functional MRI

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**TITLE**

**Development of a model for alcohol addiction in rats**

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**ABSTRACT**

Alcoholism, characterized by a loss of control over alcohol intake, is a disease that affects over 76 million people worldwide (WHO, 2004). The risk for alcoholism is conferred by an interplay of genetic and environmental factors which remain incompletely understood. Recent studies have shown that rodents display continued drug seeking despite aversive consequences, which is an important behavioural characteristic of addiction in humans. Rats and mice develop alcoholism-like behaviour in that they become insensitive to adulteration with the bitter tastant quinine. The overarching goal of this project is to identify behavioural and neural characteristics that confer the vulnerability for alcoholism. To that aim, we determined whether Lister-Hooded rats develop alcoholism-like behaviour. We subjected the animals to two home-cage drinking schedules (intermittent and continuous access), and subsequently tested their motivation for alcohol in an operant setup. We hypothesized that rats with a history of intermittent access to alcohol consume more alcohol, are less sensitive to quinine adulteration and show greater motivation to consume alcohol in the operant settings. Two separate groups of rats consumed 20% ethanol in water in the home-cage using a two-bottle choice paradigm on an intermittent (3 times a week access to alcohol) or a continuous access schedule. Next, rats were trained to respond for alcohol under fixed ratio and progressive ratio schedules to assess their motivation for alcohol. Our preliminary findings show that rats on an intermittent access schedule consumed more alcohol than rats that had continuous access to alcohol. Moreover, within groups, we observed marked individual variation in alcohol intake and preference. The effect of quinine adulteration on alcohol intake and the motivation to consume alcohol in operant boxes are currently analyzed.

**KEY WORDS:** Alcohol, addiction, motivation

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**TITLE**

**The role of the intermediate filament GFAP in mechanotransduction in reactive astrocytes**

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**ABSTRACT**

Like all cells, brain cells are sensitive to mechanical cues. When an external force is exerted, for instance by brain trauma or neurodegenerative diseases such as Alzheimer's and Parkinson's Disease, astrocytes become reactive, which is hallmarked by an increased expression of the intermediate filament (IF) protein GFAP. This can leave a scar that is thought to have a protective effect at first, but may inhibit brain repair at a later stage. Thus studying the GFAP IF-network and its role in astrocyte mechanics is important for understanding brain trauma and brain pathology models. In addition, multiple isoforms of GFAP have been identified, which seem to be differentially expressed in functionally different subtypes of astrocytes.

IFs are emerging as a key component of the cytoskeleton, integrating the full spectrum of cellular responses to biochemical, biomechanical and cellular stresses. We study the role of GFAP and the IF network in the molecular response of cells to mechanical forces.

We plan to study the effect of mechanical forces on astrocytes by growing them on a laminin coated PDMS-membrane and stretching the cells at different strain amplitudes and frequencies. Laminin is the most abundant extracellular matrix constituent of the brain. We identified all integrins that bind laminin on primary mouse astrocytes by qPCR analysis. After a relevant stretch regime has been found, the astrocyte's transcriptome will be analyzed through microarrays, to identify the difference in response between primary wild type and GFAP knock-out astrocytes and to elucidate the role of IFs in mechanotransduction.

**KEY WORDS:** GFAP, astrocytes, mechanotransduction

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## **TITLE**

**fMRI as a diagnostic tool for early dementia**

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## **ABSTRACT**

### Introduction

The diagnosis of dementia at an early stage of the disease is challenging. Symptoms are often mild and unspecific and atrophy, as observed in established dementia, may not yet show on conventional MRI. Functional MRI (fMRI) has the potential to detect changes in memory function preceding structural atrophy and may therefore be better suited to support definitive diagnosis at an earlier stage. This study aims to assess fMRI of memory processing as a diagnostic tool for dementia.

### Methods

Eleven patients suspected of dementia (mean age 58.5y, 5 males, mean Mini Mental Status Examination (MMSE) 27.7) and 6 controls (mean age 55.5y, 3 males, mean MMSE 29.9) performed a visual learning and recall task (adaptation of Gron et al. 2001) at 3T, involving repetitive memorization with subsequent immediate active recall of geometric patterns. Conditions were alternated five times. After 20 minutes, a delayed recall session was performed. In-scanner performance was monitored and tested for significant differences between patients and controls with 2-sample tests.

Data were pre-processed and analyzed using SPM8 (London, UK). Individual t-contrast images were calculated using the general linear model for all immediate recall sessions combined and for the delayed recall session and subsequently entered into a second level analysis comparing patients with controls using 2-sample t-tests with a threshold of  $p < .001$ .

### Results

In-scanner performance was significantly better in controls than patients ( $p=0.001$ ). Compared to controls, during immediate recall patients showed enhanced activation in the left temporal and bilateral parietal regions, bilateral superior frontal and cingulate gyrus.

During delayed recall, patients showed increased activation in the right hippocampus, bilateral superior temporal gyrus, right middle temporal gyrus and bilateral parietal regions in comparison to controls.

### Conclusion

Patients with suspected dementia show increased activation in memory and attention networks, reflecting compensatory changes. Furthermore, patients show a reverse pattern of neural activation during immediate and delayed recall, because the hippocampus is normally implicated in immediate but not delayed recall. The fact that we observe hippocampal activation in delayed recall in our patient population indicates an impairment in the consolidation of information from episodic to long term memory.

Such findings may serve as a diagnostic marker of early dementia, indicating the potential contribution of fMRI to the definitive diagnosis of dementia even before atrophy becomes apparent on conventional MRI. Future efforts are directed at the differentiation between several types of dementia by increasing the sample size of both patient and control groups.

**KEY WORDS:** Early dementia, fMRI, memory

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**TITLE**

**Glutamate receptors and spontaneous recovery after cue-extinction of nicotine seeking**

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**ABSTRACT**

Pharmacological intervention and behavioral therapy can support an attempt to quit smoking. In an animal model of self-administration, an operant drug seeking behaviour can be reduced by repeated exposure to cues that were previously paired with the drug, but now in the absence of drug, a process termed extinction. Extinction is thought to involve the formation of a new inhibitory memory, which is supported by the fact that the mere passing of time (spontaneous recovery), or a return to the drug-paired context (renewal), causes a resurgence in operant behaviour.

The duration of the extinction session and therefore the amount of exposure to nicotine-paired cues affects the time-lime of extinction. Among rats, there are individual differences in resistance to extinction training and level of spontaneous recovery. Spontaneous recovery is an indication of extinction memory failure and can be used to assess extinction memory strength.

A molecular mechanism involved in learning is the glutamatergic NMDA receptor. The effect of the partial NMDA agonist D-cycloserine (DCS) and the NMDA antagonist 3-(2-Carboxypiperazin-4-yl)propyl-1-phosphonic acid (CPP) on early nicotine extinction learning and spontaneous recovery will be discussed. Proteomics will be used to identify and quantify protein level changes in early nicotine extinction. Markers of neuronal activation can be applied to the mapping of neuronal substrates involved in nicotine extinction, spontaneous recovery and renewal. Combining behaviour, proteomics and immunohistochemistry, we aim elucidate the molecular and cellular mechanisms of nicotine extinction with the ultimate goal of developing novel treatment methods for nicotine addiction.

**KEY WORDS:** NMDA receptors, extinction, nicotine addiction

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**TITLE**

**The role of the hippocampus in consolidating information containing regularities**

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**ABSTRACT**

Studies have shown that with time, memories become less specific and detailed and instead have a more abstract and general character. Another line of research points at the reduced involvement of the hippocampus as memories grow older; shifting towards a more cortical memory trace – perhaps including the medial prefrontal cortex. Thus far, there has been no study that specifically looked at the differential consolidation pathways for information that does and that does not contain regularities. It was hypothesized that especially when information contains regularities, the hippocampus will be decoupled from the retrieval network, and medial prefrontal regions will instead become involved in retrieving these memories.

Subjects learned face-location associations; half of the associations were created in such a way that specific facial features determined the location of the face, the other associations were completely random. The regularities were complicated and could therefore only be extracted over several encoding blocks, thereby simulating the learning of rules over separated episodic events. Subjects retrieved the associations in the fMRI scanner after 2 intervals; one scan was scheduled ½ hour after the encoding session, the other followed 48 hours later.

Behavioral analyses showed that rule associations were retrieved more accurately and also showed a slower forgetting rate than the random associations. Contrary to our hypotheses, it was found that the random associations showed reduced hippocampal activity over time whereas the rule associations showed persistent hippocampal activity. Connectivity analyses revealed that this persistent activity could be related to the crosstalk between the hippocampus and several prefrontal areas; the left middle frontal gyrus and the anterior cingulate cortex. In addition, connectivity analyses showed that the retrieval of remote rule associations are less dependent on the connectivity between the representational areas and the hippocampus.

It can be concluded that, especially over time, regularities help against forgetting. This benefit is related to a brain network that still includes the hippocampus, but the connectivity it shows shifts from the representational areas towards frontal areas. This hippocampal-frontal crosstalk might represent the incorporation of rule related information into a frontal-based retrieval network, that with time, might become totally independent of the hippocampus.

**KEY WORDS:** Memory, regularities, hippocampus

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**TITLE**

**Impact of single-cell stimulation in rat barrel somatosensory cortex on the detection of whisker movements**

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**ABSTRACT**

A single whisker stimulus activates a significant fraction of the ~10.000 neurons within a somatosensory cortex barrel column. How do the resulting action potentials (APs) in these neurons relate to perception? To address this question we explored the effects of electrical single-cell stimulation on psychophysical detection performance in head-immobilized rats. Animals were first trained to report via tongue licks a small (10 deg) ramp-and-hold deflection applied to a single whisker. The animals' detection thresholds for whisker movement decreased over several daily sessions to deflection velocities around 125 deg/s. Once animals had reached stable asymptotic performance, trials with whisker deflections near the detection threshold (with response rates of ~50%) were randomly mixed with near-threshold whisker deflection trials in which we simultaneously evoked short (200 ms) trains of 5-20 APs in a single barrel cortical neuron using juxtacellular nanostimulation. Recordings were targeted to neurons located in the barrel column corresponding to the deflected whisker. Our preliminary data show that animals respond more often in whisker deflection trials paired with single-cell stimulation than in whisker deflection-only trials. Control experiments show that this sensory effect is dependent on the generation of APs by nanostimulation. Specifically, application of 2-4 times the average stimulation current into extracellular space does not affect the animal's behavior. These preliminary data suggest that adding APs in individual cortical sensory neurons can bias the perception of single whisker movements. Ongoing experiments aim at quantifying the sensory effect of single-cell stimulation for different physiologically identified cell types.

**KEY WORDS:** Barrel cortex, electrical stimulation, neural coding

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**TITLE**

**Identification of neurodegenerative and neuroprotective mechanisms in a MPTP marmoset model for idiopathic Parkinson's disease**

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**ABSTRACT**

Parkinson's disease (PD) is the second most common progressive neurodegenerative disorder of the central nervous system. PD is initially characterized by movement disorders due to the progressive loss of dopamine neurons in the substantia nigra. Current pharmaceutical treatments focus on the relief of disease symptoms, but have little effect on the progression of the disease and do not prevent neurodegeneration. In order to design treatment strategies that are aimed at targeting the cause of neurodegeneration or neurodegeneration itself, it is important to better understand the neurodegenerative mechanisms underlying PD; which genes and proteins in the brain play a role during the development of this disease, how do these genes and proteins contribute to disease progression, and how can the expression and function of these genes and proteins be manipulated in order to treat the disease efficiently?

Here we will use intermittent low dose MPTP injection in marmosets as a clinically relevant model for PD. Animals will be weekly injected with 0.5 mg/kg MPTP and monitored over time using a multilevel integrative approach consisting of behavioral assessment, brain imaging, histology, biochemistry and immunology. The integrative nature of this project and the human clinical validity of a marmoset model will contribute to a better understanding of the development of PD. In addition, we will analyze brain tissue of animals from all disease stages using genomics and proteomics approaches, which may reveal novel molecular targets in the development and progression of the disease.

Interestingly, and unlike humans, MPTP-injected marmosets also show spontaneous recovery of PD symptoms after discontinuing of MPTP treatment. This uniquely also provides an opportunity to identify novel neuroprotective mechanisms that may be used to design treatment strategies for human PD patients.

**KEY WORDS:** Parkinson's disease, behaviour, transcriptomics and proteomics

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**TITLE**

**Effects of focus and expectancy on processing of nociceptive information in the rat using somatosensory evoked potentials**

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**ABSTRACT**

Pain management in veterinary medicine is hampered by lack of self-report by the subject and relative large interobserver variability of pain-scoring systems. Components of the somatosensory evoked potential (SEP), a time-locked recording of the electroencephalogram (EEG) associated with nociceptive (electrical) stimulation, have been demonstrated to correlate well with self report in humans and with analgesic intervention in experimental nociception in animals, thus providing a promising objective parameter for studying nociception. With this study we evaluate if the SEP can be used to detect altered nociceptive brain processing in rats induced by previous experience and expectancy of aversiveness of nociceptive stimuli.

First, a baseline measurement was carried out using SEP-evoking stimuli as an unconditioned stimulus (US). The US consisted of 32 square wave 2 ms electrical pulses of 3 mA with a constant inter-stimulus-interval (ISI) of 2 seconds administered on the tail base. Subsequently animals were randomly divided into either a high-expectancy (HE) or low-expectancy (LE) group in which expectancy was manipulated differently by means of a training with a modified Pavlovian fear-conditioning paradigm (PFCP). The PFCP consisted of 10 coupling of a conditioned stimulus (CS; tone 1500 Hz, 40 sec) with a high (5 mA) or low (1 mA) intensity US in the HE and LE, respectively. The next day (day 2), animals of both groups were administered the baseline US (3 mA) on two separate occasions, preceded by the CS only on the first occasion.

Initial analysis of recorded SEPs revealed a significant difference in overall SEP amplitude between groups during the PFCP training as well as in the late SEP components in the first session of day 2 (US preceded by CS), but not the second session (only US). Both sessions on day 2 showed a higher amplitude SEP signal compared to the baseline measurement.

These results combined indicate that the PFCP training was successful and a general increase in SEP amplitude over time occurred. In analogy with human findings with expectancy, differences in late SEP components between groups on day 2 following the CS were expected, yet could not be confirmed as this SEP was not significant different from the internal control SEP without CS recorded afterwards.

The absence of detectable neurophysiological effects of expectancy created by the CS may be explained by the constant ISIs within an US. Although the CS predicted the onset of either a high or low intensity US, constant ISIs of stimuli of the same intensity within one US could have abolished the predictive effect of the preceding CS.

**KEY WORDS:** Pain, neurophysiology, rat

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**TITLE**

**Seeing without knowing: neural signatures of a visual illusion in the absence of report**

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**ABSTRACT**

Every day, we experience a rich visual world in front of our eyes. Much of our visual world however, is not actually present in the physical stimulation. The brain continuously alters and adds percepts to the scene to let it make sense: gaps are filled in and objects are completed when not fully in view. Do these processes take place even when we do not attend to these objects and therefore are not able to report about what we are seeing? Here we make use of the Kanizsa illusion to show that the neural signature of perceptual completion can be present in the absence of report, using functional magnetic resonance imaging and a task in which observers were rendered blind for the illusion due to attentional demands. The neuronal activity associated with perceptual completion was evident in lower and higher visual areas, irrespective of the ability to report about it. This suggests that report is not necessary for a process such as perceptual completion to be accomplished, but might merely be a mechanism to communicate about the perceptual information derived from a scene.

**KEY WORDS:** fMRI, visual perception, consciousness

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**TITLE**

**Retinoic acid-dependent and -independent gene-regulatory pathways of Pitx3 in meso-diencephalic dopaminergic neurons**

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**ABSTRACT**

Development of meso-diencephalic dopamine (mdDA) neurons requires the combined actions of the orphan nuclear receptor Nurr1 and the paired-like homeobox transcription factor Pitx3. Whereas all mdDA neurons require Nurr1 for expression of Th and survival, dependence on Pitx3 is only displayed by the mdDA subpopulation that will form the substantia nigra (SNc). Previously, we demonstrated that Pitx3<sup>-/-</sup> embryos lack the expression of the retinoic acid (RA)-generating enzyme Ahd2, which is normally selectively expressed in the Pitx3-dependent DA neurons of the SNc. Restoring RA-signaling in Pitx3<sup>-/-</sup> embryos revealed a selective dependence of SNc neurons on the presence of RA for differentiation into Th-positive neurons and maintenance throughout embryonic development. Whereas these data are suggestive of an important developmental role for RA in neurons of the SNc, it remained unclear whether other Nurr1 and Pitx3 target genes depend on RA signaling in a manner similar to Th.

In search for genes that were affected in Pitx3-deficient mdDA neurons and restored upon embryonic RA treatment, we provide evidence that Delta-like 1, D2R (Drd2) and TH are regulated by Pitx3 and RA signaling, influencing the mdDA terminal differentiated phenotype. Furthermore, we show that regulation of Ahd2-mediated RA-signaling represents only one aspect of the Pitx3 downstream cascade, since Vmat2, Dat, Ahd2 (Aldh1a1), En1, En2 and Cck were unaffected by RA treatment and are (subset) specifically modulated by Pitx3. In conclusion, our data reveal several RA-dependent and -independent aspects of the Pitx3-regulated gene cascade suggesting that Pitx3 acts on multiple levels in the molecular subset-specification of mdDA neurons.

**KEY WORDS:** Dopamine, midbrain, retinoic acid

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**TITLE**

**The aging synapse: synapse proteomic alterations in mouse models of aging**

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**ABSTRACT**

As we grow older we will all experience memory loss and cognitive decline, which can have serious impact on the quality of our daily life. Cognitive decline can affect various brain functions including perceptual speed, inductive reasoning, verbal ability and verbal memory, and possibly the most important, hippocampus-dependent learning and memory. Understanding age-related cognitive decline is of great importance. Most elderly people experience some form of age-related neuropathology, and as the life expectancy of our population increases, the prevalence of age-related cognitive decline and neurodegenerative disorders such as Alzheimer's disease (AD) will only increase. Cognitive decline will become a serious burden in health care in the coming decades, with nearly 50% of adults over the age of 85 in the United States affected with AD. The greatest risk for cognitive decline and AD is age itself. Therefore, understanding the cellular and molecular processes underlying normal and pathological aging of the brain is of crucial importance to develop therapeutic treatment for cognitive decline.

Here, we identified alterations in the protein composition of hippocampal synapses that could potentially underlie age-related cognitive decline. As a model for aging we used *Ercc1* mutant mice, which are impaired in multiple DNA repair systems and consequently show many features of accelerated aging, including progressive memory deficits. In addition, we used two Alzheimer mouse models, APP<sub>swe</sub>,PS1<sub>dE9</sub> and APP<sub>swe</sub>,PS1<sub>M146V</sub>, tau<sub>P301L</sub>. These mouse models have been developed to mimic the major neuropathological hallmarks of AD, namely amyloid plaques and neurofibrillary tangles and they recapitulate many, although not all, features of AD, and are widely used in AD research. Comparing these different mouse models allows us to identify common pathways and distinct pathways that define normal and pathological aging that ultimately lead to cognitive decline.

Comparative quantitative hippocampal synapse proteomics have been performed to study the in vivo molecular composition of synapses and to detect changes in synapse composition and function. Many deregulated proteins were found in the different aging models, in which we could detect both common and model-specific pathways, ultimately leading to aging.

**KEY WORDS:** Aging, synapse, proteomics

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**TITLE**

**Network structure of the ventral tegmental area**

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**ABSTRACT**

The ventral tegmental area (VTA) is an important midbrain nucleus, whose activity levels have been implicated in pathologies like schizophrenia. Dopaminergic VTA neurons display spontaneous rhythmic activity that is extensively studied at the single-unit level in particular in relation to pharmacological manipulation. On a 64 channel micro electrode array (MEA) we could simultaneously record multiple single-units (often more than 20 in the same slice) and investigate the relations underlying these firing patterns. Based on their action potential shape, activity pattern and response to the D2 receptor agonist quinpirole (1 nM) dopaminergic neurons were selected for further analysis. All dopaminergic neurons were oscillators firing within a frequency range of 1-4 Hz under baseline conditions. Bath application of different concentrations of  $K^+$  increased the firing rate by more than 30 % (n = 35) We considered the VTA neurons as a large collection of weakly coupled oscillators and used various methods to quantify the coupling strength and underlying functional connectivity pattern. In addition we determined how these parameters are dependent on the mean firing level induced by elevated  $K^+$ . The analysis will be further expanded to study manipulation by dopaminergic and serotonergic pharmacology at the level of the network.

**KEY WORDS:** Network, VTA, pharmacology

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**TITLE**

**A fine mapping study of 7 top scoring genes from a GWAS for major depressive disorder**

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**ABSTRACT**

Major depressive disorder (MDD) is a psychiatric disorder that is characterized by, amongst others, persistent dysphoria, loss of interest and pleasure and psychomotor retardation. Environmental circumstances have proven to influence the aetiology of the disease, but MDD also has an estimated 40% heritability. However, MDD is a polygenic disorder and does not have a single genetic cause. In 2009, a genome wide association study (GWAS) was performed on the Dutch GAIN-MDD cohort but genome-wide significance was not yielded, but the non-synonymous coding single nucleotide polymorphism (SNP) rs2522833 in the *pcl* gene became nominally significant after post-hoc analysis with an Australian cohort which used similar ascertainment. The absence of genome-wide significance in many GWA studies may be caused by low SNP coverage of genes, insufficient specificity of a phenotype, lack of power or several other causes. To increase SNP coverage to 100% in the scenario of common variation ( $maf=0.1$ ,  $r^2=0.8$ ) and find a more strongly associated variant we selected seven genes from the GAIN-MDD GWAS: *pcl*, *gzm*, *anep*, *afap111*, *st3gal6*, *fgf14* and *ptk2b*. We genotyped 349 SNPs using the Taqman OpenArray system. After genotyping, none of the SNPs showed genome-wide significance, with the lowest P-value for rs2715147 in *pcl* at  $P=6.8E-7$ . We imputed missing genotypes and after imputation, rs2715147 and rs 2715148 showed the lowest P-value at  $P=1.2E-6$ . When we created a haplotype of these SNPs together with the non-synonymous coding SNP rs2522833, the P-value decreased to  $9.9E-7$ . We can conclude that using common variation in order to fine map the results of the GAIN-MDD GWAS does not result in a more strongly associated variant. However, *anep*, *gzm* and *ptk2b* may still be interesting candidate genes based on their function. The results for *pcl* suggest that an unknown causal variant may exist in high LD with rs2715147, rs2715148 and rs2522833.

**KEY WORDS:** Major depressive disorder, single nucleotide polymorphism, fine mapping

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## TITLE

Initial PET studies in rats with novel radiotracer [<sup>11</sup>C]D617 and comparison with (R)-[<sup>11</sup>C]verapamil

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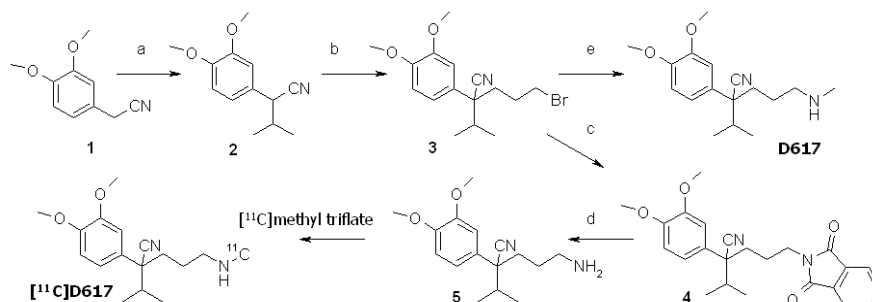
## ABSTRACT

### Objectives

The aim of this study is to develop the synthesis of [<sup>11</sup>C]D617 and to study its brain distribution in rats with and without pre-treatment with P-gp inhibitor tariquidar and compare the results with previous data obtained with (R)-[<sup>11</sup>C]verapamil.

### Methods

Two consecutive PET-scans were obtained in eight rats. Tariquidar, 15 mg/kg, was administered 20-25 min prior to the second scan. Blood samples were obtained in parallel to the PET-scans. The brain distribution volume (V<sub>T</sub>) was estimated for each rat and scan using Logan analysis.



**Scheme 1.** The precursor for labelling D617 with C-11, compound **5**, was synthesized in 4 steps and subsequently reacted with [<sup>11</sup>C]methyl triflate to give [<sup>11</sup>C]D617. a: DMF, NaH, isopropyl bromide, RT, 5 hours, 77%; b: THF, n-BuLi, 1,3-dibromopropane -78°C, 2 hours, 83%; c: toluene, 18-Crown-6, potassium phthalimide, reflux 6 hours, 86%; d: THF, EtOH, hydrazine·H<sub>2</sub>O, RT, 2 hours, 74%; e: methylamine, toluene/H<sub>2</sub>O, 56%.

## Results

Precursor, **5**, was synthesized in 41% overall yield. Reference D617 was synthesized in >98% purity and 56% yield starting from **3**. The labelled product, [<sup>11</sup>C]D617, was synthesized in 62-68% yield and with >99% (radio)chemical purity. Synthesis time was 50 minutes and specific activity was 70-94 GBq/μmol at end of synthesis.

The V<sub>T</sub> was (average ± standard deviation) 1.1 ± 0.16 and 2.3 ± 0.44 for the two respective scans, i.e. the tariquidar treatment increased the V<sub>T</sub> 2-fold. This can be compared to (R)-[<sup>11</sup>C]verapamil, which under the same experimental design show a 11-fold increase in V<sub>T</sub> after tariquidar treatment. Further comparison of the two tracers shows that the baseline V<sub>T</sub> is similar, around 1 for both tracers.

## Conclusion

[<sup>11</sup>C]D617 was synthesized with good yield and SA. [<sup>11</sup>C]D617 appeared to be a weaker P-gp substrate than (R)-[<sup>11</sup>C]verapamil. When modeling the transport of (R)-[<sup>11</sup>C]verapamil into the brain, it may be possible to lump (R)-[<sup>11</sup>C]verapamil and its metabolite, [<sup>11</sup>C]D617, together in baseline PET studies when P-gp is uninhibited. However, this should not automatically be done in P-gp interaction studies, since P-gp inhibition has a different effect on the distribution of [<sup>11</sup>C]D617 vs. (R)-[<sup>11</sup>C]verapamil in the brain.

## Acknowledgments

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**KEY WORDS:** [<sup>11</sup>C]Verapamil, [<sup>11</sup>C]D617 and PET

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**TITLE**

**Increase in endoplasmic reticulum-associated tissue transglutaminase and enzymatic activation in a cellular model of Parkinson's Disease**

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**ABSTRACT**

Parkinson's disease (PD) is characterized by accumulation of  $\alpha$ -synuclein aggregates and degeneration of melanised, catecholaminergic neurons. The tissue transglutaminase (tTG) enzyme catalyzes molecular protein cross-linking. In PD, tTG levels are increased and cross-linking has been identified as an important factor in  $\alpha$ -synuclein aggregation. In our quest to link tTGs distribution in the human brain to the hallmarks of PD pathology, we recently reported that catecholaminergic neurons in PD disease-affected brain areas display typical endoplasmic reticulum (ER) granules showing tTG immunoreactivity. In the present study, we set out to elucidate the nature of the interaction between tTG and the ER in PD pathogenesis, using retinoic-acid differentiated SH-SY5Y cells exposed to the PD-mimetic 1-methyl-4-phenylpyridinium (MPP<sup>+</sup>). Alike our observations in PD brain, MPP<sup>+</sup>-treated cells displayed typical TG-positive granules, that were also induced by other PD mimetics and by ER-stress inducing toxins. Additional immunocytochemical and biochemical investigation revealed that tTG is indeed associated to the ER, in particular at the cytoplasmic face of the ER. Upon MPP<sup>+</sup> exposure, additional recruitment of tTG towards the ER was found. In addition, we observed that MPP<sup>+</sup>-induced tTG activity results in transamidation of ER membrane proteins, like calnexin. Our data provide strong evidence for a, so far unrecognized, localization of tTG at the ER, at least in catecholaminergic neurons, and suggests that in PD activation of tTG may have a direct impact on ER function, in particular via post-translational modification of ER membrane proteins.

**KEY WORDS:** Tissue transglutaminase, Parkinson's Disease, SH-SY5Y

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**TITLE**

**Rules and mechanisms of spike timing-dependent plasticity at adult human neocortical synapses**

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**ABSTRACT**

Activity-dependent changes in neuronal connection strength enable our brain to refine neural circuits and learn based on experience. Synapses can bi-directionally alter strength, and the magnitude and sign of these changes depend on the precise millisecond timing of pre- and postsynaptic action potentials.

This phenomenon, termed spike-timing-dependent plasticity (STDP), is typically studied in young laboratory animals, as it appears the ability of synapses to undergo STDP decreases with age. It is unknown whether STDP also occurs in the adult human brain and what synaptic learning rules may exist at these synapses.

Here, using human brain slices made from tissue removed for surgical treatment of epilepsy patients, we investigate whether adult human temporal cortex synapses can change strength in response to pairing presynaptic input with a single postsynaptic action potential, and compare this to adult rat temporal cortex synapses. Furthermore, we explore the mechanisms that underlie STDP in humans and rats using pharmacology.

**KEY WORDS:** Synaptic plasticity; human neocortex; learning and memory

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**TITLE**

**Inhibitory synapse turnover in adult ocular dominance plasticity**

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**ABSTRACT**

Plasticity in the cerebral cortex is fundamental to our ability to learn and adapt to altered sensory experience, both during development and in adulthood. Functional changes are often reflected by the restructuring of synaptic connections. For example, monocular deprivation (MD) leads to a shift in responsiveness of the adult visual cortex to the open eye, which is accompanied by increased turnover of dendritic spines in layer V, but not in layer II/III. Most studies have focused on the excitatory synapses located on dendritic spines. It is known, however, that inhibition also plays an important role in ocular dominance plasticity. To which extent inhibitory synapses themselves show structural plasticity is still unclear. Therefore, we have developed a novel approach to visualize inhibitory synapses by chronic two-photon imaging. We monocularly deprived adult mice and imaged inhibitory synapses on layer II/III pyramidal neurons of the binocular cortex. We find that, in contrast to excitatory synapses, inhibitory synapses show considerable turnover in layer II/III, indicating that their structural changes might play a role in ocular dominance plasticity.

**KEY WORDS:** Plasticity of inhibitory synapses, chronic 2photon imaging

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**TITLE**

**Sleep shapes small-world properties of subsequent spontaneous waking brain activity**

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**ABSTRACT**

It is not yet well known which brain regions are affected by sleep, or conversely, sleep loss. Previous work reported that sleep deprivation changes the parameters that describe overall resting state functional connectivity within the Graph Theory framework. Here, we extend these findings to investigate whether there are topographical differences in the sensitivity of graph theoretical network parameters to sleep deprivation, reflecting regional rather than network-wide brain changes. In balanced order, resting state EEG was obtained in 8 healthy participants, during a day following normal sleep and during a day following total sleep deprivation. Topographical maps of Graph Theory parameters describing local clustering and path length characteristics were obtained from functional connectivity matrices, based on synchronization likelihood in five different frequency bands. A non-parametric permutation analysis with cluster correction for multiple comparisons was applied to assess significance of topographical changes in cluster coefficient (local specialization) and path length (global integration).

Topographical significant changes after sleep deprivation were exclusively found in the eyes-closed condition. The cluster coefficient decreased frontally in the alpha frequency band and the path length increased frontally in the theta frequency band. These effects seemed unrelated to volume conduction.

The results indicate that sleep deprivation most strongly affects the graph theoretical properties of frontal brain regions in the theta and alpha frequency band. While previous studies showed a frontal dominance of EEG power changes induced by prolonged wakefulness, our findings add that also the maintenance of functional connectivity of frontal regions is strongly dependent on sleep.

**KEY WORDS:** Sleep, EEG, functional connectivity

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**TITLE**

**Wnt5a in peripheral neuroregeneration**

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**ABSTRACT**

In contrast to the central nervous system (CNS), the peripheral nervous system (PNS) has the remarkable ability to regenerate, and regrow severed axons, following injury. Although this is a robust effect, often PNS injury is characterized by a lack of (complete) functional recovery. Therefore, the current notion is that there is much to gain in PNS regeneration.

A clinical model to study hampered PNS regeneration is the neuroma-in-continuity condition. A neuroma is a swelling of the PNS after traumatic injury. The neuroma contains a myriad of neuro-repulsive growth cues, which impede axon outgrowth through the injury site to the proper end organ. When a neuroma is formed, surgery is required to remove the affected portion of the nerve, and connect the proximal and distal stumps using a graft.

Microarray analysis, comparing the proximal nerve-stump to the neuroma, has identified Wnt5a as one of the main up-regulated transcripts. Next to neuro-injury Wnt5a also plays a prominent role in CNS development. In both these contexts, Wnt5a has been portrayed as a repulsive axon guidance cue, but its exact role and downstream signalling pathway remain largely unclear.

This project aims to characterize the contribution of Wnt5a signalling in unsuccessful axon outgrowth after injury. We attempt to achieve this by assessing Wnt5a and its receptors expression in human neuroma tissue. Followed by, characterization of the repulsive axon growth properties of Wnt5a signalling in cell-cultures. Finally, *in vivo* analysis of Wnt signaling after peripheral nerve injury, will assist to determine if interfering with specific members of the Wnt5a pathway will prove beneficial to axon regeneration.

**KEY WORDS:** Wnt5a, neuroregeneration, neuroma

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**TITLE**

**Characterization of  $^{123}\text{I}$ - and  $^{18}\text{F}$ -labeled pharmaceuticals for imaging dopamine D<sub>2</sub>- receptors in the high-affinity state**

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**ABSTRACT**Introduction

It is well known that dopamine D<sub>2</sub> receptors play a central role in CNS disorders such as schizophrenia, addiction and Parkinsonism. There are 3 subtypes of the D<sub>2</sub> family which all belong to the family of G<sub>i</sub>-coupled 7 transmembrane receptors, which typically exhibit high and low affinity states for agonists. This high affinity state is the active state of the receptor, and alterations of receptors in high-affinity state are possibly more important for disorders than those of overall receptor density. Therefore, the overall aim of the project is to develop novel PET and SPECT ligands for the labeling of the high affinity state of the D<sub>2</sub> receptor. Here we report the initial findings in determining the affinity of potential ligands at various dopamine receptor subtypes.

Methods

A series of 9 potential dopaminergic ligands were synthesized and iodinated or fluorinated with the cold isotopes. Our initial experiments were based on human dopamine receptors stably expressed in Chinese hamster ovary cells obtained from Dr. Zhiwei Wang (UC Irvine). Saturation and competition binding studies were performed using [ $^3\text{H}$ ]spiperone as the radioligand for the D<sub>2</sub> receptors and [ $^3\text{H}$ ]SCH23390 for the D<sub>1</sub> receptors. An assay volume of 500  $\mu\text{l}$  was chosen to avoid ligand depletion with 1  $\mu\text{M}$  (+)butaclamol to determine non-specific binding. A pre-incubation of 15 minutes and an incubation time and temperature of 2 hours and 25 °C were used on a 96 well plate platform. Incubations were terminated by rapid vacuum filtration over GF/C glass fiber filters.

Results

The [ $^3\text{H}$ ]spiperone saturation studies detected a D<sub>2</sub> receptor density of 78 fmol/mg protein with an affinity ( $K_D$  value) of 24 pM (n = 12). The [ $^3\text{H}$ ]SCH23390 saturation studies detected a D<sub>1</sub> receptor density of 45 fmol/mg protein and a  $K_D$  of 327 pM. Competition studies with the novel compounds showed that several of these have an affinity in the target range ( $pK_i > 8$ ) and that are highly selective for the D<sub>2L</sub> over the D<sub>1</sub> receptor. Competition studies with the endogenous agonist dopamine however yielded biphasic curves with approximately 32% high affinity sites.

Conclusion

These data show that the initial experiments seem promising for some compounds regarding affinity and selectivity. The degree of agonism of these compounds needs to be tested.

**KEY WORDS:** Dopamine D<sub>2</sub> receptor, radioligand binding, method

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**TITLE**

**Activity-dependent clustering of functional synaptic inputs on developing hippocampal dendrites**

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**ABSTRACT**

During brain development, before sensory systems become functional, neuronal networks spontaneously generate synchronized bursts of neuronal activity. Such activity patterns have been described in detail on the level of the network, but what synaptic inputs patterns are received by an individual neuron during such events remains unclear. By combining local calcium imaging with whole-cell electrophysiology we were able to identify the activity of individual synaptic sites during spontaneous activity in CA3 pyramidal neurons in organotypic slice cultures from neonatal rats. Analysis of the spatio-temporal activity patterns revealed that synapses that were located close together on the dendritic branch (<20  $\mu\text{m}$ ) were significantly more activated in concert than synapses that were farther apart. These findings indicate that the dendritic tree is organized with subcellular precision and that local clusters of synaptic inputs tend to carry functionally related information. We speculated that this clustering could be an activity-dependant sorting process during development, where neighboring synapses with presynaptic axons that spike simultaneously get specifically stabilized. We therefore investigated whether spontaneous activity during development is necessary for of fine-scale organization to arise. To achieve this we blocked all spiking activity by application of TTX during incubation. We found that in control cells neighboring synapses indeed showed higher levels of co-activity than synapses that were farther apart. However, prior incubation with TTX completely abolished this spatio-temporal organization of synaptic inputs. Our findings show that spontaneous network activity acts as an important component in the precise wiring of neural networks and demonstrated for the first time that it is capable of connecting neurons with subcellular precision. Long term imaging experiments are currently being performed to investigate the precise plasticity mechanisms involved in the observed organization of synaptic inputs during development.

**KEY WORDS:** Development, synaptic connectivity, spontaneous activity

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## TITLE

**Response inhibition in obsessive-compulsive disorder patients and their unaffected siblings**

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## ABSTRACT

### Introduction

Patients with obsessive-compulsive disorder (OCD) are known to have impaired response inhibition. Studies on the exact neural underpinnings of this deficit have been inconclusive. Also, the extent to which the neural correlates of inhibition dysfunction are an endophenotype of OCD remains unclear. The aim of this study was to assess the neural correlates of inhibitory control in a large group of unmedicated adult OCD patients, their unaffected siblings and healthy controls. We hypothesised 1) decreased performance in OCD; 2) impaired recruitment of inhibitory control regions (pre-supplementary motor area (pre-SMA) and right inferior frontal gyrus (IFG)); 3) an, at least partly, similar response in the unaffected sibling group.

### Methods

Forty-two OCD patients, 18 unaffected siblings, and 39 healthy controls performed a visual stop-signal task during functional magnetic resonance imaging (fMRI). For each subject the stop-signal reaction time (SSRT), a behavioural measure of inhibitory control, was calculated. fMRI data were pre-processed and analysed using SPM8 software. Brain BOLD activity related to inhibitory control was assessed on first level in the contrast Stop Success versus Go. Two second-level analyses were done: one analysis comparing OCD patient group with controls, and another comparing the siblings with their OCD patient family-members and a matched control group.

### Results

Behaviourally, the SSRT was significantly increased in OCD patients versus controls (mean  $\pm$  SEM:  $210 \pm 8$  ms and  $187 \pm 7$  ms, respectively;  $p < 0.02$ ). The SSRT of the siblings ( $198 \pm 9$  ms) did not significantly differ from both groups.

Healthy controls activated right IFG (BA 46) and right parietal cortex (BA 40) significantly more than OCD patients. OCD patients, on the other hand, activated left pre-SMA (BA6) more than controls during inhibition success. Comparing patients, siblings and controls showed a significant effect of group in left pre-SMA and right parietal cortex. Post-hoc tests revealed that siblings shared the increased pre-SMA activity during inhibition with the OCD patient group. Moreover, in siblings the increased pre-SMA activity was bilateral. On the other hand, like the controls, siblings also had more right parietal cortex activation during inhibition compared with the OCD patients.

### Discussion

In line with our hypothesis, patients showed impaired inhibitory control (increased SSRT) related to decreased activation of right IFG and parietal cortex. The pre-SMA is known to be of critical importance for the inhibition of motor-actions. Increased pre-SMA activation in patients and siblings during inhibition might reflect a compensatory mechanism in the face of a less efficient inhibition network. This might explain why siblings, with their more extended bilateral pre-SMA hyperactivity, did not show a behavioural deficit. We are the first to show this endophenotype of compensatory pre-SMA activity during inhibition in OCD.

**KEY WORDS:** Obsessive-compulsive disorder, functional Magnetic Resonance Imaging, endophenotype

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**TITLE****Coding in the cerebellar nuclei****AUTHORS**Laurens Witter, S. Ozcelik, C.I. de Zeeuw**DEPARTMENT/INSTITUTE**

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**ABSTRACT**

The cerebellar nuclei are located at the output stage of the olivo-cerebellar system. All information processed in the cerebellar cortex needs to pass the cerebellar nuclei (CN). Tens to hundreds of Purkinje cells (PCs: the main output neuron of the cerebellar cortex) converge on a single cerebellar nuclei neuron. Together with excitatory afferents, the PC inputs control the timing of intrinsically generated action potentials of CN neurons. Even though textbooks show an oversimplified CN that contains only one or a few classes of neurons, anatomical recent electrophysiological investigations have revealed several types of neurons. The roles of these neurons however remain elusive. Here we used in-vivo whole cell recordings in anesthetized mice to shed light on the information processing in several classes of CN neurons. We recorded from each neuron for several minutes while trying to stimulate the afferent Purkinje fibers. Cell-physiological data was collected and used to identify cell classes. A subset of neurons was also stained with neurobiotin for histological analysis and group identification. Putative GABAergic (pGABA) cells showed the greatest propensity for post-inhibitory rebound firing while putative glutamatergic cells (pGlut) and putative glycinergic cells (pGly) showed little to no rebound responses. In addition to a difference in rebound response, we also identified several differences between pGABA, pGlut and pGly neurons with respect to their input resistance, firing frequency and regularity and electrotonic coupling.

**KEY WORDS:** Cerebellum, electrophysiology, in-vivo**TELEPHONE NUMBER:** 06-21996151**E-MAIL-ADDRESS:** l.witter@nin.knaw.nl

**TITLE****Piccolo/PCLO effect on neural correlates of emotional encoding****AUTHORS**

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**ABSTRACT**

Emotional information processing, such as word encoding and recognition, is thought to be related to anhedonia, one of the core symptoms of major depressive disorder (MDD). Emotional word processing is thought to be abnormal in MDD and is believed to be associated with monoamine levels (Merens et al., 2007). A GWA study for MDD implicated piccolo (PCLO), which protein product is thought to be involved in monoaminergic neurotransmission. (Sullivan et al., 2008). We hypothesize that the piccolo risk allele (PCLO+) is associated with altered brain activity during an emotional word encoding and recognition task.

Functional MRI was acquired during an event-related version of an emotional word memory task. fMRI data were analyzed using SPM5. Response times were calculated and used in an ANOVA with genotype as independent factor. Threshold for significance was set at  $p < .05$ .

To test whether PCLO+ is associated with altered brain activity in task related regions, we conducted an ANOVA with genotype as an independent factor ( $p < .05$  FDR corrected).

No effect of genotype was detected on performance during encoding. For negative word encoding we found six significant regions of difference between genotype groups: bilateral insular cortex, medial frontal, anterior cingulate, caudate nucleus, and striatum. This represented hypoactivity in the PCLO+ group relative to the PCLO- group. A special genotype effect occurred in the striatum, where we found reduced activity in the PCLO+ group relative to the PCLO- group within MDD patients, which was absent in healthy controls. We have shown piccolo genotype dependent changes in activity in the bilateral insular cortex, medial frontal, anterior cingulate, and striatum during encoding of negative words, without performance differences. This indicates an increased effort of the PCLO+ group on performance level despite the decreased brain activity relative to the PCLO- group.

**KEY WORDS:** Neuroimaging genetics, PCLO, word encoding

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**TITLE**

**The reliability of coding in thalamocortical relay cells**

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**ABSTRACT**

The thalamus modulates the information flow to and from the cortex. The basal ganglia provide the thalamus with inhibitory input, whereas the cortex sends excitatory connections to the thalamus. Thalamocortical relay (TCR) neurons can fire both single spikes and bursts: the latter ones after a low threshold T-type calcium current is activated from a sufficiently hyperpolarized membrane potential. The question what these spikes and bursts code for and how the basal ganglia can influence this coding still remains open.

Information processing of TCR neurons was investigated in a computational model and compared with data recorded from TCR neurons in-vitro patched in rat thalamic brain slices. Gaussian noise superimposed on a DC current that put the neuron in different firing regimes (bursting or spiking) was injected into the TCR neuron and the reliability and precision of the recorded output spike trains was analyzed.

The results of the analysis show that the two firing regimes of the TCR neurons, bursting mode and spiking mode, represent different coding strategies. At a low membrane potential, the neuron is in bursting mode, in which bursts phase-lock preferentially to low frequencies in the input signal, up to about 20 Hz, and single spikes show little reliability. At more depolarized membrane potentials the neuron shows a gradual change from a bursting to a spiking regime. In this second regime single spikes are more precise and more reliable, earlier in time, and phase lock to input frequencies up to 60 Hz.

The mechanisms that could be responsible for the changes in output reliability are assessed in a computational model. It was found that the reliability of the output consists of two parts: a part that depends on the input-output relation of the neuron and an 'intrinsic' part. The experimental results can now be re-interpreted: single spikes become more reliable with increasing holding potential because of the increasing input-output curve. However, in the bursting regime the burst frequency is more variable than one would expect from this curve, resulting in a lower reliability. This is probably caused by slow adaptation.

The dependence of the reliability on the input-output curve shows that there is a tradeoff between reliability and sensitivity: a 'rate coder' will have a steep input-output curve whereas a 'spike coder' will have a flat one. These results show also that even at the single neuron level the thalamus can use different coding strategies depending on whether it is in a more hyperpolarized state, for instance as a consequence of strong basal ganglia input, or in a more depolarized state, induced by strong cortical input. This implies that single neurons can change their encoding regime depending on the background activity of the surrounding network.

**KEY WORDS:** Computational neuroscience, burst and spike coding, reliability

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**TITLE****Alpha-synuclein and its role in Parkinson disease****AUTHORS**Sjirk-Jan Zijlstra, F. Mela, S. Jain, P. Heutink**DEPARTMENT/INSTITUTE**

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**ABSTRACT**

Parkinson disease (PD) is one of the most common neurodegenerative disorders affecting aging people, with more than 500.000 people suffering from this disease in Europe. The disease is characterized by motor and cognitive disturbances and accompanied by characteristic neuronal inclusions (Lewy bodies and Lewy neurites) consisting mainly of  $\alpha$ -synuclein proteins. Genetic risk factors together with environmental increase risk for developing PD. Alpha-synuclein (SNCA) point mutations, together with duplication and triplication of the gene, have been reported as PD-causing mutations. Also, recent GWAS studies show SNCA to be associated with increased risk for sporadic PD. At present it is thought that increased expression of SNCA and amino acid substitutions can lead to SNCA aggregation and neuronal death.

This project has three aims. The first is to study the mechanism of SNCA regulation; in particular which transcription factors affect the expression of SNCA. Our second aim is to elucidate the consequences of SNCA overexpression on the metabolic equilibrium of the cell and monitor the mechanisms and the dynamics of SNCA aggregates formation. Our third aim is to do a genome-wide functional screen for genes that can regulate alpha-synuclein aggregation.

To study SNCA regulation we are constructing a human neuronal cell line in which the level of endogenous transcription of SNCA can be monitored in a high throughput manner with a GFP protein co-expressed from the same mRNA by an Internal Ribosome Entry Site (IRES). We will use this cell line and our automated cell culture facility to undertake a broad screening effort which will systematically assess the effect of knocking down all the transcription factors annotated in the human genome on SNCA transcription level.

To study the effect of overexpressing SNCA we are working towards the construction of a human neuronal inducible cell line, in which SNCA is under the control of a tetracycline-inducible promoter. This cell line will be exploited to assess the consequences of an up-regulated expression of the wild type SNCA gene, as well as six aggregation-prone mutated forms of SNCA (A30P, A53T, E46K, S129A, S129D and S129E). The changes induced in the cellular transcriptional profile by this overexpression will be captured by microarray analysis. The changes induced in the micro RNA profile will be studied by sequencing.

Also, we will use the same inducible SNCA expression cell line in which SNCA is able to bind to GFP, thereby allowing us to follow the cellular distribution of SNCA. One, we will use this setup to monitor the dynamics of SNCA aggregates formation. Second, using this system we will screen for regulators of SNCA aggregation using a genome wide human shRNA library. With this library and our automated cell culture facility, we can systematically knock-down single genes and image the effect of these genes on alpha-synuclein aggregation in a high-content high-throughput way.

The results of this project will help us to understand better the molecular network in which SNCA is embedded and the event that accompany SNCA aggregation, helping us to identify new potential drug targets.

**KEY WORDS:** Alpha-synuclein, Parkinson's Disease, aggregation**TELEPHONE NUMBER:** 020-5988829**E-MAIL-ADDRESS:** s.zijlstra@vumc.nl

**TITLE**

**The inflammatory molecules IL-1 $\beta$  and HMGB1 can rapidly enhance focal seizure generation in rat entorhinal cortex**

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**ABSTRACT**

Inflammatory signalling pathways are recognized to increase the susceptibility to epileptic seizures through different mechanisms. A phosphorylation of subunit 2B of the N-Methyl-D-aspartate (NMDA) receptor (Maroso et al., 2010; Balosso et al., 2008) has been proposed to mediate the action of both Interleukin-1 beta (IL-1 $\beta$ ) and high mobility group B1 (HMGB1). We here investigated the role of these proinflammatory molecules in a rat entorhinal cortex slice model of focal epilepsy in which a double NMDA pulse can evoke a propagating, seizure-like discharge in low Mg<sup>2+</sup> and 4-aminopyridine (4-AP, Losi et al., 2010). By monitoring seizure discharge generation through patch-clamp recordings from layer V-VI pyramidal neurons coupled to simultaneous calcium imaging from tens of neurons and astrocytes, we could reveal that upon local applications with either IL- $\beta$  or HMGB1 the probability that a single NMDA pulse evokes a fID was significantly increased (from 10% in control, to 50-60% after IL- $\beta$  or HMGB1 applications). We also found that HMGB1 could enhance the direct response of neurons to NMDA stimulation, but only after the neuronal network experienced a sustained epileptiform activity. Our findings suggest that both IL-1 $\beta$  and HMGB1, locally applied to the epileptogenic site, can rapidly lower the threshold of fID generation in the neuronal network. A full clarification of the underlying mechanism may be fundamental to develop new therapeutic strategies for human focal epilepsies.

**KEY WORDS:** Inflammation, focal seizure, HMGB1

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