16th Annual PhD Meeting

Graduate School Neurosciences
Amsterdam Rotterdam

19 and 20 November 2009

Woudschoten Conference Center, Zeist
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Cover: Caroline Bruinsma
Dear PhD student,

Welcome to the 16th Annual Meeting of PhD-students of the Graduate School Neurosciences Amsterdam Rotterdam at Woudschoten Conference Center in Zeist. This year’s meeting is organized in collaboration with the Rudolf Magnus Institute of Neuroscience in Utrecht.

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 1st and 2nd year will present their work as a poster, PhD-students in their 3rd year will present a blitz-presentation in addition to a poster, and PhD-students in their 4th year will give an oral presentation.

The two-day program includes research topics on both fundamental and clinical neuroscience. 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 Rogier Poorthuis, will then decide which poster presenter will win the award. The best blitz-presentation and the best oral presentation will be chosen by the audience. Prizes will be awarded on Friday. We are pleased that Tim Tully will give the Swammerdam Lecture on Thursday afternoon. Dr. Tully has studied the genetic basis of memory for his entire career. Dr. Tully discovered that a gene called CREB plays an important role in regulating the conversion of short-term memory to long-term memory. He was a professor at Cold Spring Harbor Laboratory until he became Chief Scientific Officer of Dart Neuroscience LLC in 2007. He also is an Adjunct Professor at Tsinghua University in Beijing China and at the National Tsing Hua University in Hsin Chu Taiwan. It is a great honour to have him as a speaker at the 2009 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 meeting in Woudschoten will give you a scientifically satisfactory exchange as well as a pleasant stay.

The organizing committee:

Caroline Bruinsma  Dept Neuroscience, ErasmusMC, Rotterdam
Felisa van Hasselt  Center for NeuroScience, SILS, UvA, Amsterdam
Erika van Hell  RMI, Utrecht
Elly Hol  NIN, Amsterdam
Diane Nijholt  Dept. Neurogenetics, AMC, Amsterdam
Marie Orre  NIN, Amsterdam
Jeroen Pasterkamp  RMI, Utrecht
Evelien Platje  Dept. Child- and Adolescent Psychiatry, VUmc, Amsterdam
Zsuzsika Sjoerds  Dept. Psychiatry, VUmc, Amsterdam
Nelleke Verhave  TNO, Rijswijk, and Dept. MCN, CNCR, VU, Amsterdam
Jelte Wouda  Dept. Anatomy and Neuroscience, CNCR, VUmc, Amsterdam
Esther van der Zwaal  RMI, Utrecht

Els Borghols  ONWAR, Amsterdam
Program Annual Meeting 2009  
Thursday, 19 November

<table>
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<tr>
<th>Time</th>
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<tr>
<td>09:00 – 09:45</td>
<td>Registration/ coffee and tea</td>
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<td>09:45 – 10:00</td>
<td>Words of welcome: Jelte Wouda</td>
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<td><strong>Didactic comments:</strong> Chris de Zeeuw</td>
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<td>10:00 – 11:15</td>
<td><strong>Session 1: Development, degeneration and regeneration</strong> Chair: Jeroen Pasterkamp</td>
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<td>Laura Smit-Rigter</td>
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<td>Prental fluoxetine exposure causes life-long abnormalities in cortical development</td>
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<td>Kasper Roet</td>
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<td>Identification of novel glial proteins with the capacity to stimulate the extension of neurites in vitro</td>
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<td>Myrrhe van Spronsen</td>
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<td>Goldberg Shprintzen Syndrome resolved: KBP inhibits kinesin-dependent cargo trafficking</td>
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<td>Marleen Sta</td>
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<td>Innate and adaptive immunity in amyotrophic lateral sclerosis (ALS): evidence of complement activation</td>
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<td>11:15 – 11:30</td>
<td>Coffee and tea</td>
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<td>11:30 – 13:00</td>
<td><strong>Session 2: Genetic Models</strong> Chair: Martien Kas</td>
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<td>Nutabi Camargo</td>
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<td>Deletion of SCAP in astrocytes: the implication of compromised lipid metabolism in the mouse brain</td>
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<td>Hemi Malkki</td>
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<td>Genetic background of the behavioural traits relevant for operant learning in mice</td>
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<td>Jean-Pierre Sommeier</td>
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<td>The role of the GABA receptor alpha 1 subunit in Ocular Dominance Plasticity</td>
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<td>Ofir Betsalel</td>
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<td>Novel variations and pathogenic mutations in the SLC6A8 gene: validation of splice prediction tools</td>
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<td>Sabine Schmitz</td>
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<td>Role of Cyclin-Dependent Kinase 5 Phosphorylation of Munc18 in synaptic transmission</td>
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<td>13:00 – 14:00</td>
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<td>Blitz Session I</td>
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<td>Poster Session I / Printers market</td>
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<td>15:45 – 17:00</td>
<td><strong>Session 3: Parkinson's disease</strong> Chair: Peter Burbach</td>
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<td>Simone van den Berge</td>
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<td>Neurogenesis in the Parkinson's disease brain</td>
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<td>Hayriye Cagnan</td>
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<td>Frequency Selectivity of a Thalamocortical Relay Neuron</td>
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<td>Joanna Korecka</td>
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<td>Differentiated SH-SY5Y cells as a good in vitro model for Parkinson disease studies</td>
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<td>Nelleke Verhave</td>
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<td>Evaluation of Riluzole treatment in a marmoset MPTP model for early Parkinson's disease</td>
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<td>17:00 – 17:30</td>
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<td>17:30 – 17:45</td>
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<tr>
<td>17:45 – 18:45</td>
<td><strong>Swammerdam Lecture by Dr. Tim Tully</strong> (CSO of Dart Neuroscience LLC)</td>
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<td>Enhancing memory</td>
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<td>19:00 – 20:30</td>
<td>Dinner</td>
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<td>20:30 – 21:30</td>
<td>Scientific Bingo</td>
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Program Annual Meeting 2009

Friday, 20 November

08:00 – 09:00 Breakfast

Didactic comments: Matthijs Verhage

09:00 – 10:15 Session 4: Electrophysiology Chair: Cyriel Pennartz

Hans-Rüdiger Geis
Frequency response areas of neighboring neurons in the mouse dorsal inferior colliculus studied with double in vivo whole-cell recordings
Felisa van Hasselt
Programming effects of individual within-litter maternal care on gene expression, function and structure in the adult rat hippocampus
Jasper Poort
The role of Attention in Figure-Ground segregation in V1 and V4
Margreet Ridder
MLC1 is involved in brain water homeostasis and is associated with chloride channel activity

10:15 – 10:45 Coffee and tea

10:45 – 12:15 Session 5: Imaging Chair: Dick Veltman

Hans Buiter
[11C]AF150(S), an agonist pet ligand for in vivo imaging of the M1 muscarinic acetylcholine receptor
Oswald Bloemen
White matter markers for psychosis in a prospective ultra high risk cohort
Rana Al Hussainy
[18F] labelled fluoromethylnorbrnynl WAY. A new radiopharmaceutcal to visualize the 5-HT1A receptor
Willem Huijbers
The relation between the brain’s default mode network, memory, and attention: A combined resting-state/task-based fMRI study
Sebastian Schagen
The effects of puberty suppression and the consecutive addition of cross-sex hormones on brain activation during mental rotation in transsexual adolescents: an fMRI study

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: Sleep and Behavior Chair: Tommy Pattij

Nico Romeijn
Sensing the future: skin temperature predicts lapses in vigilance
Cathalijn Leenaars
The effects of 12 hours of full sleep deprivation by means of mild forced locomotion on a novel switch-task paradigm
Priyanka Rao
Role of AMPA receptor trafficking in extinction of conditioned contextual fear of mice
Jelte Wouda
Long-term effects of chronic and binge-like alcohol exposure during adolescence on motivational and cognitive behavior

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

16:30 – 16:45 Closing remarks (Esther van der Zwaal)
Federation of European Neuroscience Societies presents
FENS-Forum Socials:

**JUMP the FENS**

The Amsterdam Version
International PhD-Student PARTY!

July 5th 2010, Club MELKWEG

BE THERE!!!

More info: jumpthefens2010@gmail.com
www.jumpthefens.eu

FENS-Forum: 3-7 JULY 2010,
RAI Congress Centre Amsterdam, The Netherlands
Blitz Session I 19 November, 14:00 – 14:30
Ruth van Holst
Henrique Cabral
Thaís Rizzi
Rebecca Schutte
Yasmin Namavar
Yael Reijmer
Katja Ritz
Sandra van der Salm
Ling Shan
Laurens Witter
Linda Holtman
Albertine Scheltema Beduin
Pieter van Bokhoven
Marise Machielsen
Martijn Wokke
Natalia Goriounova
Qiluan Schaafsma-Zhao
Asiya Giniatullina

Blitz Session II 20 November, 13:15 – 13:45
Joost Wiskerke
Erika van Hell
Patricia Klemmer
Jiun Youn
Diana Nijholt
Jolanda Prins
Xin Qiao
Evelien Platje
Mirjam van Tricht
Jochem Cornelis
Jorrit van Asselt
Pieter Goltstein
Fleur Zeldenrust
Rhea van de Bospoort
Juliane Lauks
Evert-Jan Kooi
Jeroen Melief
Marjan Steenweg
Maarten Witte
Poster Session I  
19 November, 14:30 – 15:45

Group: A (Addiction 1)
1) Ruth van Holst  
Cognitive flexibility in alcohol and gambling addiction
2) Nienke Broos-Boersma  
Stable impulsive decision making predicts extinction resistance and cue-induced relapse of cocaine self-administration
3) Ping Gao  
Immediate early gene expression in cocaine addiction
4) Zsuzsika Sjoerds  
The hijacked brain; an fMRI study on the neural correlates of habit formation in alcohol dependency

Group: C (Cognition 1)
9) Henrique Cabral  
Coding of trajectories in hippocampal CA1 place cells in normal and NMDA NR-1 mutant mice
10) Thais Rizzi  
Fatty Acids and intelligence
11) Rebecca Schutte  
Sleep and Daytime Functioning in Childhood – a Meta-Analysis
12) Sandra Cornelisse  
Psychosocial stress enhances emotional long-term memory and working memory in men, but not in women
13) Giovanni Piantoni  
Reactivation during sleep of recently acquired memory

Group: E (Degeneration 1)
19) Yasmin Namavar  
tRNA Splicing Endonuclease mutations cause Pontocerebellar Hypoplasia
20) Anna Carrano  
Neuropathological characteristics of capillary cerebral amyloid angiopathy with dyshoric changes
21) Ewout Groen  
FUS mutations in familial ALS in The Netherlands
22) Jinte Middeldorp  
GFAP splice variant expression in astrocytes is associated with amyloid plaques in Alzheimer’s disease

Group: F (Degeneration 2)
23) Yael Reijmer  
MRI correlates of cognitive decline in type 2 diabetes
24) Lyn Setchell  
Development of C-11-labelled agents for in vivo detection of tissue transglutaminase activity
25) Marie Orre  
Proteasomal inhibition by Aβ in astrocytes in Alzheimer’s disease
26) Robin Verhaar  
Tissue transglutaminase (tTG) and neurodegeneration: added value of a cell based assay for selection of effective inhibitors in preclinical development
27) Marlene Végh  
Aging in the central nervous system of DNAge mouse models

Group: H (Motor systems)
33) Katja Ritz  
Alternative splicing of epsilon-sarcoglycan in Myoclonus-Dystonia
34) Sandra van der Salm  
Voluntary motor preparation in tics and psychogenic movement disorders
35) Ling Shan  
Histamine Production in Parkinson’s Disease Brain: relationship with Lewy bodies and gender
36) Laurens Witter  
Differential olivo-cerebellar cortical control of rebound activity in the cerebellar nuclei
37) Justus Groen  
THAP1 mutations in focal dystonia
38) Froukje de Vries  
Amphetamine induced striatal dopamine release in Tourette’s syndrome using positron emission tomography
Group: I (Pharmacology 1)

39) Linda Holtman  
**Effects of cox-2 inhibition with SC-58236 on epileptogenesis, seizures and antiepileptic drug therapy with phenytoin**

40) Albertine Scheltema Beduin  
**Rocksan: clozapine versus olanzapine in patients with schizophrenia and substance abuse**

41) Elodie Girault  
**Acute intragastric administration of the antipsychotic olanzapine increases the plasma glucose concentration and induces hepatic insulin resistance in male rats**

42) Lianne Schmaal  
**Neural substrates of impulsivity in addiction and the effects of pharmacological challenges**

43) Joost Verbeek  

Group: K (Psychiatric disorders 1)

49) Pieter van Bokhoven  
**Social defeat stress and subsequent antidepressant or behavioral therapy: a hippocampal focus**

50) Marise Machielsen  
**Cannabis use in patients with a psychotic disorder and patients at Ultra High Risk of psychosis: impact on psychotic- and pre-psychotic symptoms**

51) Anouk den Braber  
**A DTI study of monozygotic twins discordant for obsessive-compulsive symptoms**

52) Xin-nui Qi  
**Expression of stress-related genes in the human prefrontal cortex in mood disorders**

53) Stella de Wit  
**Emotion regulation in obsessive-compulsive disorder patients and healthy controls: preliminary data**

Group: M (Receptors 1)

60) Cleo Crunelle  
**Varenicline increases striatal D2/3 receptor binding in rats**

61) Elsmarieke van de Giessen  
**Striatal dopamine D2 receptor availability in diet induced obese rats: developing an obesity model**

62) Martine Groen  
**Modulation of calcium spikes by metabotropic glutamate receptors**

63) Marijn Kuipers  
**Mechanisms of AMPA receptor trafficking in hippocampal neurons**

64) Qian Wang  
**Glucocorticoid receptor expression in the human hippocampus**

Group: O (Sensory system 1)

70) Martijn Wokke  
**Figure-ground signals in early and object specific visual areas: A combined fMRI, EEG and TMS study**

71) Jeroen Bos  
**Touch and See, multisensory integration**

72) Zimbo Boudewijns  
**Axonal projections of Layer 5 neurons in primary somatosensory cortex: an in vivo labeling study**

73) Rubén Saavedra  
**A study on the phagocytic properties of Olfactory Ensheathing Glia cells. Is the olfactory ensheathing glia cell a professional phagocyte?**

Group: Q (Signalling 1)

79) Natalia Goriounova  
**Adolescent nicotine exposure induces lasting changes in presynaptic mGluR2 function in excitatory synapses in prefrontal cortex**

80) Qiluan Schaafsma-Zhao  
**Cellular Endocannabinoid signalling in the rat prefrontal cortex**

81) Asiya Giniatullina  
**Biochemical methods for studying regulation of membrane fusion**

82) Rocío Diez Arazola  
**The calcium-binding protein DOC2B mediates spontaneous neurotransmitter release**

83) Marieke Meijer  
**Munc18-1 binding to the SNARE complex in not a prerequisite for synaptic vesicle fusion**
Group: B (Addiction 2)
5) Joost Wiskerke
An important role for mesolimbic mu-opioid receptors in amphetamine-induced inhibitory control deficits
6) Janna Cousijn
Craving modulates brain responses to cannabis cues
7) Bart Lubbers
Characterization of brevican+/- mice as a model for relapse vulnerability
8) Rogier Poorthuis
Nicotine effects neuronal network functioning in multiple layers of the prefrontal cortex through desensitization

Group: D (Cognition 2)
14) Erika van Hell
The acute effects of THC on brain activity during working memory
15) Patricia Klemmer
Quantitative synaptic proteomics in a mouse model of Fragile X Syndrome
16) Jiun Youn
Determination of substrain differences between C57BL/6J and C57BL/6N mice in behavioral flexibility using two spatial learning tasks
17) Tara Arbab
Hippocampal codes of space and episodic memory in a mouse model of Fragile-X mental retardation
18) Guilherme Testa Silva
Synaptic properties, dynamics and connectivity in the Fmr1-KO mouse: a model for Fragile X mental retardation

Group: G (Degeneration 3)
28) Diana Nijholt
Endoplasmic reticulum stress in Alzheimer’s disease: Effects on cellular proteolysis
29) Hyung Elfrink
The function of Rab6 during ER stress and Alzheimer’s disease
30) Elizabeth Moloney
Towards using neuropilin-1 receptor-bodies as scavengers to neutralise semaphorin 3A function in ALS
31) Kerstin Wirz
Functional characterization of target genes involved in Alzheimer’s disease development and progression
32) Simon-Shlomo Poil
The Neurophysiological Biomarker Toolbox for Pre-clinical EEG/MEG research

Group: J (Pharmacology 2)
44) Jolanda Prins
The triple reuptake inhibitor DOV216,303, a putative new antidepressant, decreases ICSS thresholds without producing withdrawal effects
45) Xin Qiao
Anti-epileptic drugs bind to α-subunits of voltage-gated Na+ channels with different binding kinetics
46) Tessa Douma
Differential effects of mood stabilizers on prepulse inhibition deficits in mice with corticotrophin-releasing factor over-expression
47) Anne Klomp
Fluoxetine and the developing brain: a pharmacological MRI study in rats
48) Anouk van Loon
The effect of a pharmacological intervention on recurrent processing

Group: L (Psychiatric disorders 2)
54) Evelien Platje
Neurobiological correlates of Disruptive Behaviour Disorder in a normal population; differences between boys and girls
55) Mirjam van Tricht
Reduced Parietal P300 preceding a first psychotic episode
56) Addy van Dijk
Deep brain stimulation in Sapap3-mutant mice: an animal model for OCD
57) Eva Verbeek
Resequencing of candidate genes for major depressive disorder
58) Saskia Woudstra  
Analysis plan for the association of the PCLO SNP with functional neuroimaging data

59) Hetty Boleij  
Judgment bias in a mouse model for "pathological anxiety"

**Group: N (Receptors 2)**

65) Jochem Cornelis  
The neuronal regenerating role of PPAR-γ

66) Marlies Oostland  
The role of the 5-HT3 receptor in the postnatal development of the cerebellum

67) Roxanna Volcu  
Feed-forward and feed-back connections target different glutamate receptor configurations in macaque primary visual cortex

68) Emanuele Zurolo  
Cannabinoid receptors, CB1 and CB2, expression during human cortical development and in epileptogenic developmental pathologies

69) Nils Zuiderveen Borgesius  
A non-enzymatic role of CaMKIIβ in hippocampal synaptic plasticity

**Group: P (Sensory system 2)**

74) Jorrit van Asselt  
Characterization of Zebrafish Horizontal Cell

75) Pieter Goltstein  
Orientation and motion tuning in the mouse visual cortex after visual conditioning: an in vivo 2-photon imaging study

76) Henrique Alves  
Functional analysis of Crumbs proteins in retina and brain

77) Timo van Kerkoerle  
Multiple short-term memory signals in monkey primary visual cortex

78) Manon Schaap  
Development of a rat model to study the emotional component of pain using the somatosensory-evoked potential

**Group: R (Signalling 2)**

84) Fleur Zeldenrust  
Two forms of feedback inhibition determine the dynamical state of a small hippocampal network

85) Rhea van de Bospoort  
Identifying molecular mechanisms underlying dense core vesicle release in neurons

86) Anouk Marsman  
Glutamatergic neurotransmission in schizophrenia: what happens?

87) Juan Zhao  
An imbalance of GABA and glutamate in Alzheimer Disease but not in Depression in the superior gyrus of the prefrontal cortex and anterior cingulated cortex

88) Natasha Pasricha  
Effects of corticosterone pulsatility on the excitability (mEPSCs) of granular neurons of dentate gyrus

**Group: S (Signalling 3)**

89) Juliane Lauks  
Neurobeachin: an AKAP involved in the regulation of glutamatergic receptors

90) Femke den Boon  
Cannabinoid-mediated modulation of synaptic transmission in the rat prefrontal cortex

91) Tony Cijszew  
Lifetime at the membrane: The molecular factors that influence tethering and docking of secretory vesicles at their target

92) Arthur de Jong  
Visualization of synaptic heterogeneity in hippocampal neurons

93) Daniëlle van Versendaal  
Chronic 2-photon imaging in vivo to study synaptic remodelling

**Group: T (White matter)**

94) Evert-Jan Kooi  
Lesion-independent hippocampal pathology in multiple sclerosis: markers for cognitive decline?
95) Jeroen Melief
Characterization of human post mortem microglia isolated by FACS sorting

96) Marjan Steenweg
MRI pattern recognition in defined hypomyelinating disorders

97) Maarten Witte
Mitochondrial alterations in MS brains

98) Laura van Berge
Cell type specific differences in mRNA splicing as potential explanation for neural specificity of symptoms in LBSL

99) Mark Mizee
MDR efflux transporter expression in Multiple Sclerosis lesions
F-18 LABELLED FLUOROMETHYL-NORBORNYL-WAY. A NEW RADIOPHARMACEUTICAL TO VISUALIZE THE 5-HT1A RECEPTOR

Rana Al Hussainy, J. Verbeek, H.J.C. Buiter, R.P. Klok, J.D.M. Herscheid

Department of Nuclear Medicine & PET Research, VU University medical center, Amsterdam

ABSTRACT

Objectives: In the last few years, we suggested that problems such as defluorination of radiopharmaceuticals can be solved by having the radiolabel on a bridgehead position of e.g. the cubyl moiety, to prevent E2-elimination of HF. In vivo investigations showed that [18F]CH2F-cubyl-WAY binds to the HT1A receptor in rat brain. Unfortunately, the same studies also indicated some defluorination, presumably as a result of the pseudo-aromatic character of cubane. Furthermore, the precursor of this compound was unstable and has to be stored in a refrigerator. For these reasons, we approach to synthesize another WAY derivative; [18F]CH2F-norboryl-WAY. Interestingly, initial in vitro screening to investigate the binding affinity showed that CH2F-cubyl-WAY and CH2F-norboryl-WAY were having almost the same Kd-value. Unlike the precursor of CH2F-cubyl-WAY, the precursor of this novel compound seems to be stable at room-temperature.

Methods: Saponification of (1) with KOH in methanol gave (2) in 60% yield. Treatment with SOCl2 in dry acetonitrile to give the acid chloride and subsequently adding WAY100634 and NEt3 gave (3) in 90% yield. Reduction of (3) with LiBH4 in 1,2-dimethoxyethane followed by a reaction with tosylchloride in dichloromethane gave (4) in 45% overall yield. Finally, a straightforward radiofluorination of (4) was done under standard fluorination conditions in dry acetonitrile for 30 minutes. Separation from the precursor was easily preformed using prep HPLC yielding a (radio)chemically pure product in a radiochemical yield of over 22%.

Biodistribution; Four male wister rats received an injection of 15MBq(300µL) of this [18F]fluoromethylbicyclo[2.2.1]heptyl-WAY, in the tail vein. Rats were sacrificed 45 min post injection. Several tissues and distinct brain regions were dissected, weighed and counted for radioactivity.

Results: In vivo biodistribution studies revealed that this [18F]CH2F-norboryl-WAY has a relatively high uptake in the ROI of the rat brain (hippocampus and cortex). The ratio’s of tissue of interest/cerebellum at 45 minutes were: Striatum/ Cerebellum = 1.31, OccCortex/ Cerebellum = 1.80, and Hippocampus/ Cerebellum = 5.56. We also found that [18F]CH2F-norboryl-WAY has a higher brain uptake and less liver and kidney uptake compared to [18F]CH2F-cubyl-WAY. Furthermore, no abnormal bone uptake was detected.

Conclusions: Radiofluorinated norboryl-WAY is easily accessible and has a stable carbon-radioisotope bond. Further studies using PET-imaging are planned to confirm our findings. Finally, this study showed that [18F]CH2F-norboryl-WAY is a promising radiopharmaceutical to visualize the 5-HT1A receptor.

KEY WORDS
Norboryl-WAY, cubyl WAY, 5-HT1A, fluorination

TELEPHONE-NUMBER: 020-4449704
E-MAIL-ADDRESS: r.alhussainy@vumc.nl
FUNCTIONAL ANALYSIS OF CRUMBS PROTEINS IN RETINA AND BRAIN

C. Henrique Alves, B. Park, A. Sanz Sanz, J. Wijnholds

Department of Neuromedical Genetics, Netherlands Institute for Neuroscience, Amsterdam

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. The gene is necessary for the production of a functional CRB1 protein of which the functions are not well known. Making use of mice that lack CRB1 we could show that a) CRB1 resides in a region, called the subapical region, or SAR, adjacent to adherens junctions between Müller glia cells and photoreceptors, b) CRB1 localizes specifically at the SAR of Müller glia cells but not of photoreceptors, c) CRB1 is required to maintain adhesion between Müller glia cells and photoreceptors, d) family members CRB2 and CRB3 reside at the SAR of Müller glia cells as well as photoreceptors. 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 created Crb2 conditional knockout mice. Using recombineering in bacterial artificial chromosomes and CRE/loxP technology a conditional gene targeting construct for Crb2 was successfully generated. The Crb2 gene is expressed from 2 different promoters that are far apart, therefore, the Crb2 targeting vector was designed to flank the last four coding exons with loxP sites. The targeting construct also contained a neomycin resistance cassette, flanked by frt recombination sites, in an intron. The targeting vector was used to generate Crb2flox/wt mouse embryonic stem (ES) cells by homologous recombination. The Crb2flox/wt conditional knockout mice were generated by blastocyst injections of Crb2flox/wt ES cells. Chimaeric mice gave germ line transmission, where after the neomycin cassette was successfully removed by crossing the Crb2flox/wt mice with a transgenic mouse that expressed FLP recombinase in the germ line. The conditional knockout mice were crossed with Chx10-cre transgenic mice expressing CRE in a mosaic pattern in all cell types of the retina; with Rx3-cre transgenic mice expressing CRE in a field of cells from which the retina and the ventral hypothalamus develop; and with Emx1-cre transgenic mice in which the expression of CRE is restricted to the neurons in the developing and adult cerebral cortex and hippocampus. Furthermore, triple mutant Crb1-/-;Crb2flox/flox;Chx10-Cre/+ and Crb1-/-;Crb2flox/flox;Rx-Cre/+ mice are being bred for future analysis of overlapping and unique functions for CRB2 and CRB1 in the retina. The analysis of Crb2flox/flox;Chx10-Cre/+, Crb2flox/flox;Rx-Cre/+, Crb2flox/flox;Emx1-Cre/+ mice and their controls started recently. In addition, we are setting-up Crb2 gene silencing protocols to study the effect of reducing CRB2 levels in retina and brain.

In summary, Crb2 mutant mice were generated and are ready to study the functions of Crumbs proteins in retina and brain.

KEY WORDS
Leber congenital amaurosis, Crumbs homolog1, conditional knockout

TELEPHONE-NUMBER: 020-5664687
E-MAIL-ADDRESS: h.alves@nin.knaw.nl
Fragile X syndrome (FRX) is the most common form of inherited mental retardation (Bagni and Greenough, 2005, Nat Rev Neurosci 6, 376-387). Its symptoms include low IQ test scores, learning difficulties, epilepsy, attention-deficit, autism, and hyperactivity. FRX is caused by a mutation that silences the Fragile X Mental Retardation 1 gene (Fmr1), which lies on the X-chromosome (Verkerk et al., 1991, Cell 65, 905-914). The mutation gives the X-chromosome a "fragile" appearance, hence the name.

A mouse model for this condition has been developed, in which the Fmr1 gene is knocked out (Fmr1-KO) (Mientjes et al., 2006, Neurobiol Dis 21, 549-555). Mice with genetic mutations regarding the Fragile X mental retardation gene differ from normal mice under several neurophysiological aspects, such as altered hippocampal and prefrontal signaling and plasticity. Impaired spatial learning and deficits in reversal learning have been reported in these animals. On the other hand, nothing is known about how the neural networks of the hippocampus are affected by the mutation.

A hippocampal place cell is a neuron that fires strongly when the animal is at a specific location of the environment that corresponds to the cell's place field. The ensemble of place cells in the hippocampus provides a full representation of the environment. As environmental conditions change, hippocampal spatial representation is correspondingly modified: with experience in the new environment, hippocampal neurons undergo synaptic modification, (re)acquiring stable place fields that represent the environment. Coordination of place cell firing and the refinement of place fields are therefore believed to be essential for spatial learning.

This study will focus on how Fmr1-KO mice use spatial information of (visual cues in) their surroundings to determine their location within the environment (place cell activity), and how this spatial representation is changed when the spatial cues are altered. To investigate this point, we will implant control and Fmr-1 mice with an electrophysiological device capable of simultaneously record tens of hippocampal neurons, while the animal moves freely through an enclosed environment. Hippocampal place cell behavior will be tested as the environment changes. As under these conditions normal animals display re-organization of the hippocampal neural code, the way this code is affected by the Fmr-1 mutation will possibly provide a link between the genetic aspect of the disease and its cognitive and behavioral phenotype.

**KEY WORDS**
Fragile X, hippocampus, in vivo mouse physiology

**TELEPHONE-NUMBER:** 020-5258372 / 06-21598150

**E-MAIL-ADDRESS:** t.arbab@uva.nl
CHARACTERIZATION OF ZEBRAFISH HORIZONTAL CELL CONNEXINS

Authors
Jorrit B. van Asselt, J. Klooster, Y. Claassen, M. Kamermans

Department/Institute
Netherlands Institute for Neuroscience, Amsterdam

Abstract
Introduction
Connexins play a major role in horizontal cell (HC) function, both as gap junctions for cell-cell communication and as hemichannels in a feedback pathway from HCs to cones. Eastman et al. (2006) showed that four Cxs (Cx52.6, Cx52.7, Cx52.9 and Cx55.5) form a genetically homologous group of proteins. Previously, it was shown that both Cx52.6 and Cx55.5 were localized in HCs of zebrafish, and that Cx52.6 reduced its conductance with hyperpolarization (Bruzzone), whereas Cx55.5 had a large, non-voltage dependent conductance (Shields et al., 2007). The present study addresses the question whether 1) the Cx52.7 and Cx52.9 are also localized in HCs, and 2) what their current/voltage relations were.

Methods
Antibodies were raised against Cx52.7 and Cx52.9, and immunohistochemical (IHC) techniques were applied on the zebrafish retina. In addition, two electrode voltage clamp (TEVC) experiments were performed on Xenopus laevis oocytes injected with RNA coding for either Cx52.6, Cx52.7, Cx52.9 or Cx55.5.

Results
Double labeling experiments show that all connexins potentially co-localize. The TEVC-experiments show that these four connexins form functional hemichannels when individually expressed in oocytes. Analysis of current/voltage relations of these four different Cx hemichannels indicate different electrophysiological characteristics for each Cx.

Discussion
Although these four Cxs are genetically highly homologous, the different localization and different electrophysiological properties together imply specific and most likely different functions for each of these Cxs in the HCs. Expressing four different connexins in one cell type indicates a great need for diversity in Cxs by HCs. Possibly, different Cxs are needed for a very fine level of control over the tuning of 1) the gap junctions for cell-cell communication, and/or 2) feedback pathway from horizontal cells to cones.

Keywords
Retina, connexins, hemichannels, zebrafish

Telephone-Number: 020-5664422
E-mail-Address: j.van.asselt@nin.knaw.nl
Leukoencephalopathy with brainstem and spinal cord involvement and elevated lactate (LBSL) is a childhood white matter disorder clinically characterized by slowly progressive signs of pyramidal, cerebellar and dorsal column dysfunction. MRI shows inhomogeneous signal abnormalities in the cerebral white matter and selective involvement of specific brain stem and spinal cord tracts. LBSL is caused by mutations in the gene \textit{DARS2}, which encodes mitochondrial aspartyl-tRNA synthetase. This protein is necessary in every cell for protein synthesis in mitochondria. The selective involvement of specific white matter tracts in LBSL is therefore striking. All patients are compound heterozygous for two mutations in \textit{DARS2} and almost all patients have one mutation affecting the splicing of exon 3. Constructs of \textit{DARS2} were made that upon transfection into cells allowed the quantification of the splicing of exon 3. These constructs were transfected into cell types of various origin, neural and non-neural. The effect of the mutations on exon 3 splicing differed between cell types. The mutations had a larger effect in cells from neural origin, especially in neuronal cell types. This could partly explain the selective vulnerability of white matter tracts in LBSL patients.
NEUROGENESIS IN THE PARKINSON'S DISEASE BRAIN

Simone A. van den Berge1, Lieneke Kooijman1, C. Eleana Zhang1, Machiel R. Zandvliet1, Wilma D.J. van de Berg2, Elly M. Hol1

1 Astrocyte Biology & Neurodegeneration, Netherlands Institute for Neuroscience, Amsterdam
2 Dept. of Pathology, VU University medical center, Amsterdam

The adult human brain contains two main regions where constitutive neurogenesis takes place, the subgranular zone and the subventricular zone (SVZ). In the SVZ, neural stem cells proliferate to produce fast-dividing neural progenitor cells, which migrate through the rostral migratory stream and differentiate to form new inter-neurons in the olfactory bulb. Studies in both rodent and human brain provide evidence that a subgroup of astrocytes, lining the ventricles, is the stem cell pool in the SVZ.

In our group, we have found a specific marker for the neurogenic astrocytes, GFAP-delta. Our aim is to study these neurogenic astrocytes in Parkinson’s disease (PD), since in previous studies it has been shown that dopamine depletion of the striatum, such as in PD, may lead to a decrease in neurogenesis.

We studied the expression of the proliferation marker PCNA and of GFAP-delta by immunohistochemistry in the caudate nucleus of incidental and sporadic PD patients and of age and sex-matched controls. The number of proliferating cells and neurogenic astrocytes will be correlated to dopaminergic cell death in the substantia nigra of these patients. Also, we have analyzed GFAP-delta expression in the olfactory bulb of PD patients and controls. In addition, we aim to replicate in vitro experiments modulating dopamine in neurosphere cultures. Such studies have been performed in rodent cultures only and need further validation in human neural stem cell cultures. For this purpose, we will use neurosphere cultures from post mortem human brain, which were set up in our lab. This project will help us understand the proliferative capacity of neural stem cells in Parkinson’s disease, which may be very important for repair strategies in this disease.

References
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Human brain material provided by the Netherlands Brain Bank

KEY WORDS
Parkinson’s disease, neural stem cells, astrocytes, subventricular zone

TELEPHONE-NUMBER: 020-5665508
E-MAIL-ADDRESS: s.van.d.berge@nin.knaw.nl
NOVEL VARIATIONS AND PATHOGENIC MUTATIONS IN THE SLC6A8 GENE: VALIDATION OF SPLICE PREDICTION TOOLS

AUTHORS
Ofir T. Betsalel, E.H. Rosenberg, L.S. Almeida, C. Jakobs, G.S. Salomons

DEPARTMENT/INSTITUTE
Metabolic Unit, Clinical Chemistry, VU University medical center, Amsterdam

ABSTRACT
Since the identification of the X-linked creatine transporter defect in 2001 we detected in the SLC6A8 gene 14 synonymous variants and 48 unclassified variants (UVs) located outside the open reading frame. We explored the use of prediction tools of splice-site and exonic splice enhancers (ESE) to assess putative effects of given UVs by validating these tools on the wildtype sequence and 18 UVs that we stratified as (non) disease causing by cDNA analysis (n=13), and/or by the presence of the UVs in unaffected male controls (n=5). Secondly, these tools were applied on 10 (including 6 novel) splice error mutations and 44 (including 29 novel) UVs. Seven of these variants were selected for further research. Four were classified as pathogenic as three of these were predicted to have a splice effect by at least four splice tools. Surprisingly the fourth pathogenic mutation was suggested to have a small deleterious effect by one splice tool only, but at the cDNA level erroneous splicing was detected. Three other variants were classified as non disease causing variants since SLC6A8 deficient fibroblasts showed no splicing errors, when transiently transfected with minigenes with a partial SLC6A8 segment containing the variant.
All SLC6A8 variants are included in a newly being developed LOVD DNA variation database (http://www.LOVV.nl/SLC6A8). In conclusion, predictor tools can be used to select UVs for further research, but for final conclusions as to the disease causing nature, usually further laboratory studies are warranted.

KEY WORDS
SLC6A8, splicing, ESE, LOVD

TELEPHONE-NUMBER: 020-4442418
E-MAIL-ADDRESS: o.betsalel@vumc.nl
WHITE MATTER MARKERS FOR PSYCHOSIS IN A PROSPECTIVE ULTRA HIGH RISK COHORT


Department of Psychiatry, Academic Medical Center, Amsterdam

Background
Subjects at “Ultra High Risk” (UHR) for developing psychosis have differences in white matter (WM) compared to healthy controls. WM integrity has not yet been investigated in UHR subjects in relation to the development of subsequent psychosis. Hence we investigated a prospective cohort of UHR subjects and compared whole brain fractional anisotropy (FA) of those who would later develop psychosis (UHR-P) to those who would not (UHR-NP).

Methods
We recruited 37 subjects who fulfilled UHR criteria, and 10 healthy controls. 3 Tesla MRI scans and PANSS ratings were obtained at baseline. UHR subjects were assessed at 9, 18 and 24 months for development of frank psychosis. We compared baseline FA of UHR-P to controls and UHR-NP subjects. Furthermore, we related clinical data to MRI outcome in the patient population.

Results
Of the 37 UHR subjects, 10 had transition to psychosis. UHR-P subjects showed significantly lower FA values than control subjects in medial frontal lobes bilaterally. UHR-P subjects had lower FA values than UHR-NP subjects, lateral to the right putamen and in the left superior temporal lobe. UHR-P subjects showed higher FA values, compared to UHR-NP, in the left medial temporal lobe. In UHR-P, positive PANSS negatively correlated to FA in the left middle temporal lobe. In the total UHR group positive PANSS negatively correlated to FA in the right superior temporal lobe.

Conclusions
UHR subjects who later develop psychosis have differences in WM integrity, compared to UHR subjects who do not develop psychosis and to healthy controls, in brain areas associated with schizophrenia.

KEY WORDS
UHR, schizophrenia, transition, DT-MRI, white matter, FA

TELEPHONE NUMBER: 06-24148589
E-MAIL-ADDRESS: o.j.n.bloemen@amc.nl
SOCIAL DEFEAT STRESS AND SUBSEQUENT ANTIDEPRESSANT OR BEHAVIORAL THERAPY: A HIPPOCAMPAL FOCUS

Pieter van Bokhoven, J.E. van der Harst, T.S. Heistek, P.J. Lucassen, W.J.G. Hoogendijk, A.B. Smit, S. Spijker

1 Dept. of Molecular & Cellular Neurobiology, 3 Dept. of Experimental Neurophysiology and 5 Dept. of Psychiatry, Center for Neurogenomics & Cognitive Research, VU University and VU University medical center, Amsterdam, 2 Deltaphenomics B.V., Wageningen, 4 SILS Centre for Neuroscience, University of Amsterdam, Amsterdam

Altered hippocampal function has been implicated in patients suffering from depression based on reduced hippocampal volume and cognitive performance in hippocampus-dependent tasks. Also, rodents show stress-induced morphologic changes in this brain region (decreased neurogenesis and neuronal atrophy). Chronic stress induced by social defeats (SD) in rat inter-male confrontations followed by subsequent long-term individual housing has been shown to produce a unique behavioral phenotype with high face validity for depression. This includes impaired cognitive behavior and reduced sensitivity to reward (anhedonia). Both phenotypes are rescued by chronic antidepressant therapy. Additionally, anhedonia is reduced by behavioral therapy (BT) consisting of regular transfer to an enriched environment. In this study, we showed that SD animals show reduced hippocampal LTP and that this was restored by antidepressant therapy (imipramine 20 mg/kg) as well as by BT. To see whether attenuated LTP was associated with a reduced neurogenesis in the dentate gyrus, quantitative stereological techniques were performed to analyze doublecortin (DCX) positive neurons as a post-mitotic marker of immature neurons. Finally, we are studying differentially expressed proteins in hippocampal synaptic membranes after social defeat stress and after subsequent antidepressant or behavioral therapy by performing quantitative iTRAQ proteomics. By correlating synaptic protein expression with observed depressive symptoms, as well as with data on synapse plasticity and cell proliferation, we aim to identify neuroplastic mechanisms that lie at the basis of depressive phenotypes and thereby reveal new potential molecular targets for drugs that can alleviate these symptoms.

Depression, stress, social defeat, antidepressants

06-41880326
pieter.van.bokhoven@cncr.vu.nl
JUDGMENT BIAS IN A MOUSE MODEL FOR “PATHOLOGICAL ANXIETY”

Hetty Boleij

Utrecht University, Faculty of Veterinary Medicine, Department of Animals in Science and Society & Rudolph Magnus Institute, Utrecht

ABSTRACT

Animal models are broadly used in preclinical research to investigate mechanisms of anxiety disorders. Notably, these models are based on biologically adaptive behaviour, i.e. normal anxiety. To obtain relevant knowledge on pathological anxiety, animal models showing pathological, i.e. non-adaptive, behaviour are needed. To identify such an animal model, we aim at characterising pathological anxiety in mice. Behavioural adaptation in animals can be assessed for example by changes in behavioural responses over time, i.e. habituation. Thus pathological anxiety, as it is non-adaptive, might be characterised as impaired behavioural habituation.

Previously we have found that mice from the inbred strain 129P3 show impaired habituation of avoidance behaviour in the modified hole board test (mHB), while mice from another inbred strain, BALB/c, show normal habituation in the same test. In addition, it was found that there was a decrease in the expression of the immediate early gene c-fos (indicating a decreased neural activity) in the lateral septum and prefrontal cortex of 129P3 mice compared with BALB/c mice. This suggests that 129P3 mice have difficulties with adequately integrating environmental stimuli with emotional processes (Salomons et al. in press). Together these results in the mHB indicate that these animals may be pathologically anxious, but further research is necessary to support this idea.

Anxiety is associated with cognitive processes that help the animal react appropriately in situations of threat. Pathological anxiety might be caused by impaired cognitive control over emotional processes. One cognitive characteristic that is known to occur in human anxiety patients and has recently also been found in animals is a so-called judgment bias; anxiety patients interpret ambiguous stimuli more negatively compared to normal controls. Accordingly, to improve the face validity of 129P3/J mice as a model for pathological anxiety we here investigate whether these mice show a more negative judgement of ambiguous stimuli in comparison with the normally anxious BALB/c mice.

In this study mice from both the BALB/c and 129P3 strains will be trained to associate one odour stimulus (CS+) with a piece of almond and another odour stimulus (CS-) with a bitter piece of almond (aversive quinine taste). After acquisition the mice are tested for their response to ambiguous odour stimuli, i.e. odour mixtures in comparison with the reaction to the pure CS+ or CS-. Judgment bias will be indicated by the latency to approach and the latency to eat the almond piece, but also other behavioural parameters are scored. It is expected that 129P3 mice have a more negative interpretation of the ambiguous stimuli and will therefore have a higher latency to approach and eat the piece of almond if ambiguous odours are presented.

KEY WORDS
Pathological anxiety, 129P3/J mice, BALB/cJ mice, cognitive bias, odour conditioning

TELEPHONE-NUMBER: 030-2534149
E-MAIL-ADDRESS: h.boleij@uu.nl
CANNABINOID-MEDIATED MODULATION OF SYNAPTIC TRANSMISSION IN THE RAT PREFRONTAL CORTEX

AUTHORS
Femke S. den Boon, T.R. Werkman, P.J.P. Chameau, W.J. Wadman

DEPARTMENT/INSTITUTE
Centre for Neuroscience-Swammerdam Institute for Life Sciences, University of Amsterdam, Amsterdam

ABSTRACT
The endocannabinoid (eCB) system plays a major role in the modulation of neurotransmission in the central nervous system. The modulatory actions of eCB’s appear to be achieved through retrograde signaling; eCBs are released from the postsynaptic cell and bind to CB receptors present on the presynaptic neuron. Biochemical imbalances in the eCB system in brain structures like the prefrontal cortex (PFC) may cause or exacerbate pathological disorders such as addiction, schizophrenia and mood disorders.

Preliminary immunohistochemical data show positive staining for cannabinoid 1 receptor (CB1R) in layers II, III and V of the prelimbic area of the rat PFC. In vitro field potential recordings performed in these layers of the prelimbic area show that the two CB1R agonists WIN-552122 and CP-55940 (0.01 µM 0.3 µM) dose dependently decrease the fEPSP amplitude. High frequency stimulation (4 trains of 100Hz stimulation) in the presence of WIN-552122, CP-55940 (0.1µM) or SR141716 (1 µM, CB1R antagonist) leads to a short term increase of the fEPSP amplitude, compared to vehicle treated slices. Together, these data suggest an important role for CB1R-mediated signaling in the PFC.

KEY WORDS
Endocannabinoids, prefrontal cortex, field potentials, high frequency stimulation

TELEPHONE-NUMBER: 020-5257642
E-MAIL-ADDRESS: f.s.denboon@uva.nl
TITLE
TOUCH AND SEE, MULTISENSORY INTEGRATION

AUTHORS
Jeroen J. Bos, M.A. Vinck, C.M.A. Pennartz

DEPARTMENT/INSTITUTE
Swammerdam Institute of Life Sciences - Cognitive Neuroscience, Cognitive & Systems Neuroscience (SILS-CNS CSN), Amsterdam

ABSTRACT
The outside world is perceived as a single dynamic multisensory percept, this despite the classical view in which all different senses are initially processed individually. This raises two basic questions; 1) How unimodal are these primary sensory areas? 2) How does the initially separated sensory information get reintegrated to form this single percept we experience?
In our current study we will address these two questions by looking into the pathway of multisensory processing. We will do simultaneous tetrode recordings in primary sensory areas (vision, V1 and touch, barrel cortex), an early sensory integration area (perirhinal cortex) and a memory region (CA1 of the hippocampus). During recordings rats will perform a sensory discrimination task in which they have to indicate if the patterns they see and feel are the same or if they are different from each other. To allow full control of the experimental conditions a new set-up was made. Initial testing indicates that the rats are able to discriminate between the different visual patterns. Another innovation is the new larger multi-tetrode drive. This new drive holds 32 tetrodes allowing us enough leads to do the recordings in four different brain areas.

KEY WORDS
Multisensory integration, vision, touch, electrophysiology

TELEPHONE-NUMBER: 020-5258373
E-MAIL-ADDRESS: j.j.bos@uva.nl
IDENTIFYING MOLECULAR MECHANISMS UNDERLYING DENSE CORE VESICLE RELEASE IN NEURONS

Rhea van de Bospoort, Sabine K. Schmitz, Ruud Toonen

Deprtment of Functional Genomics, Neuroscience Campus Amsterdam, VU University, Amsterdam

Dense core vesicles (DCVs) contain neuromodulatory peptides and hormones that play critical roles in many biological processes, ranging from neuronal survival to memory formation. In contrast to synaptic vesicles, DCV release is not restricted to the nerve terminal. Relatively little is known about the proteins involved in the targeting and capture of DCV vesicles. We have used Semaphorin 3A as a DCV marker coupled to pH-sensitive EGFP (Semaphluorin), to study DCV release in mouse hippocampal neurons using live cell imaging. To elucidate the molecular pathway and to identify potential differences with synaptic vesicle release, we focused on the role of a set of proteins (SNARE proteins, Munc13, Munc18 and SynaptotagminI) that are involved in synaptic vesicle secretion. We show that in contrast to synaptic vesicles, the SNARE protein synaptobrevin, Munc13 and Munc18 are not essential for DCV release. These results suggest a different requirement for synaptic proteins in DCV secretion compared to synaptic vesicle release.

KEY WORDS
Dense core vesicle, exocytosis, SNARE, Munc13, Munc18

TELEPHONE-NUMBER: 020-5987792
E-MAIL-ADDRESS: rhea.van.de.bospoort@cncr.vu.nl
AXONAL PROJECTIONS OF LAYER 5 NEURONS IN PRIMARY SOMATOSENSORY CORTEX: AN IN VIVO LABELING STUDY


*authors contributed equally

Department/Institute
# Department of Integrative Neurophysiology, Center for Neurogenomics and Cognitive Research, VU University, Amsterdam
$ Max-Planck institute of Neurobiology, Martinsried, Germany

ABSTRACT
Rats have the ability to actively scan objects with their whiskers, similar to the way humans use their fingertips during active sensation. Successful identification of objects through whisker movements involves processing of both touch and movement information. In the thalamus, sensory-related neurons can be found that either encodes touch, movement, or both, and these neurons are located in distinct anatomical and functional domains of the thalamus. Using anterograde tracing, we show that this segregated flow of sensory information is maintained in the projections from the thalamus to the barrel cortex. In addition, the contribution of different cell-types to processing various aspects of sensory information can also be shown physiologically in the barrel cortex. More specifically, L5B neurons are activated upon passive movement of the whiskers in anesthetised rats (resembling touch) while L5A neurons are activated during active (self-initiated) movement of the whiskers in the awake rat. Here, we addressed the question whether the functional differences in L5 are further maintained in the cortex by characterizing the intracortical projections of these L5 neurons. We found that L5A neurons show extensive axonal projections with abundant projections to L2/3. In contrast, the axonal projections of L5B neurons are less extensive and are confined to L5B with only few projections to supragranular layers. These results suggest that the segregation between touch and movement related processing remains preserved by intracortical (downstream) projections.

KEY WORDS
Barrel cortex, in vivo labelling, axonal reconstruction

TELEPHONE-NUMBER: 020-5987099
E-MAIL-ADDRESS: zimbo.boudewijns@cnrc.vu.nl
A DTI STUDY OF MONOZYGOTIC TWINS DISCORDANT FOR OBSESSIVE-COMPULSIVE SYMPTOMS

AUTHORS
Anouk den Braber¹, D. van ‘t Ent¹, D.C. Cath², D.I. Boomsma¹, M. Barysheva³, A.D. Lee³, L.C. Foland-Ross³, J.L. Stein³, P.M. Thompson³, E.J.C. de Geus¹

DEPARTMENT/INSTITUTE
¹ Department of Biological Psychology, VU University Amsterdam, Amsterdam, ² Utrecht University, Utrecht, ³ UCLA School of Medicine, Los Angeles, CA, United States

ABSTRACT
Introduction
Obsessive-compulsive disorder (OCD) affects approximately 2-3% of the population and is characterized by recurrent, persistent, and intrusive anxiety-provoking thoughts or images (obsessions) and subsequent repetitive behaviors (compulsions). A clinical diagnosis of OCD requires impairment, but OC symptoms (OCS) are highly prevalent in the general population (70-80%). Structural magnetic resonance imaging studies indicated several gray matter abnormalities in OCD patients compared to unaffected controls that overall point to a deficit in cortico-striatal-thalamo-cortical circuits. Recent diffusion tensor imaging (DTI) studies also find white matter abnormalities generally overlapping with the reported gray matter findings. However, there are still inconsistencies regarding the brain structures involved and the direction of anatomical changes. A reason for this could be the differential impact of genetic and environmental risk factors for OCD that do not necessarily lead to identical underlying neurobiological changes. Heritability for OCS ranges from 27-47% in adults and 45-65% in children. If genetic factors explain 27-65% of the variability in OCS, as much as 35-73% should be accounted for by environmental stressors. The discordant monozygotic (MZ) twin design can reveal brain differences specifically due to influences of environmental risk factors. MZ twins are genetically identical; therefore differences in behavior must reflect exposure to individual-specific environment. Here we used DTI to scan twin pairs discordant for OCS to highlight white matter brain regions linked to OCS that are particularly susceptible to environmental factors.

Methods
20 MZ twin pairs discordant for OCS (age range 18-60 years) were scanned on a 3.0 T Intera MR system (Philips, Medical Systems). DTI data were acquired (33 directions) with the following parameters: slice thickness=3mm, TR=4863 ms, TE=94 ms, matrix =256x256, FOV=230 mm, b-value=1000 s/mm². One volume without diffusion-weighting was also acquired. DTI analysis was performed using software from the Laboratory of Neuro Imaging, UCLA, including fluid registration of DTI data to a common template. Voxelwise comparison, using a paired t-test, was performed to indicate regions of significantly altered fractional anisotropy (FA) in the OCS high-scoring twins compared to their OCS low-scoring co-twins.

Results
Compared to OCS low-scoring twins, OCS high-scoring twins exhibited significantly decreased FA in right temporal lobe, right insula, right sensory motor area, left occipital lobe and left frontal lobe. Increased FA for the OCS high-scoring twins compared to their OCS low-scoring co-twins was observed in the left corpus callosum (genu) and left insula.

Conclusions
These results generally overlap with white matter abnormalities reported in previous DTI studies of OCD patients. Our findings in the MZ discordant twins suggest that the white matter changes found in OCD patients are caused in part by environmental risk factors acting independently from genetic risk factors.

KEY WORDS
Obsessive compulsive disorder, diffusion tensor imaging, fractional anisotropy, twin study

TELEPHONE-NUMBER: 020-5982723
E-MAIL-ADDRESS: a.den.braber@psy.vu.nl
STABLE IMPULSIVE DECISION MAKING PREDICTS EXTINCTION RESISTANCE AND CUE-INDUCED RELAPSE OF COCAINE SELF-ADMINISTRATION

AUTHORS
Nienke Broos, Anton N.M. Schoffelmeer, Leontien Diergaarde, Tommy Pattij*, Taco J. de Vries*
* contributed equally

DEPARTMENT/INSTITUTE
Department of Anatomy and Neurosciences, VU University medical center, Neuroscience Campus Amsterdam, Amsterdam

ABSTRACT
Introduction
Epidemiological studies in humans suggest that impulsivity is related to addiction. The direction and nature (type of impulsivity and aspect of addiction) of this relationship is unclear. This study examines specifically whether impulsive choice (delay discounting) predicts subsequent propensity to exhibit addictive behaviors in a rodent model of cocaine self-administration and relapse. The (in)stability of impulsive decision making was monitored throughout the study.

Methods
Ninety-six rats were screened on the delay discounting task to measure impulsive choice. Subsequently, the upper and lower quartiles were trained to self-administer cocaine or saline. Sensitivity to and motivation for cocaine were measured at the end of acquisition, prior to initiating extinction. Following extinction, relapse was provoked by either cocaine associated cues, cocaine itself or the pharmacological stressor Yohimbine. During the course of the experiment impulsive choice behavior was monitored weekly.

Results
Impulsive choice remained stable within individuals during cocaine self-administration, extinction and relapse. In addition, impulsive choice did not determine cocaine intake, sensitivity or motivation to respond for cocaine. However high impulsive rats show a strong resistance to extinction and a higher propensity to relapse following re-exposure to cocaine-associated stimuli.

Conclusion
Impulsive choice is a highly stable trait that is not influenced by prolonged cocaine intake. Rather, high levels of impulsive choice act as a predisposing factor for prolonged cocaine seeking and enhanced relapse vulnerability.

KEY WORDS
Delayed reward task, impulsivity, cocaine, addiction, cue induced relapse

TELEPHONE-NUMBER: 020-4445677
E-MAIL-ADDRESS: n.broos@vumc.nl
TITLE
[11C]AF150(S), AN AGONIST PET LIGAND FOR IN VIVO IMAGING OF THE M1 MUSCARINIC ACETYLCHOLINE RECEPTOR

AUTHORS
Hans J.C. Buiter¹, J.E. Leysen¹, A. Fisher², M.C. Huisman¹, D.L. Knol³, A.A. Lammertsma¹, A.D. Windhorst¹

DEPARTMENT/INSTITUTE
¹ Nuclear Medicine and PET Research, VU University medical center, Amsterdam
² Israel Institute for Biological Research, Ness-Ziona, Israel
³ Epidemiology and Biostatistics, VU University medical center, Amsterdam

ABSTRACT
Purpose
The M1 muscarinic acetylcholine receptor (M1ACh-R) is enriched in basal ganglia, hippocampus, olfactory bulb and cortical areas, and plays a role in motor control and cognition. The aim of this study was to evaluate [11C]AF150(S) a M1ACh-R agonist PET ligand for its in vivo binding to the high-affinity state of the M1ACh-R.

Procedures
The regional distribution of [11C]AF150(S) in rat brain was assessed by dynamic PET imaging in rats after iv injection, under baseline conditions as well as after pre-treatment with various compounds.

Results and discussion
Moderate uptake of [11C]AF150(S) was observed, with a brain region over cerebellum ratio of 1.4 for striatum and 1.2 for cerebral cortex. This binding could be significantly and specifically reduced after pre-treatment with M1ACh-R antagonists and haloperidol. Haloperidol is a dopamine D₂ receptor blocker that causes an increase in extracellular acetylcholine, which may compete with [11C]AF150(S) binding.

Conclusion
[11C]AF150(S) revealed a small, significant M1ACh-R related signal in vivo and deserves further evaluation as a M1ACh-R agonist PET ligand.

KEY WORDS
[11C]AF150(S), M1 muscarinic acetylcholine receptor, agonist, rodent, brain biodistribution, in vivo, Positron Emission Tomography, high-affinity state, agonist, HRRT

TELEPHONE-NUMBER: 020-4445698
E-MAIL-ADDRESS: hjc.buiter@vumc.nl
Coding of trajectories in hippocampal CA1 place cells in normal and NMDA NR-1 mutant mice

Henrique O. Cabral¹, C. Fouquet², T. Arbab¹, C. M. A. Pennartz¹, L. Rondi-Reig², F. P. Battaglia¹

Departments/Institute
1 SILS-Center for Neuroscience, University of Amsterdam, Amsterdam
2 Lab. Neurobio. of Adaptive Processes, Univ. P et M Curie, Paris, France

Abstract
In addition to the current position, hippocampal CA1 place cells are known to encode information on the path the animal has previously taken and on its future trajectory. These correlates are thought to be important for route planning, a major function of the spatial navigational system. Previously we studied self-localization and path finding in a novel starmaze task which allowed disambiguation of allocentric (based on spatial representation of distal cues), and egocentric (depending on the temporal organization of self-motion information associated with choice points) strategies. This task was composed of 5 alleys configured in a pentagon, plus 5 alleys coming out radially from the pentagon vertices. Mice could make use of different strategies to reach the goal alley, which were assessed during probe trials. We found a specific impairment in path finding and sequential organization of behavior in mice lacking the NR-1 NMDA receptor subunit in CA1, with respect to control animals (Rondi-Reig et al. 2006, J. Neurosci. 26:4071).

We recorded and are in the process of analyzing 120 hippocampal neurons from the dorsal hippocampal CA1 subfield of 3 mice, implanted with a miniature tetrode array. Food restricted mice performed an appetitive version (food reward) of the same task, on a dry version of the same maze. Each mouse performed 15 sessions with 15 to 18 trials each, depending on the training stage. In the process of learning the optimal trajectories, the animals committed several mistakes, directing to alleys leading away from the goal. Many cells appeared to modify their spatial correlates, depending on whether the mouse made mistakes. To quantify this effect, we are currently computing the average firing activity of each cell on each of the alleys, separating the trials in which the mouse made a "correct" turn (leading closer to the goal) or a "mistake" turn in the same direction. Similarly, we are analyzing the activity on each alley depending on the alley of provenience. In addition, we will compare the firing patterns of each cell during the normal trials with the firing of the same cells during the probe trials, in which the strategy used by the animals are identified.

Key words
Spatial navigation, in-vivo electrophysiology, place cells, NMDA-receptors

Telephone-Number: 020-5257637
E-mail-address: h.deoliveiracabral@uva.nl
FREQUENCY SELECTIVITY OF A THALAMOCORTICAL RELAY NEURON

AUTHORS
Hayriye Cagnan1,2, Wytse J. Wadman2, Pascal Chameau2, Hubert C.F. Martens1

DEPARTMENT/INSTITUTE
1 Philips Research, High Tech Campus, Eindhoven
2 University of Amsterdam, Swammerdam Institute for Life Sciences, Amsterdam

ABSTRACT
Parkinson’s disease is a neurodegenerative movement disorder. Parkinson’s disease motor symptoms are linked to synchronized oscillatory activity patterns observed in the Basal Ganglia nuclei in the theta and beta frequency bands, and alleviation of the motor symptoms due to treatment (i.e. pharmacological or surgical) are correlated with a decrease in these activity patterns (Brown, Movement Disorders, 18, 357-363, 2003).

Deep Brain Stimulation (DBS) is a surgical technique used in the treatment of late – stage Parkinson’s disease. DBS involves delivery of high frequency (i.e. 120-180 Hz stimulus frequency) pulses to target nuclei such as the subthalamic nucleus (STN), globus pallidus internum and ventral intermediate nucleus of the thalamus and gives rise to suppression of Parkinson’s disease motor symptoms. It has been reported that clinically effective DBS frequency and stimulus amplitude are inversely related (Benabid et al., Lancet, 337, 403-406, 1991).

We investigated 1) effects of synchronized oscillatory Basal Ganglia activity patterns on a thalamocortical relay neuron 2) effects of STN - DBS on the thalamocortical relay neuron; and 3) the underlying mechanism for the inverse relationship between DBS frequency and stimulus amplitude, required to alleviate Parkinson’s disease motor symptoms.

KEY WORDS
Deep Brain Stimulation, Parkinson’s disease, Basal Ganglia, Thalamocortical relay neuron, theta and beta band

TELEPHONE-NUMBER: 040-2749181
E-MAIL-ADDRESS: hayriye.cagnan@philips.com
DELETION OF SCAP IN ASTROCYTES: THE IMPLICATION OF COMPROMISED LIPID METABOLISM IN THE MOUSE BRAIN

AUTHORS
Nutabi Camargo, August B. Smit, Mark H.G. Verheijen

DEPARTMENT/INSTITUTE
Dept. of Molecular & Cellular Neurobiology, Center for Neurogenomics and Cognitive Research, Neuroscience Campus Amsterdam, VU University, Amsterdam

ABSTRACT
The brain is remarkably different in lipid composition than other organs. It is enriched in polyunsaturated fatty acids (PUFAs) and cholesterol; the brain with 2% of the total body weight contains about one quarter of the total amount of cholesterol in the body. Many neurodevelopmental and neurodegenerative diseases are associated with disrupted lipid metabolism in the brain, e.g. Niemann-Pick and Alzheimer disease. Astrocytes have been shown to play a pivotal role in the maturation of neuronal circuitry and in synaptic function. Here, we ask the question whether lipid supply by astrocytes plays a role in this.

The synthesis of cholesterol and fatty acids is controlled by a family of transcription factors called Sterol Regulatory Element Binding Proteins (SREBPs). Activation of these transcription factors is dependent on the activity of SCAP (SREBP cleavage activated protein). In this study, we generated Cre-lox SCAP mice, from which the SCAP gene was deleted in astrocytes by driving Cre from the human GFAP promoter (from E14 on). These animals were used to study the role of SCAP-mediated activation of SREBPs in the supply of lipids from astrocytes to neurons during development and in the adult brain.

We found that SCAP knockout animals show an inducible startle-like behavior (hyperekplexia/dystonia) with an onset at 2 months. The number of startles increased with age and was correlated with a decrease in survival. In addition, SCAP mutant mice had smaller brains, whereas no difference was found in overall body weight. Current research is aimed at revealing the cellular and synaptic changes that occur in SCAP mutant brains in more detail.

KEY WORDS
Astrocytes, neuron glia interaction, myelination SCAP, SREBP, GFAP

TELEPHONE-NUMBER: 020-5987122
E-MAIL-ADDRESS: nutabi.camargo@cnhr.vu.nl
TITLE
NEUROPATHOLOGICAL CHARACTERISTICS OF CAPILLARY CEREBRAL AMYLOID
ANGIOPATHY WITH DYSHORIC CHANGES

AUTHORS
Anna Carrano¹, J. van Horssen², E. Richard³, J.J.M. Hoozemans¹, H.E. de Vries², J.M. Rozemuller¹

DEPARTMENT/INSTITUTE
¹ VU University medical center, Pathology, Amsterdam
² VU University medical center, Molecular Cell Biology and Immunology, Amsterdam
³ Academic Medical Center, Neurology, Amsterdam

ABSTRACT
Cerebral amyloid angiopathy (CAA) is a common neuropathological feature of Alzheimer's disease (AD) pathology, characterized by deposition of amyloid beta (Aβ) in the meningeal and cortical arteries, arterioles and capillaries of the brain. The amyloid in the vessels can extend into the parenchyma referred to as dyshoric angiopathy. The aim of this study was to delineate neuropathological characteristics of subjects with severe capillary CAA (CAA type 1) with dyshoric changes.

Neuropathologically CapCAA has been found to contain both Aβ40 and Aβ42 which show similar distribution features in the capillaries, nevertheless we could observe that the vessels wall is mostly affected by Aβ40 deposition, while in the dyshoric component both species are present. Interestingly Aβ42, and not Aβ40, was also observed forming bulb-like structures protruding from the capillaries wall. Remarkably, no diffuse or classical plaques were found in blocks with numerous Aβ laden capillaries.

Immunoreactivity for both activated microglia and astrocytes was observed associated with virtually all Aβ laden microvasculature, showing a stronger activation where Aβ deposition spreads into the parenchyma with a correlation between gliosis and progression of the pathology. Amyloid deposition in the capillaries and activation of microglia (innate immunity) is associated also with local increase of ubiquitin-immunoreactive neuritic dystrophy and in some case in co-presence with hyperphosphorylated tau accumulation in neuropil threads and in dystrophic neurites. In addition these patients showed also an alteration in the expression of tight junction proteins: claudin-5 expression was significantly decreased in Aβ-laden capillaries.

KEY WORDS
Cerebral amyloid angiopathy, Alzheimer's Disease, amyloid

TELEPHONE NUMBER: 020-4444032
E-MAIL-ADDRESS: a.carrano@vumc.nl
LIFETIME AT THE MEMBRANE: THE MOLECULAR FACTORS THAT INFLUENCE TETHERING AND DOCKING OF SECRETORY VESICLES AT THEIR TARGET

Tony Cijsouw, Matthijs Verhage, Ruud F.G. Toonen

Department of Functional Genomics, CNCR, VU University, Amsterdam

Secretory vesicles in the nervous system and endocrine tissue contain a large variety of signalling molecules for cell-cell and humoral communication. These signalling molecules are released upon fusion of the vesicle with the target membrane. This fusion process is a tightly regulated process and many genes have been implicated in its regulation. A number of upstream events have to take place before a vesicle is fusion-competent. The first event is that vesicles become stably docked at the target by unknown molecular mechanisms. Several years ago, our lab was the first to identify a molecular factor essential for this first docking step, the \textit{munc18-1} gene (see Voets et al 2001). Since this discovery, we have designed an assay to monitor tethering and docking of individual vesicles in living secretory cells, based on total internal reflection fluorescence (TIRF) imaging. Using this assay we aim to analyse the consequences of genetic and pharmacological manipulations of genes that may be involved in the tethering and docking of secretory vesicles.

SECRETION, chromaffin cells, Total Internal Reflection Fluorescence (TIRF) microscopy, vesicle fusion

TIRF imaging
TITLE
A ROLE OF PPAR-γ IN NEURONAL REGENERATION

AUTHORS
Jochem Cornelis, Geert Geeven, Harold D. MacGillavry, Mathisca C. M. de Gunst, Joost Verhaagen, August B. Smit, Ronald E. van Kesteren

DEPARTMENT/INSTITUTE
Dept. of Molecular and Cellular Neurobiology, Center for Neurogenomics and Cognitive Research, Neuroscience Campus Amsterdam, VU University, Amsterdam

ABSTRACT
Neuronal processes in the central nervous system (CNS) do not spontaneously regenerate after damage, which is the main reason for the irreversible and devastating effects of for instance spinal cord injuries. Damaged axons in the peripheral nervous system (PNS) on the other hand do spontaneously regenerate. Understanding the intrinsic differences in regenerative capacity between central and peripheral neurons will help to develop intervention strategies to promote neuronal regeneration in the CNS.

To identify neuron-intrinsic mechanisms underlying neuronal regeneration we performed gene expression profiling of forskolin-stimulated F11 cells. F11 cells are derived from rat embryonic DRG neurons, and we previously showed that raising intracellular cAMP levels in F11 cells mimics the gene regulatory events in DRG neurons under regenerating conditions (MacGillavry et al).

One gene that was significantly upregulated after forskolin stimulation is the transcription factor PPAR-γ, which is implied in various cellular processes, such as adipose tissue development, inflammatory response, glucose metabolism and also neuronal differentiation. In a parallel study we found PPAR binding sites to be overrepresented in genes that are repressed during successful regeneration in vivo. Together, these data suggests that PPAR-γ may be responsible for part of the transcriptional differences underlying successful regeneration.

Here we used specific PPAR agonists and blockers to show that PPAR-γ activation indeed stimulates outgrowth of F11 cells and DRG neurons through repression of several growth-inhibiting genes. We currently hypothesize that PPAR-γ may be involved in detecting changes in fatty acid signaling at the site of injury, and translating these signals into an appropriate gene expression response. We are now using genome-wide chromatin immunoprecipitation analysis and in vivo intervention of PPAR-γ signaling to further address the transcriptional regulatory role of PPAR-γ in neuronal regeneration.

References

KEY WORDS
Neuronal regeneration

TELEPHONE-NUMBER: 020-5982527
E-MAIL-ADDRESS: jochem.cornelis@cnccr.vu.nl
PSYCHOSOCIAL STRESS ENHANCES EMOTIONAL LONG-TERM MEMORY AND WORKING MEMORY IN MEN, BUT NOT IN WOMEN

Sandra Cornelisse¹, A.H. van Stegeren², C.G. van Stuijvenberg², M. Joëls¹

DEPARTMENT/INSTITUTE
¹ SILS-Center for Neuroscience and Cognitive Science Center Amsterdam, University of Amsterdam, Amsterdam
² Department of Clinical Psychology and Cognitive Science Center Amsterdam, University of Amsterdam, Amsterdam

ABSTRACT
Exposure to stress activates the autonomic nervous system (ANS) and the hypothalamic-pituitary-adrenal (HPA) axis. The activation of these systems leads to a rapid release of the catecholamines adrenaline and noradrenaline, and a somewhat slower release of glucocorticoids, respectively. These stress hormones are known to differentially modulate memory function. Memory can be impaired or strengthened by stress, depending on different factors like the memory type and -phase under study, the emotional value of the learned information and the sex of the subjects.

Here, we investigated the impact of psychosocial stress on long-term memory for neutral and emotional pictures and working memory in both men and women. 77 Subjects were exposed to either the Trier Social Stress Test (TSST) or a control condition, after which they were exposed to neutral and emotionally arousing pictures and subsequently performed a working memory task (N-back task). Incidental memory for the pictures was tested one week later with a free recall and a recognition test. During the experiment salivary cortisol and alpha-amylase levels, as well as subjective affect state, were assessed at several time points to validate stress effects. Additionally, the effects of the individual neuroendocrine stress responses on memory were explored.

Results show that stress hormone concentrations as well as subjective negative affect states increased significantly in response to the stress task. As expected, in men as well as women memory for emotional arousing information was better than for neutral information, in both the stress and control condition. In addition, stress enhanced recognition memory for emotional versus neutral pictures only in male subjects. The individual cortisol response to stress correlated positively with the difference between emotional and neutral recognition performance. Our results furthermore indicate that stress enhanced working memory, particularly in men, during the first block of a 2-back task.

The results of the current study provide additional insights in the influence of stress on different memory functions, especially emphasizing the role of gender differences.

KEY WORDS
Psychosocial stress, declarative memory, working memory, Cortisol (CORT), Salivary Alpha-Amylase (sAA)

TELEPHONE-NUMBER: 020-5258463 / 06-53123688
E-MAIL-ADDRESS: S.Cornelisse@uva.nl
CRAVING MODULATES BRAIN RESPONSES TO CANNABIS CUES

Janna Cousijn1,2, Anneke E Goudriaan1, Dick J. Veltman1, Wim van den Brink1, Reinout W. Wiers2

1 Amsterdam Institute for Addiction Research, Department of Psychiatry, Academic Medical Center, University of Amsterdam, Amsterdam
2 Department of Developmental Psychology, University of Amsterdam, Amsterdam

Craving is thought to play an essential role in the development and maintenance of addictive behaviors. When exposed to cannabis related cues, heavy cannabis users report higher levels of craving accompanied by physiological changes reflecting increased arousal. Neuroimaging studies showed that many different drug-related cues activate central limbic areas, e.g. the brain reward system, with a greater activation seen in subjects with higher levels of craving. It is not known, however, if this is also true for cannabis. With functional magnetic resonance imaging the neural correlates of craving were investigated in heavy cannabis users (n = 33) compared to sporadic cannabis users (n = 21) and controls (n = 22). Visual cannabis cues were found to activate various structures associated with the reward pathway like the amygdale, nucleus accumbens, hippocampus, medial frontal cortex, and orbitofrontal cortex in heavy users compared to sporadic users and controls. Activity in the hippocampus, medial frontal cortex, and orbitofrontal cortex was found to correlate positively with levels of subjective craving in heavy cannabis users as indicated with the Cannabis-Craving Questionnaire. The superior- and inferior frontal gyrus showed less active with higher levels of craving. Activation in the precuneus and inferior frontal gyrus was negatively correlated with problem scores derived from the Cannabis Use Disorder Identification Task (CUDIT). These results show that cannabis cues, just as many other drug related cues, activate areas associated with addiction pathology. Both subjective craving and cannabis use related problems modulate these brain responses. These findings are important for understanding the neural underpinnings of cannabis addiction.

KEY WORDS
Cannabis, craving, fMRI, addiction

TELEPHONE-NUMBER: 020-5256729
E-MAIL-ADDRESS: j.cousijn@uva.nl
TITLE
VARENICLINE INCREASES STRIATAL DOPAMINE D_{2/3} RECEPTOR BINDING IN RATS

AUTHORS
Cleo L. Crunelle\textsuperscript{1, 2}, Michelle L. Miller\textsuperscript{1, 2}, Kora de Bruin\textsuperscript{2}, Wim van den Brink\textsuperscript{1}, Jan Booij\textsuperscript{2}

DEPARTMENT/INSTITUTE
\textsuperscript{1} Amsterdam Institute for Addiction Research and Department of Psychiatry, Academic Medical Center, University of Amsterdam, Amsterdam
\textsuperscript{2} Department of Nuclear Medicine, Academic Medical Center, University of Amsterdam, Amsterdam

ABSTRACT
Introduction/Purpose: Disruptions in the dopaminergic system are implicated in the etiology of drug addiction, including drug craving and impulsivity, and may contribute to relapse in cocaine addicts. Striatal D\textsubscript{2} receptor availability in chronic cocaine users has been shown to be lower than in controls and low D\textsubscript{2} receptor availability promotes cocaine self-administration in non-human primates and rats [1]. Therefore, increasing striatal D\textsubscript{2} receptor availability could be a possible treatment for drug addiction. Varenicline is a selective alpha4 beta2 nicotinic receptor partial agonist and a full alpha7 agonist, and treatment with varenicline shows a reduction of withdrawal symptoms and craving for nicotine [2]. Nicotine receptors are involved in modulating the DA signal by altering dopamine release. The purpose of the current study was to determine whether and to what extent varenicline can modulate striatal DA D\textsubscript{2/3} receptor availability.

Methods: The effects of varenicline on striatal D\textsubscript{2} availability was tested in two groups of rats (varenicline, n=10; placebo, n=10). Varenicline or saline was administered daily for 14 consecutive days, and then ventral and dorsal striatal D\textsubscript{2/3} availability was ascertained by means of ex vivo phosphor storage imaging using the D\textsubscript{2/3} tracer \textsuperscript{123}I-IBZM.

Results: In controls and varenicline treated rats, IBZM binding was higher in the dorsal than in the ventral striatum. Additionally, specific striatum-to-cerebellum binding ratios in both dorsal (13.9%; \( p = 0.028 \)) and ventral (nucleus accumbens; 14.7%; \( p = 0.008 \)) striatum were significantly higher for the varenicline treated group compared to the placebo group.

Conclusions: Our experiment shows the ability of a nicotinic receptor partial agonist to increase striatal DA D\textsubscript{2}-like receptors. These findings may also explain the mechanism of action of varenicline in decreasing craving in cigarette smokers. Moreover, \textsuperscript{123}I-IBZM-binding uptake in the dorsal striatum was higher than in the ventral striatum, which is in line with previous rat work on reward. Consequently, from our experiment we hypothesize that varenicline could be a potential new aid in the treatment of addictions other than nicotine dependence, through its indirect action on DA D\textsubscript{2} receptor availability. Our findings are unique to conclude that a nicotine receptor partial agonist such as varenicline is able to influence DA D\textsubscript{2} receptor availability. Future investigation of the effects of varenicline as a pharmacotherapy is therefore warranted. Specifically, research is planned to investigate whether varenicline successfully increases DA D\textsubscript{2} receptors in a group of rats receiving daily cocaine injections.

References:

KEY WORDS
\textsuperscript{123}I-IBZM phosphor storage imaging, dopamine, nicotinic partial agonist, receptors, striatum, varenicline

TELEPHONE-NUMBER: 020-8913763
E-MAIL-ADDRESS: c.l.crunelle@amc.uva.nl
THE CALCIUM-BINDING PROTEIN DOC2B MEDIATES SPONTANEOUS NEUROTRANSMITTER RELEASE

AUTHORS
Rocio Díez Arazola¹, A.J.A. Groffen¹, L.N. Cornelisse¹, S. Martens², H.T. McMahon², M. Verhage¹

DEPARTMENT/INSTITUTE
¹ Department of Functional Genomics, Center for Neurogenomics and Cognitive Research, Neuroscience Campus Amsterdam, VU University and VU University medical center, Amsterdam
² MRC Laboratory of Molecular Biology, Hills Road, Cambridge, CB2 0QH, UK

ABSTRACT
By coupling Ca²⁺ 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 Ca²⁺-sensitivity in the sub-μM 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.

KEY WORDS
DOC2A/B, spontaneous fusion, calcium sensor, C2 domains

TELEPHONE-NUMBER: 06-16395240
E-MAIL-ADDRESS: rocio.diezarazola@gmail.com
DEEP BRAIN STIMULATION IN SAPAP3- MUTANT MICE: AN ANIMAL MODEL FOR OCD

AUTHORS
Addy van Dijk, G. Collin, A. Schott, M. Verhage, A.A. Klompmakers, M. Feenstra, G. Feng, D. Denys

DEPARTMENT/INSTITUTE
Department of Psychiatry, AMC, Netherlands Institute for Neuroscience, Amsterdam
Department of Neurobiology, Duke University Medical Center, Durham, USA

ABSTRACT
Obsessive compulsive disorder (OCD) is a psychiatric disorder that is characterised by obsessions and compulsions which affects about 2% of the population. First-choice treatments for OCD are SSRIs and cognitive behavioural therapy. If unsuccessful, clomipramine or combinations of SSRIs with an atypical antipsychotic are the best options. Nevertheless about 10% of OCD patients are therapy-resistant, of whom half have a severe form of OCD. For the latter Deep Brain Stimulation (DBS) is a new and novel treatment.

DBS is a proven successful treatment for Parkinson’s disease. Recent studies/publications have shown very promising results for OCD patients as well. (Greenberg et al, Mol Psych 2008) However the working mechanism of DBS is still uncertain. For this reason we are investigating the effect of Deep Brain stimulation in the Sapap3 knockout mouse, a new animal model for OCD. (Welch et al. Nature 2007)

The Sapap3 knockout mouse exhibits increased anxiety and compulsive grooming behaviour. Treatment with SSRIs reduces these behaviours. Sapap3 is a postsynaptic scaffolding protein at excitatory synapses that is highly expressed in the striatum. (Welch et al. Nature 2007) The striatum is part of the cortico-striato-thalamo-cortical circuit of which the involvement in the pathophysiology of OCD has been hypothesized.

So far we have bilaterally targeted two brain areas with two bipolar electrodes in the Sapap3 knockout mice. High frequency stimulation in the nucleus accumbens, the brain area targeted for treatment of OCD patients, causes a significant increase in the frequency of grooming rituals of the Sapap3 knockout mice. However it does not increase the percentage of time spent grooming which suggests that the time spent per ritual is reduced.

High frequency stimulation at different coordinates in the Caudate nucleus has not yet shown a conclusive result. However, a reduction in the time spent grooming in the Sapap3 knockout mice was observed in two mice with electrodes at medial locations, suggesting that a more extended search for stimulation targets will be successful.

KEY WORDS
Obsessive compulsive disorder, deep brain stimulation, Sapap3 knockout mice

TELEPHONE-NUMBER: 020-8913576
E-MAIL-ADDRESS: a.vandijk@amc.uva.nl
DIFFERENTIAL EFFECTS OF MOOD STABILIZERS ON PREPULSE INHIBITION DEFICITS IN MICE WITH CORTICOTROPHIN-RELEASING FACTOR OVER-EXPRESSION

AUTHORS
Tessa N. Douma, L. Groenink

DEPARTMENT/INSTITUTE
Psychopharmacology/Utrecht Institute for Pharmaceutical Sciences, Utrecht

ABSTRACT
Corticotropin-releasing factor (CRF) is implicated in the induction and/or expression of psychotic symptoms in schizophrenia and bipolar depression. These disorders are characterized by deficits in prepulse inhibition (PPI) of the acoustic startle response. Since anti-epileptic drugs (mood stabilizers) are therapeutically useful for treating schizophrenia and bipolar disorder, we evaluated the influence of mechanistically-distinct mood stabilizers on sensorimotor gating deficits displayed by transgenic mice over-expressing CRF.

The effects of valproic acid (60-240 mg/kg, i.p.), lamotrigine (3-27 mg/kg, i.p.) and carbamazepine (20-80 mg/kg, i.p.) were determined in transgenic mice overexpressing CRF as compared to their wild-type littermates, employing standard procedures. Valproic acid dose-dependently, and at 240 mg/kg completely, restored the disrupted PPI in CRF-overexpressing mice, whereas it had no significant effect in wild-type mice. Lamotrigine increased PPI both in CRF transgenics and wild-type mice. In CRF transgenics, this effect was most marked at 9 mg/kg, and at lower prepulse intensities, whereas the effects of lamotrigine in wild-type mice were most prominent at 27 mg/kg and at higher prepulse intensities. By contrast, carbamazepine had no significant effects on PPI in CRF transgenics and, at a dose of 80 mg/kg, disrupted PPI at higher intensities in WT mice.

The mood stabilizers tested had differential effects on sensorimotor gating deficits induced by excess central CRF levels. Valproic acid was the only drug that markedly improved the disrupted PPI in CRF transgenics. The major differences in mechanism of action between valproic acid and the other mood stabilizers are its enhancement of GABAergic transmission and its inhibition of histone deacetylases. These findings suggest that the detrimental effects of CRF on PPI may be related to such mechanisms.

KEY WORDS
CRF, prepulse inhibition, mood disorders, GABA

TELEPHONE-NUMBER: 030-2533038
E-MAIL-ADDRESS: t.n.douma@uu.nl
Alzheimer's disease (AD) is regarded as a neurodegenerative protein misfolding disorder. Accumulation of misfolded proteins in the endoplasmic reticulum (ER) activates the unfolded protein response (UPR), which results in upregulation of ER stress responsive genes and an overall inhibition of translation. The UPR is aimed to restore homeostasis in the ER, but prolonged ER stress results in cell death. Our lab has shown that the UPR is upregulated in the early stages of AD pathology. We have shown that the increase of BiP in brain material is strongly correlated with the upregulation of the small GTP-ase Rab6. This GTP-ase is mainly involved in retrograde vesicle transport from the Golgi complex to the ER. Although the levels of Rab6 correlate strongly with the extent of ER stress, the increase of Rab6 is not a direct consequence of the ER stress response. In this study we investigate in more depth the function of Rab6 during ER stress in Alzheimer's disease. To this end we established cellular PC12 models expressing human wild type, constitutive active (Q72R) and dominant negative (T27N) Rab6 under control of an inducible tet-off system. Q-PCR experiments indicate that the expression level of ER stress responsive genes, elicited by culturing in the presence of tunicamycin, is lower in Rab6 overexpressing cells compared to uninduced cells. In addition, the level of ER stress responsive genes is lower in PC12 cells overexpressing constitutive active Rab6 as compared to PC12 cells overexpressing wildtype Rab6. In contrast, overexpression of dominant negative Rab6 increases the induction of ER stress responsive genes. We are currently investigating whether the inhibitory effect of Rab6 on ER stress is caused by alleviation of ER stress by increased degradation or whether Rab6 directly intervenes with the ER stress response.
IMMEDIATE EARLY GENE EXPRESSION IN COCAINE ADDICTION

Ping Gao, J.H.W. Limpens, S. Spijker, L.J.M.J. Vanderschuren, P. Voorn

Dept. of Anatomy and Neurosciences, Neuroscience Campus Amsterdam, Amsterdam

Cocaine is one of the most reinforcing drugs of abuse known to date. Casual use of the drug may progress to compulsive drug seeking. However, the mechanism of this process is still unclear. The effect of cocaine in the ventral striatum has been well established, and recently there has been a surge of interest in the role of the dorsal striatum. Previous research showed that immediate early genes play a critical role in mediating drug-induced neuronal plasticity. In this study, we have therefore examined whether cocaine-self administration affects the expression of immediate early genes in medial prefrontal cortex, ventral and dorsal striatum. In ten daily sessions rats self administered cocaine or sucrose for two hours. After the final session, the animals were placed back in their home cage for thirty minutes, and sacrificed immediately therefore. A panel of twenty immediate early genes was tested by using RT-PCR. We found several immediate early genes, including c-Fos, MKP-1, Arc and Egr2, showing a higher expression in cortico-striatal systems in the cocaine/sucrose groups compared to the control group. Interestingly, some genes were increased in the dorsal striatum but not in the ventral striatum. This might suggest there is a differential role of the ventral and dorsal striatum in cocaine self-administration. In the further experiment, more details about the expression of these several immediate early genes will be detected by using the in situ hybridization or immunohistochemistry.

KEY WORDS
Cocaine, immediate early gene(IEG), RT-PCR

TELEPHONE-NUMBER: 06-43832148
E-MAIL-ADDRESS: p.gao@vumc.nl
The inferior colliculus is an almost obligatory relay in the ascending auditory pathway. The functional organization of the central inferior colliculus has been well characterized, but much less is known about the dorsal inferior colliculus. Here, we studied the micro-architecture of the dorsal inferior colliculus of the mouse by simultaneously recording from neighboring neurons in the whole-cell configuration. Thus we wanted to learn if local interconnections contribute to sound processing in the dorsal inferior colliculus and if neighboring neurons show a common organization of frequency response areas.

Neurons were shadowpatched at depths between 50 and 200 μm from the dorsal surface of the inferior colliculus. We assessed the presence of synaptic connections by monitoring the membrane potential of one neuron while applying suprathreshold current injections to the second neuron. Frequency response areas were probed with short tone bursts and FM sweeps, presented contra- and ipsilaterally under closed field conditions.

We did not find evidence for the presence of monosynaptic connections between neighboring neurons. This suggests that integration of afferent signals plays a more important role than local processing. Frequency response areas were often complex, showing several bands of excitation and/or inhibition. Frequency response areas could be highly correlated between adjacent neurons, suggesting a patterned organization of inputs to the dorsal inferior colliculus.

**KEY WORDS**
Inferior colliculus, frequency response areas, in vivo, intracellular recording

**TELEPHONE-NUMBER:** 010-7044526  
**E-MAIL-ADDRESS:** h.geis@erasmusmc.nl
STRATIAL DOPAMINE D2 RECEPTOR AVAILABILITY IN DIET INDUCED OBESE RATS: DEVELOPING AN OBESITY MODEL

AUTHORS
Elsmarieke van de Giessen, Kora de Bruin, Jan Booij, Wim van den Brink

DEPARTMENT/INSTITUTE
Department of Nuclear Medicine, Academic Medical Center, Amsterdam

ABSTRACT
Overeating of highly palatable and caloric foods is thought to play a major role in the pathophysiolozy of obesity. The limbic brain reward system plays an important role in the reinforcing effects of food and therewith overeating. An important structure of the limbic brain reward system is the striatum. It has been shown that obese people have decreased striatal dopamine D2 receptor (DRD2) availability. Also genetically modified obese animals, i.e. the Zucker rat and the OLETF rat, and diet induced obese mice show decreased DRD2 levels in the striatum. These low DRD2 levels are assumed to be an indication of an impaired limbic dopaminergic system, resulting in impaired reward signalling, which is thought to sustain overeating. The goal of this study is to develop a diet induced obese rat model with decreased striatal DRD2 availability. This model will be used for further research on anti-obesity treatments that might upregulate striatal DRD2.

METHODS
30 male Wistar rats are randomized in 3 groups. For four weeks, group 1 (n=10) is fed a high fat high sugar (HFHS) choice diet, group 2 is fed a high fat (HF) choice diet and group 3 is fed a standard diet. At day 28, the rats receive intravenous administration of the selective dopamine D2/D3 tracer [123I]-IBZM. Ninety minutes after the [123I]-IBZM injection, animals are sacrificed. Brains are removed and sliced into 50 μm slices. The 50 μm brain slices are exposed to Fuji BAS-MS IP phosphor plates for 24 hours to measure striatal DRD2 availability. Additional outcome measures are food intake, body weight, and weight of abdominal fat stores as a measure of obesity.

RESULTS
Preliminary results indicate that the DRD2 availability is decreased in the nucleus accumbens of rats on the HF diet compared to rats on standard diet. The striatal DRD2 availability of rats on HFHS diet does not differ significantly from the rats on standard diet. Both rats on HF diet and on HFHS diet have significantly increased food intake and increased abdominal fat stores compared to rats on standard diet. Final results will be shown at the conference.

KEY WORDS
Obesity, dopamine D2 receptor, nucleus accumbens, striatum

TELEPHONE-NUMBER: 020-5668323
E-MAIL-ADDRESS: e.m.vandegiessen@amc.uva.nl
TITLE
BIOCHEMICAL METHODS FOR STUDYING REGULATION OF MEMBRANE FUSION

AUTHORS
Asiya Giniatullina, S. Groffen, M. Verhage

DEPARTMENT/INSTITUTE
Dept. of Functional Genomics, CNCR, VU University, Amsterdam

ABSTRACT
The main objective of this study is to gain insight into the mechanisms regulating the secretion of synaptic vesicles. Fusion of vesicles in cells is mediated by the SNARE proteins that provide the force to bring two membranes together. In addition, there are a large number of proteins that assist the assembly of the SNAREs, act as triggers or inhibitors of fusion, or mediate the interaction with other proteins and cell membranes. The proteins studied here are characterized by having so-called C2 domains, protein structures found in more than 200 human proteins. Many of these proteins are involved in regulating fusion, are responsive to calcium signals, interact with SNARE proteins and membranes. They are often fundamental for survival and play a very important role in neurotransmission; moreover, variations in C2 domains of several proteins have been reported to be cause of human disease. We use biochemical methods to investigate protein-protein and protein-membrane interactions, calcium sensitivity and ability to promote fusion of artificial vesicles. In addition to biochemical characterization, we employ bioinformatics to analyse and predict function and structure of the C2 domains. One of the final goals is to be able to characterize the effect of mutations or single nucleotide polymorphisms found in diseases on protein function.

KEY WORDS
Synaptic vesicle fusion, phospholipid membranes, SNAREs, C2 domains

TELEPHONE-NUMBER: 020-5987792
E-MAIL-ADDRESS: asiya.giniatullina@cnr.vu.nl
ACUTE INTRAGASTRIC ADMINISTRATION OF THE ANTIPSYCHOTIC OLANZAPINE INCREASES THE PLASMA GLUCOSE CONCENTRATION AND INDUCES HEPATIC INSULIN RESISTANCE IN MALE RATS

AUTHORS
Elodie M. Girault, E. Foppen, M.T. Ackermans, E. Fliers, A. Kalsbeek

DEPARTMENT/INSTITUTE
1 Netherlands Institute for Neuroscience, Amsterdam
2 Department of Endocrinology and Metabolism and
3 Laboratory of Endocrinology, Department of Clinical Chemistry, Academic Medical Center, University of Amsterdam, Amsterdam

ABSTRACT
Atypical antipsychotic drugs such as Olanzapine are associated with weight gain and metabolic changes that increase the risk of developing type II diabetes. The mechanisms underlying these undesired side-effects are currently unknown. Therefore, we decided to investigate the effects of Olanzapine administration on glucose metabolism in more detail. All experiments were performed in undisturbed, awake and freely moving male Wistar rats.

In the first experiment, we administered Olanzapine via an intragastric cannula directly in the stomach. We chose this method to be as close as possible to the therapeutic use of this drug (i.e., oral administration) and in order to be able to administer the drug without having to handle the experimental animals. Rats were also implanted with two silicon catheters, one in the carotid artery for blood sampling and one in the jugular vein to enable the infusion of a stable glucose isotope in order to measure endogenous glucose production. During the isotope infusion, Olanzapine was administered continuously at 3 mg/kg/h during 160 min and blood samples were taken every 20 minutes. We measured plasma glucose concentrations, isotope enrichment and plasma corticosterone, insulin and glucagon levels.

Plasma glucose concentrations in the Olanzapine infused animals showed an increase from ±5.5 mmol/l to ±8.5 mmol/l at 140 min after the start of Olanzapine administration, i.e., an increase of more than 50%. Vehicle treated animals showed no significant increase of plasma glucose concentrations (always around 5.5 mmol/l). Endogenous glucose production on the other hand only showed a minor change in the Olanzapine treated animals, i.e., less than 5% increase. Therefore, the increased plasma glucose concentrations upon the intragastric administration of Olanzapine apparently are not solely due to an increased hepatic glucose production.

In the second experiment, we investigated the effects of an acute administration of Olanzapine on insulin sensitivity and glucose tolerance by using the hyperinsulinemic euglycemic clamp technique. During the clamp circulating plasma insulin concentrations were elevated to approximately 2x normal basal levels. In the vehicle-treated animals the hyperinsulinemic conditions caused the expected decrease in the endogenous glucose production of ±60%. The animals treated with Olanzapine, on the contrary, showed almost no changes in endogenous glucose production (stable around 60 µmol/kg/min), i.e., the Olanzapine treatment completely prevented the insulin induced inhibition of glucose production. Glucose uptake showed a slight decrease in Olanzapine group compared to controls. Obviously, acute intragastric administration of Olanzapine induces severe (hepatic) insulin resistance in rats.

The next experiment will be to investigate the effects of the same treatment on muscle insulin sensitivity.

KEY WORDS
Glucose metabolism, type II diabetes, insulin resistance, olanzapine

TELEPHONE-NUMBER: 020-5664524
E-MAIL-ADDRESS: e.girault@nin.knaw.nl
ORIENTATION AND MOTION TUNING IN THE MOUSE VISUAL CORTEX AFTER VISUAL CONDITIONING: AN IN VIVO 2-PHOTON IMAGING STUDY

Pieter M. Goltstein, E.B.J. Coffey, C.M.A. Pennartz

Cognitive and Systems Neuroscience, Center for Neuroscience, SILS, University of Amsterdam, Amsterdam

ABSTRACT

During development, the visual cortex is subject to large-scale organization of the synaptic matrix resulting in the ability of neurons to detect a variety of features in the environment and ultimately leading to visual perception. This process is thought to be fundamentally driven by mechanisms of self-organization and winds down during adulthood. Some recent studies point out that also in adulthood, neurons in the rodent visual cortex are subject to plasticity and can be modulated by the timing of upcoming reward (Shuler and Bear, 2006, Science 311, 1606).

In the present study we investigated how reinforcement signals modulate the functional microorganization and feature tuning of ensembles in the primary visual cortex of the adult mouse by in vivo 2-photon imaging under anesthesia. We hypothesized that tuning of individual neurons and stimulus representation at the population level would be modified over time by a previously learned association between a visual stimulus and reinforcement.

To assess this, we trained twelve mice to associate a drifting square wave grating (100% contrast, ± 2 Hz) moving in a certain direction with delivery of a food reward while a different direction was not followed by reward. The presence of a learned association was assessed by probe trials in which stimuli were presented in the absence of reinforcement. Seven out of twelve mice learned the association and displayed behavior in accordance with the predictive value of the conditioned stimuli.

After a substantial amount of one-hour training sessions (40 to 50), we used two-photon laser scanning microscopy in combination with multi-cell bolus loading of a fluorescent intracellular calcium indicator (Oregon Green BAPTA 1-AM), to measure orientation tuning of approximately 8000 neurons in primary visual cortex. We are currently in the process of analyzing whether visual cortex neurons are significantly more tuned to the grating orientation and motion direction that had been paired with reward during previous training. Subsequent analysis steps will be 1) Calculate descriptive statistics for single neuron orientation tuning (circular variance, bandwidth, direction index, etcetera). 2) Compare the descriptive statistics of neurons with optimal tuning to the trained orientations versus neurons that are tuned to non-trained orientations. 3) Contrast the population of orientation-tuned neurons of the conditioned mice with the naive mice.

Acknowledgement

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KEY WORDS

Calcium imaging, reinforcement learning, primary visual cortex, mouse physiology, two-photon laser scanning microscopy

TELEPHONE-NUMBER: 020-5257605 / 06-25022656
E-MAIL-ADDRESS: p.m.goltstein@uva.nl
TITLE
ADOLESCENT NICOTINE EXPOSURE INDUCES LASTING CHANGES IN PRESYNAPTIC MGLUR2 FUNCTION IN EXCITATORY SYNAPSES IN PREFRONTAL CORTEX

AUTHORS
Natalia A. Goriounova1, Danielle S. Counotte2, Sabine Spijker2, August B. Smit2, Huibert D. Mansvelder

DEPARTMENT/INSTITUTE
1 Department of Integrative Neurophysiology, Center for Neurogenomics and Cognitive Research, VU University, Amsterdam
2 Department of Molecular and Cellular Neurobiology, Center for Neurogenomics and Cognitive Research, VU University, Amsterdam

ABSTRACT
Adolescence is a time period of increased vulnerability for developing drug addiction, with the majority of adult smokers starting the habit at this age. Brain maturation is not complete at adolescence, and in particular the prefrontal cortex (PFC) continues developing during this period, as does cognitive performance. In rodents, nicotine exposure during adolescence results in decreased attention performance and augmented impulsivity during adulthood, suggesting reduced prefrontal function. Here, we asked the question whether adolescent nicotine exposure induces lasting effects on the adult prefrontal cortex neuronal circuitry and function. We used a combination of synaptic proteomics and electrophysiological techniques to study the mechanisms underlying the cognitive deficits resulting from adolescent nicotine exposure. We find that nicotine injections during adolescence (P34-43) affect the levels of only a handful of synaptic proteins in PFC of adult rats, and most prominently reduced levels of synaptic metabotropic glutamatergic receptor 2 (mGluR2) protein. This reduction in receptor levels is paralleled by diminished presynaptic mGluR2 modulation of excitatory transmission in layer V pyramidal neurons. Activation of mGluR2 receptors in these neurons leads to decreased evoked EPSCs, reduced frequency of mEPSCs and increased paired-pulse ratio, indicating that mGluR2s act presynaptically by inhibiting glutamatergic transmission. In conditions of high glutamate release such as during high frequency stimulation mGluR2 action would be most prominent. Accordingly, we find that adolescent nicotine exposure also leads to reduced short-term depression at excitatory synapses suggesting decreased filtering of excitatory inputs necessary for selective attention. Thus, nicotine exposure during adolescence results in lasting reduction in presynaptic mGluR2 signalling and short-term plasticity in adult PFC, implicating the role of these adaptations in lasting cognitive deficits at adult age.

KEY WORDS
Nicotine, adolescence, PFC, attention

TELEPHONE-NUMBER: 020-5987099
E-MAIL-ADDRESS: natalia.goriounova@cnacr.vu.nl
FUS MUTATIONS IN FAMILIAL ALS IN THE NETHERLANDS

AUTHORS
Ewout J.N. Groen1*, Michael A. van Es1*, Paul W.J. van Vught1, Wim G.M. Spliet4, Jooyeon van Engelen-Lee4, Marianne de Visser7, John H.J. Wokke1, Helenius J. Schelhaas6, Roel A. Ophoff2,5, Katsumi Fumoto3, R. Jeroen Pasterkamp3, Dennis Dooijes2; Edwin Cuppen2,8; Jan H. Veldink1, Leonard H. van den Berg1

DEPARTMENT/INSTITUTE
1 Department of Neurology, Rudolf Magnus Institute of Neuroscience
2 DBG-Department of Medical Genetics
3 Department of Neuroscience and Pharmacology, Rudolf Magnus Institute of Neuroscience
4 Department of Pathology, University Medical Centre Utrecht
5 UCLA Center for Neurobehavioral Genetics, Los Angeles, USA
6 Department of Neurology/Clinical Neurophysiology, Donders Institute for Brain, Cognition and Behaviour, Centre for Neuroscience, Radboud University Nijmegen Medical Center, Nijmegen
7 Department of Neurology, Academic Medical Centre, Amsterdam
8 Hubrecht Institute for Developmental Biology and Stem Cell Research, Cancer Genomics Center, Royal Netherlands Academy of Sciences, Utrecht
* These authors contributed equally to this work

ABSTRACT
Recently, mutations in the FUS gene were shown to be associated with familial amyotrophic lateral sclerosis. We here investigate these mutations in cases of hereditary motor neuron disease (including ALS, PMA and SMA). We included 51 families with a family history of motor neuron disease. All patients were screened and negative for mutations in SOD1, ANG, VAPB and TARDBP, as well as deletions of SMN1 and CAG repeats in the androgen receptor gene. We used capillary sequencing of whole-genome amplified DNA using BigDye 3.1 chemistry on all 15 exons. All mutations were confirmed in a separate PCR and sequencing reaction on genomic DNA. For exons containing mutations, at least 900 neurologically normal controls were sequenced to confirm ALS specificity. We identified three mutations in four out of 52 probands. We observed two previously identified mutations (p.Arg521Cys and p.Arg521His) and one novel mutation (p.Ser462Phe). Additionally, a p.Gln210His polymorphism was identified in one proband and three healthy controls. We demonstrate FUS mutations in Dutch FALS patients and that benign variations in the gene may occur. Therefore caution is warranted when interpreting results in a clinical setting. Work on pathological findings as well as functional testing of novel mutations and phenotypic analysis is ongoing.

KEY WORDS
Amyotrophic Lateral Sclerosis, genetics, phenotype / genotype, neuromuscular disorder

TELEPHONE-NUMBER: 088-7556506
E-MAIL-ADDRESS: e.j.n.groen-3@umcutrecht.nl
The dystonias are a group of movement disorders characterized by involuntary twisting, repetitive movements and abnormal postures. Clinical presentation and genetic background of the dystonias is heterogeneous. Dystonia subtypes have been classified based on clinical presentation and genetic linkage studies and designated to a DYT number (DYT1-DYT20). Mutations in the nuclear pro-apoptotic protein THAP1 have recently been found to be associated with DYT6 dystonia, an early onset dystonia with predominant craniocervical involvement. We assessed the frequency and phenotype of THAP1 mutation carriers in a large cohort of cervical dystonia (CD) patients (n=280), early onset segmental and generalized dystonia patients (n=117) and writer’s cramp (n=70) patients. Overall, 5 new mutations were found: 3 patients with an early onset and segmental dystonia with a mutation in exon 2 (3 %), causing cervical dystonia with spread to cranial region, speech and arms. One patient has a strong family history, as his father suffers segmental dystonia of the neck and his grandfather shows complex writer’s cramp. All 3 index patients are treated with bilateral GPi stimulation, with good to moderate effect. In the cervical dystonia group 2 mutations (1%) in exon 3 of THAP1 were identified, causing late onset cervical dystonia with a negative family history. We conclude THAP1 mutations are a rare cause of late onset cervical dystonia. The frequency of DYT6 in the early onset/segmental dystonia group is comparable with DYT1, the other familial pure dystonia syndrome caused by mutations in TOR1A; however we find a higher frequency when patient selection depends on localization of disease onset in the craniocervical region.

KEY WORDS
Dystonia, DYT6, THAP1, mutation screening

TELEPHONE-NUMBER: 020-5663963
E-MAIL-ADDRESS: j.l.groen@amc.nl
Layer 5 pyramidal neurons are characterized by an elaborate dendritic tree, integrating information from both layer 5, as layer 2/3. Inputs coming in at layer 2/3 influence the soma in a two-stage way. They first induce a calcium spike at the apical tuft 600 micrometer from the soma and this calcium spike is then conducted to the soma. Somatic studies have shown that metabotropic glutamate receptors (mGluR) affect calcium currents. Here we investigate the effect of the type I mGLuR agonist 3,5-dihydroxyphenylglycine (DHPG) on the calcium spikes of a single layer 5 neocortical pyramidal cell, using simultaneously somatic and dendritic patch clamp techniques.

The effect of DHPG was twofold. Somatic excitability was dramatically increased: DHPG hyperpolarized the membrane potential, decreased input resistance, and increased spontaneous firing. In contrast the size of the calcium spikes was decreased after applying DHPG. This point to an interesting type of modulation, in which mGLuRs can enhance the effect of proximal inputs coming onto the soma, but decrease the influence of distal synapses.

KEY WORDS
L5 pyramidal cell, cortex, calcium spikes, dendritic patchclamp

TELEPHONE-NUMBER: 020-5987042
E-MAIL-ADDRESS: martine.groen@cncr.vu.nl
PROGRAMMING EFFECTS OF INDIVIDUAL WITHIN-LITTER MATERNAL CARE ON GENE EXPRESSION, FUNCTION AND STRUCTURE IN THE ADULT RAT HIPPOCAMPUS

AUTHORS
Felisa N. van Hasselt¹, Zimbo Boudewijns¹, Sandra Cornelisse¹, Els Velzing¹, Michael J. Meaney², Harm J. Krugers¹, Marian Joëls¹

DEPARTMENT/INSTITUTE
¹ SILS-Center for Neuroscience, University of Amsterdam, Amsterdam
² Douglas Mental Health University Institute, McGill University, Montréal, Canada

ABSTRACT
For several years now, the “maternal care model” in rats has been an established way to investigate the effects of early life environment on gene expression, neuroendocrine function and brain development. Thus, the amount of maternal care provided to the pups by the dam during the first postnatal week varies between litters. We and others found that, at basal levels of corticosterone, rats that received a high amount of licking and grooming (High LG) early in life have a higher degree of long-term potentiation (LTP) and more complex dendritic trees in the adult hippocampus than those that received a low amount of maternal licking and grooming (Low LG; ie. an adverse environment). Interestingly, though, the Low LG environment has been shown to ‘prepare’ the offspring for stressful conditions in their adult life. Not only do they perform better in stressful learning tasks than High LG rats, but they also show a larger degree of hippocampal LTP in the presence of the stress-hormone corticosterone.

As a refinement of the original maternal care model, we have been determining the amount of maternal care that each individual pup within a litter receives. There is a considerable variability in the amount of LG the mother provides to each of her pups, with an overall higher attendance to male versus female pups.

We found that in the hippocampal dentate gyrus the same functional differences between High and Low pups within litters occur as earlier found in pups between High and Low litters, irrespective of sex. There is a positive correlation between the amount of LG received and the degree of LTP in vehicle conditions. This correlation was inversed when the slices were incubated with corticosterone at the time of LTP induction.

In CA1 we did find a gender difference in functionality. In males, we found the same LTP pattern as described above in the DG, whereas in females the effects of LG and corticosterone on the degree of LTP seem to be reversed.

Morphology and neurogenesis data are still being analysed, but it seems that there is a difference between male and female offspring in the effect of maternal care on hippocampal dendritic complexity, spine density, proliferation and survival of newborn cells.

Our latest experiments focused on gene expression and methylation patterns in the hippocampus. Preliminary results indicate that in adult male offspring, BDNF mRNA and methylation levels vary in relation to the amount of individual LG received. At this point we have not found differences in other target genes.

KEY WORDS
Maternal care, hippocampus, epigenetics

TELEPHONE-NUMBER: 020-5258463
E-MAIL-ADDRESS: f.n.vanhasselt@uva.nl
THE ACUTE EFFECTS OF THC ON BRAIN ACTIVITY DURING WORKING MEMORY

Erika (H.H.) van Hell, M.G. Bossong, G. Jager, E. Saliasi, R.S. Kahn, N.F. Ramsey

1 Rudolf Magnus Institute of Neuroscience, Department of Neurology and Neurosurgery, University Medical Center Utrecht
2 Rudolf Magnus Institute of Neuroscience, Department of Psychiatry, University Medical Center Utrecht

Abstract

Introduction

The endocannabinoid (eCB) system consists of cannabinoid receptors and endogenous ligands that work on these receptors. The eCB system acts as a retrograde messenger system that regulates both excitatory and inhibitory neurotransmission (1). As such, the eCB system acts as a ‘fine tuning’ system and is involved in the control of many important brain functions, including learning and memory. Challenging the eCB system by administering exogenous cannabinoids such as delta9-tetrahydrocannabinol (THC), the main psychoactive component in cannabis, deteriorates all stages of memory (2), but little is known about the underlying neurophysiological mechanisms. The present pharmacological MRI study investigated the effect of THC administration on working memory (WM)-related brain function.

Methods

Twelve right-handed healthy males (age 21.7 ± 2.2) participated in a randomized, placebo-controlled, double-blind, cross-over pharmacological MRI study. All subjects underwent two functional MRI sessions, separated by two weeks, receiving THC (6 mg) or placebo using a Volcano® vaporizer (Storz-Bickel GmbH, Tuttlingen, Germany). Subjects had a history of mild cannabis use (≤ once a week) and abstained from cannabis for at least two weeks before the scanning sessions. WM was measured using a parametric Sternberg item-recognition task. Subjects were instructed to memorize target sets with different WM load, i.e. sets of 1, 3, 5, 7 or 9 consonants. After the target set a series of eight single consonants was presented, and subjects responded to targets with a button press. Performance measures were speed of response (reaction times) and accuracy. Functional imaging data were analyzed using SPM5. Five factors modelling the different memory loads were used in the regression matrix. A repeated measures GLM was performed to compare differences in brain activity between placebo and THC.

Results

THC administration increased reaction times and reduced accuracy (p<0.05). A load-effect was also observed, indicating subjects responded slower and less accurate (p< 0.01) during the higher load conditions. A load*drug interaction was found in accuracy, indicating that performance accuracy started to decrease at a lower processing load after THC then after placebo. The task activated a well-known WM network, involving the anterior cingulate cortex, the left dorsolateral prefrontal cortex, the left inferior parietal cortex, and areas in the cerebellum, the right insula, and the left inferior frontal cortex. The network was activated in a load-dependent way, i.e. brain activity was increased during the higher loads compared to the lower loads, both during placebo and THC. However, no significant main or interaction effects of THC on brain activity levels were found.

Conclusions

Maximum performal levels were reached at a lower WM load during THC than placebo, despite equal brain activity patterns. This may reflect physiological inefficiency in intermediate to high WM demands after THC administration. During THC, subjects may disengage from the task after maximum WM capacity is reached, or adopt a different strategy by disregarding some of the stimuli to maintain a reasonable level of performance.

References


Key Words

fMRI, working memory, THC, cannabis

Telephone-Number: 088-7555873
E-mail-Address: h.h.vanhell@umcutrecht
TITLE
CEREBRAL ATROPHY IN ALCOHOLICS AND PATHOLOGICAL GAMBLERS: A VOXEL-BASED MORPHOMETRY STUDY

AUTHORS
Ruth J. van Holst, A.E. Goudriaan, W. van den Brink, D.J. Veltman

DEPARTMENT/INSTITUTE
Academic Medical Center University of Amsterdam, Amsterdam Institute for Addiction Research, Amsterdam

ABSTRACT
Functional MRI studies have shown similar aberrant activation patterns between pathological gambling and substance use disorders. Alterations in brain functions can be related to abnormalities in GM volumes and have been reported in alcohol dependent (AD) subjects. However, structural imaging studies focussing on GM differences in pathological gamblers, to our knowledge, do not exist.

The purpose of our study was to investigate GM volumes using Voxel-Based Morphometry in male groups of 25 AD, 18 PG subjects and 21 controls. Except for higher depression severity scores in the PG group, no group differences existed.

Controlling for age, depression scores and total GM volumes, we found smaller GM volumes in the right inferior and medial frontal gyrus, right inferior parietal lobe, and bilateral in the lenticularis nuclei in AD compared to NC, consistent with earlier findings. In PG, smaller GM volumes were found in the right post central gyrus, bilateral parahippocampal gyrus, the right amygdala and posterior cerebellar cortex compared to NC. Smaller hippocampal and amygdala regions are associated with depression and anxiety1. However, our regression analyses between BDI score and GM volumes did not show this association and therefore this can not explain our findings.

In conclusion, we found GM differences between a substance use disorder (AD) and addictive behaviour (PG). PG was characterized by smaller parahippocampal and amygdala regions. In addition, smaller cerebellar cortex volumes, associated with executive dysfunctions and neurobehavioral syndromes such as ADHD2, are evidence of structural brain abnormalities in PG.

References

KEY WORDS
Voxel-based morphometry; pathological gambling; addiction; alcohol dependence

TELEPHONE-NUMBER: 020-8913766
E-MAIL-ADDRESS: r.j.vanholst@amc.uva.nl
EFFECTS OF COX-2 INHIBITION WITH SC-58236 ON EPILEPTOGENESIS, SEIZURES AND ANTIEPILEPTIC DRUG THERAPY WITH PHENYTOIN

AUTHORS
Linda Holtman, E.A. van Vliet, P.M. Edelbroek, E. Aronica, J.A. Gorter

DEPARTMENT/INSTITUTE
SILS – Center for Neuroscience, University of Amsterdam, Amsterdam

ABSTRACT
Purpose
Status epilepticus (SE) leads to upregulation of pro-inflammatory proteins including cyclooxygenase-2 (cox-2) which could be implicated in the epileptogenic process and epileptic seizures. Recent studies show that cox-2 can regulate expression of P-glycoprotein (P-gp) during epileptogenesis and epilepsy. P-gp could cause pharmacoresistance by reducing brain entry of anti-epileptic drugs such as phenytoin (PHT). Here we have investigated the effects of cox-2 inhibition on epileptogenesis, spontaneous seizures and PHT treatment in a rat model for temporal lobe epilepsy (TLE).

Methods
A 3-day treatment with the cox-2 inhibitor SC-58236 (SC) was started one day before electrically induced SE. Chronic epileptic rats were treated with SC for 14 days, which was followed by a 7-day period of SC/PHT combination treatment. Seizure activity was monitored continuously using electroencephalography. Brain-to-plasma ratios for PHT were determined by high performance liquid chromatography.

Results
SC treatment did not affect SE duration, but led to an increased number of rats that died during the first 2 weeks after SE. Cox-2 inhibition during the chronic period led to an increased number of seizures in the 2nd week of treatment in 50% of the rats. SC/PHT-treatment reduced seizures significantly for two days.

Conclusions
Both SC treatment that started before SE and the 14 day treatment in chronic epileptic rats led to adverse effects in the TLE rat model. Although the cox-2 inhibition improved PHT brain entry, which could be helpful in overcoming pharmacoresistance, the treatment with SC does not seem to be a suitable approach for anti-epileptogenic or anti-epileptic therapy.

KEY WORDS
Status epilepticus, neuroinflammation, temporal lobe epilepsy, p-glycoprotein, anti-epileptic drugs

TELEPHONE-NUMBER: 020-5258370
E-MAIL-ADDRESS: l.holtman@uva.nl
THE RELATION BETWEEN THE BRAIN’S DEFAULT MODE NETWORK, MEMORY, AND ATTENTION: A COMBINED RESTING-STATE/TASK-BASED FMRI STUDY

AUTHORS
Willem Huijbers\textsuperscript{1}, Cyriel Pennartz\textsuperscript{1}, Roberto Cabeza\textsuperscript{2}, Sander Daselaar\textsuperscript{1}

DEPARTMENT/INSTITUTE
\textsuperscript{1} University of Amsterdam, Swammerdam Institute for Life Sciences, Faculty of Science, Amsterdam 
\textsuperscript{2} Duke University, Center for Cognitive Neuroscience, Department for Psychology and Neuroscience, Durham, USA

ABSTRACT
The brain’s default mode network (DMN) – consisting of ventral parietal, posterior cingulate, medial frontal, and hippocampal regions – is activated during internally-oriented tasks and shows strong coherence in spontaneous rest activity. Despite a surge of recent interest, the functional role of the DMN remains poorly understood. Interestingly, the DMN tends to be activated during the retrieval of past events from memory but deactivated during the encoding of novel events into memory. One hypothesis is that these opposing effects reflect a difference between an orientation towards internal events, such as retrieved memories, vs. external events, such as to-be-encoded stimuli. Another hypothesis is that hippocampal regions are coupled with the DMN during retrieval but decoupled from the DMN during encoding. The present fMRI study investigated these two hypotheses by combining a resting-state coherence analysis with a task that measured the encoding and retrieval of both internally-generated and externally-presented events. Results revealed that the main DMN regions were activated during retrieval but deactivated during encoding. Counter to the internal/external orientation hypothesis, this pattern was not modulated by whether memory events were internal or external. Consistent with the hippocampal coupling hypothesis, the hippocampus behaved like other DMN regions during retrieval but not during encoding. Taken together, our findings clarify the relationship between the DMN and the neural correlates of memory retrieval and encoding.

KEY WORDS
Memory, attention, fMRI, default-mode network

TELEPHONE-NUMBER: 020-5257728
E-MAIL-ADDRESS: w.huijbers@uva.nl
Communication between neurons is mediated by synapses, where electric activity is translated into a chemical signal, i.e. the fusion of neurotransmitter-filled vesicles with the plasma membrane. Synaptic transmission is a probabilistic process in the central nervous system; typically about 30% of the action potentials arriving at a synapse actually trigger the fusion of a vesicle. Moreover, presynaptic terminals from the same neuron may vary substantially in their probability of neurotransmitter release. The release probability at a synapse can vary over time, depending on the pattern of neuronal activity, modulation by extracellular stimuli and the makeup of the vesicle fusion machinery. In this way, presynaptic terminals are actively involved in processing and storing information in neuronal circuits.

To study the biochemical nature of synaptic heterogeneity, we made use of the fluorescent reporter SypHy to visualize individual fusion events at synapses of cultured hippocampal neurons, combined with patch clamp electrophysiology. This experimental design can be used to determine the role of vesicle fusion proteins, presynaptic receptors and intracellular signal molecules in defining and modulating synaptic heterogeneity.

**KEY WORDS**
Synaptic vesicle release, fluorescence microscopy, electrophysiology

**TELEPHONE-NUMBER:** 020-5986931
**E-MAIL-ADDRESS:** arthur.de.jong@cncr.vu.nl
TITLE
MULTIPLE SHORT-TERM MEMORY SIGNALS IN MONKEY PRIMARY VISUAL CORTEX

AUTHORS
Timo J. van Kerkoerle, Mathew Self, Pieter R. Roelfsema

DEPARTMENT/INSTITUTE
Vision & Cognition, Netherlands Institute for Neuroscience, Amsterdam

ABSTRACT
Visual short-term memory (VSTM) is essential for cognitive behavior, bridging episodes in which visual input is no longer present. VSTM is traditionally associated with the prefrontal cortex and intraparietal sulcus (Chafee & Goldman-Rakic 1998; Edin et al. 2009). But neural correlates of VSTM have also been found in occipital and temporal areas (Pasternak & Greenlee 2005; Xu & Chun 2006), suggesting short-term memory storage takes place in the same cortical areas that are used for perceptual processing. There is even some limited evidence that primary visual cortex (V1) is involved in VSTM (Supèr, Lamme & Spekreijse 2001; Harrison & Tong 2009).

Here we used a curve tracing task (Roelfsema, Lamme & Spekreijse 1998) to study VSTM in monkey primary visual cortex. The monkey was presented briefly (150ms) with a set of curves, one of which was connected to the fixation point and was designated the target. Crucial segments of the curves revealing the target were then removed and the monkey was required to remember the location of the target curve during a 600ms memory period. After this period the monkey made a saccade to the target location. We measured multi-unit activity (MUA) from V1 using laminar electrodes which allow MUA to be measured simultaneously from the different laminae of V1. We found that MUA was increased when the target curve fell in the receptive field of the recording site, compared to the distracter curves. Furthermore this positive modulation remained high for the duration of the memory period suggesting that this modulation constitutes a memory trace. In control experiments we found that removing all visual information during the retention period or lengthening the retention period to over 1s did not remove the modulation. Likewise the modulation remained when the planned eye-movement was identical for both remembered targets and non-remembered distracters.

We divided the memory-trace into two components by presenting a 50ms duration mask during the retention period. The initial component of the trace was maskable and therefore may reflect an iconic memory trace. However the modulation returned after the mask, indicating that feedback was able to re-initiate a durable memory trace suggesting that working memory traces reach all the way back to V1. Both of these memory components were strongest in the superficial and deep layers of V1 and were weaker in layer 4 supporting the idea that they are due to feedback. The spatial specificity of these memory components varied, with the initial iconic memory trace being highly spatially specific whereas the later working memory component was more broadly tuned.

KEY WORDS
Short-term memory; working memory; iconic memory; V1

TELEPHONE-NUMBER: 020-5664905
E-MAIL-ADDRESS: t.van.kerkoerle@nin.knaw.nl
Fragile X syndrome (FXS) is the most common inherited form of mental retardation. The patients suffer from various behavioral problems such as deficits in learning and memory, cognitive abnormality, hyperactivity and autism. It is caused by the impaired expression of the RNA-binding Fragile X mental retardation protein (FMRP), leading to disturbances in dendritic protein synthesis and causing abnormalities in synaptic structure and function.

Here, we examine the hippocampal synapse proteome in the adult FMRP knockout mice in order to get insight into the molecular underpin of aberrant synaptic neurotransmission and behavioral abnormality in FXS. We used 8 independent biological replicates. The peptides derived from the trypsin digested proteins were labeled with 8-plex iTRAQ reagents for quantitative analysis. The 2D LC-tandem mass spectrometry identified about 900 proteins, from which about 630 proteins were quantified.

A distinct group of proteins necessary for cytoskeleton remodeling, synapse architecture and neuronal outgrowth was strongly regulated, including Dihydropyrimidinase-related protein 3, 4 and 5 and the PKC substrates MARCKS, BASP-1 and GAP-43. Their up-regulation may underlie the observed elongated and thin synapses in fmr1 KO mice. Furthermore Synaptic vesicle associated proteins like Synapsin 1, Synaptophysin, Synaptic vesicle glycoprotein and Synaptotagmin-1, were in general up-regulated. In addition mGluR2 and the G protein subunits beta and gamma were regulated. Together, these implicate impairments in synaptic vesicle cycle and neurotransmitter release, which may affect synaptic function and plasticity in Fragile X Syndrome.

KEY WORDS
Fragile X Syndrome, Hippocampus, Synapse, Proteomics, iTRAQ

TELEPHONE-NUMBER: 020-5982527
E-MAIL-ADDRESS: patricia.klemmer@cncr.vu.nl
TITLE
FLUOXETINE AND THE DEVELOPING BRAIN: A PHARMACOLOGICAL MRI STUDY IN RATS

AUTHORS
Anne Klomp1, J.L. Tremoleda2, M. Wylezinska-Arridge2, W. Gsell2, L. Reneman1

DEPARTMENT/INSTITUTE
1 Department of Radiology, Academic Medical Centre, Amsterdam
2 Biological Imaging Centre, Imaging Science Department, MRC Clinical Sciences Centre, Hammersmith Hospital, Imperial College London, United Kingdom

ABSTRACT
Selective serotonin reuptake inhibitors (SSRIs) are the second most commonly prescribed psychotropic drugs in children and adolescents. In 2007 over 8,500 children under age 21 were prescribed with this antidepressant in the Netherlands alone. Fluoxetine is at the moment the only SSRi registered for use in children over 8 years. Although numerous trials have shown robust safety of this SSRI in adults, limited data is available on its effects on the developing brain during (pre)adolescence. SSRIs like fluoxetine act upon the serotonergic (5-HT) system by blocking the 5-HT transporters and in this way increasing the amount of available 5-HT. Animal studies have demonstrated that pharmacological manipulations of extracellular 5-HT concentrations during early life can lead to abnormal functioning of the 5-HT system in adulthood. This is related to the key role of 5-HT in axonal outgrowth of 5-HT projections during brain development. Recent literature and ongoing studies in our group suggest that fluoxetine treatment has a stimulatory effect on the outgrowth of the 5-HT system which can only be seen in the developing brain and is still ongoing in adolescence.

Pharmacological MRI (phMRI) is a new non-invasive method to assess 5-HT function. phMRI maps the hemodynamic BOLD response to a drug challenge which can be seen as a surrogate for changes in brain activity. It can thus be used to visualize the effect of 5-HT manipulations on the animal and human brain and in this way assess 5-HT function. The aim of this study is to validate phMRI as a useful method to assess the stimulatory effect of chronic fluoxetine treatment on outgrowth of the 5-HT system which is specific to the developing brain. If so, this non-invasive method could open new horizons in the research of (pediatric) neuropsychiatric disorders involving the 5-HT system.

Young (PND21) and adult rats (PND60-66) were treated with either fluoxetine (5mg/kg, p.o.) or placebo for 21 days, followed by one medication-free week before assessment of 5-HT function by phMRI. During the phMRI experiments, hemodynamic BOLD activity was measured under gas anesthesia (isoflurane, 1.5-2%). An initial period of baseline recordings was followed by recordings of functional activity after an acute challenge with fluoxetine (5mg/kg, i.v.). Throughout the experiment, recordings of respiration rate, body temperature, invasive blood pressure and blood gas measures were kept. Data were fitted to a linear model comprising the baseline period before the injection of the fluoxetine and the “activation” period using MRI analysis program FSL. Preliminary findings show a clear transient effect of the challenge on blood pressure and a more prolonged effect on the MRI BOLD signal. Also, the activation pattern of young animals differed from the adults. Higher level analysis is ongoing. A different activation pattern in young treated vs. non-treated animals but not in adult treated vs. non-treated would support the suggested effects of chronic fluoxetine treatment on functioning of the 5-HT system specific to the developing brain.

KEY WORDS
Fluoxetine, developing brain, serotonin, pharmacological MRI

TELEPHONE-NUMBER: 020-5668322
E-MAIL-ADDRESS: a.klomp@amc.uva.nl
In multiple sclerosis (MS), 40%-60% of the patients suffer from cognitive decline, most notably memory impairment. The hippocampus plays a pivotal role in learning and memory processing. Recently, neuropathological studies revealed that the hippocampus may be heavily affected in MS, as both extensive demyelination and neuronal loss have been found in MS hippocampal tissue. However, little is known about the pathogenesis of hippocampal pathology (nor of grey matter pathology in a broader sense). In this study, several possible pathogenic pathways in the MS hippocampus are explored, including glutamate excitotoxicity, oxidative stress and selective vulnerability of neuronal subpopulations. Also, concomitant neuroprotective responses are investigated.

A total of 24 formalin-fixed MS hippocampi and 10 age- and sex-matched non-neurological control hippocampi were selected from the Netherlands Brain Bank, cut (8 μm), and immunohistochemically stained for parvalbumin (subset of GABA-ergic interneurons), excitatory amino acid transporters 1 and 2 (EAAT1/2; glutamate transporters) and choline acetyltransferase (ChaT; neurotransmitter catabolist).

Preliminary observations showed a drastic decrease of parvalbumin-positive GABA-ergic inhibitory interneurons in MS hippocampi compared to control hippocampi. Selective loss of inhibitory interneurons may lead to enhanced glutamate-mediated excitotoxicity. However, we also detected a protective increase of EAAT1 and -2 in MS hippocampi. ChaT immunopositivity was reduced in MS, indicating that cholinergic, like glutamatergic, metabolism is disturbed. Remarkably, differences in protein expression were lesion-independent: both demyelinated and non-demyelinated hippocampal areas showed changes in expression of the above described markers, which led us to propose that (hippocampal) grey matter demyelination does not necessarily predate neurodegeneration, but may instead coincide with it. The above described changes are likely to be involved in ongoing neurodegeneration in MS hippocampus and may represent a substrate for cognitive decline.

Currently ongoing work includes quantification of the immunostainings described above, as well as evaluation of hippocampal glutamate and acetylcholine receptor expression patterns, and of neural growth factors. Interindividual differences in severity of hippocampal pathology will be related to putative differences in overall brain pathology. These results will be presented at the meeting.

**KEY WORDS**
Multiple sclerosis, grey matter pathology, hippocampus, neurodegeneration, cognition

**TELEPHONE-NUMBER:** 020-4444096
**E-MAIL-ADDRESS:** e.kooi@vumc.nl
DIFFERENTIATED SH-SY5Y CELLS AS A GOOD IN VITRO MODEL FOR PARKINSON DISEASE STUDIES

AUTHORS
Joanna A. Korecka¹, E. Blaas², S.O. Spitzer¹, R.E. van Kesteren², A.B. Smit², D.F. Swaab¹, K. Bossers¹, J. Verhaagen¹

DEPARTMENT/ INSTITUTE
¹ Neuroregeneration, Netherlands Institute for Neuroscience, Amsterdam
² Center for Neurogenomics and Cognitive Research, Neuroscience Campus Amsterdam, VU University, Amsterdam

ABSTRACT
Human neuroblastoma SH-SY5Y cells are widely used in Parkinson's disease (PD) research in order to generate in vitro models for the disease. SH-SY5Y cells are used both as undifferentiated and retinoic acid (RA) differentiated cells while testing different stress responses mimicking Parkinsonian molecular changes. Although widely used, these cells have not been well characterized in terms of gene expression. We have developed a long-term culture system where cells are cultured for 8 days and differentiated by 1µM RA, forming a stable neuron-like cell population. To characterize the exact neuronal phenotype we performed a microarray study comparing undifferentiated versus RA-differentiated SH-SY5Y cells. Here we show RA-induced gene expression changes upon differentiation of these cells into a neuronal phenotype. We also demonstrate that SH-SY5Y cells display a dopaminergic phenotype since they express well-established dopaminergic markers such as Nurr1, Pitx3, TH, VMAT2 and DRD2, in addition to the production of dopamine. Detailed analysis of the array results showed that RA cell differentiation induces a clear downregulation of other transmitter phenotypes (for example: enzymes involved in the production of noradrenalin or acetylcholine), leaving us with a predominantly dopaminergic cell. We have confirmed these array results by qPCR and for some selected genes we have also shown protein expression profiles. We have also studied the expression of target genes, previously identified in a microarray study of human PD human (Bossers et al, 2008), to validate SH-SY5Y cells as a relevant in vitro model. We were able to confirm the expression of most of these genes in the RA differentiated cells also on the protein level and conclude that RA- differentiated SH-SY5Y cells cultured under our growth conditions provide a good in vitro model for research on PD. We have also analyzed the impact of the chronic low dose MPP+ toxicity treatment on these cells and considered neuroprotection mechanisms involved in this model.

Reference
Bossers et al., 2009, Brain Pathology; 19:91-107.

KEY WORDS
SH-SY5Y cells, retinoic acid differentiation, dopaminergic phenotype, Parkinson's Disease

TELEPHONE-NUMBER: 020-5665503
E-MAIL-ADDRESS: j.korecka@nin.knaw.nl
Titre: Mechanisms of AMPA receptor trafficking in hippocampal neurons

Auteurs: Marijn Kuijpers, Lukas C. Kapitein, Max A. Schlager, Myrrhe van Spronsen, Dick Jaarsma, Casper C. Hoogenraad

Département/Instituut: Department of Neuroscience, Erasmus Medical Center, Rotterdam

Résumé: The brain is a network of electrically active neurons that communicate with each other via synapses. In excitatory synapses, the presynaptic terminal usually releases the neurotransmitter glutamate, which diffuses across the synaptic cleft to bind to, and activate, glutamate receptors in the postsynaptic membrane. It is widely believed that learning and memory is mediated by long-lasting modifications in synaptic strength. In recent years it has become evident that an important way to alter the strength of synaptic transmission is to change the abundance of glutamate receptors at the postsynaptic membrane. The steady-state level of synaptic glutamate receptors is determined by their delivery and removal. Despite their importance in neurotransmission and synaptic plasticity, little is known about the mechanisms underlying glutamate receptor trafficking.

AMPAR-type glutamate receptors mediate most of the postsynaptic response in excitatory synapses and are composed of GluR1-4 subunits in heteromultimeric configuration. The GluR2 subunit has received particular attention because it participates in specific interactions with multiple cytoplasmic proteins. Of particular importance, GluR2 binds to the GRIP (glutamate receptor interacting protein) family of proteins which is an adaptor for kinesin-1 motor proteins (Hoogenraad et al., Nature Neuroscience, 2005). In this project, we examined the AMPA receptor trafficking rules in primary hippocampal neurons and investigate the role of motor proteins in neuronal receptor transport.

Mots clés: AMPA, neurons, receptor trafficking, motor proteins

N° de téléphone: 010-7043373
Adresse e-mail: m.kuijpers.1@erasmusmc.nl
Neurobeachin (nbea) is a multidomain brain-enriched putative A-kinase adaptor protein (AKAP), highly expressed in the brain during development. Nbea-null mice have severe defects in neuromuscular synaptic transmission resulting in lethal paralysis of the newborns. Although the function of nbea is still unknown, our data suggests a role in the regulation of the glutamatergic receptors (GluRs). Nbea null mutant neurons exhibit a substantial reduction of the postsynaptic current evoked by external glutamate application, which can be restored by expression of full-length NBEA-ires-EGFP in these neurons. While the bulk of endogenous nbea positive puncta are localized in close apposition to the Golgi apparatus, we found dendritic nbea to partially colocalize with fluorophore conjugated transferrin, as well as with Rab 11, both markers of recycling endosomes. No colocalization of nbea was found with early endosomal markers Rab 5 and EEA1 or with the lysosomal marker Lamp 1. The above results imply that nbea might be involved in the trafficking of glutamatergic receptors. However, further studies will be necessary to establish in which pathway precisely Nbea is involved in, e.g. direct post-Golgi to membrane traffic, recycling pathway, etc.

KEY WORDS
Neurobeachin, AKAP, BEACH, GluR
THE EFFECTS OF 12 HOURS OF FULL SLEEP DEPRIVATION BY MEANS OF MILD FORCED LOCOMOTION ON A NOVEL SWITCH-TASK PARADIGM

Cathalijn H.C. Leenaars¹, R.N.J.M.A. Joosten¹, H. Sandberg¹, A. Zwart¹, E. Ruimschotel¹, M. Dematteis², M.G.P. Feenstra¹, E.J.W. van Someren¹

¹ the Netherlands Institute for Neuroscience, Amsterdam
² Laboratoire HP2, Faculte de Medecine de Grenoble, France

ABSTRACT

Introduction
Sleep deprivation impairs cognitive performance in both humans and rats. Performance of “prefrontal” tasks appears to be particularly sensitive to sleep deprivation. Previous data from our group show that insomniacs are impaired on a switch-task that strongly involves prefrontal cortical activity.

To enable research into the neurochemical substrates underlying impairments on these kind of paradigms, we developed a switch-task for rats, and investigated the effect of 12h of sleep deprivation on task performance.

Methods
Male Wistar rats, on a diet of 15g/day and on a reversed light-dark cycle (lights OFF at 10.00h), were daily trained at dark onset to perform 2 different discrimination tasks separately (eg. light -> left lever rewarded and tone -> right lever rewarded). The 2 tasks were gradually integrated within sessions, creating a conditional discrimination task offered in blocks of 5-10 trials of one type.

Rats were sleep deprived for 12h by mild forced locomotion during the light phase. Rotational speed and alterations in direction were gradually increased during the deprivation period to compensate for increases in sleep pressure, efficiently reducing slow-wave-sleep to 1.4% of control days. In the control condition (same rats, different days), the same amount of locomotion was spread out over a 23h period, so rats had sufficient time to sleep.

Data
After 38 training sessions, both error rates and latencies were significantly different for switch and 5th trials. Approximately 85% of switch-trials were correctly performed, compared to 99% of 5th trials. Latencies were 0.8 s on switch trials and 0.6 s on 5th trials.

Error rate switch costs were significantly increased after 12 h of full sleep deprivation compared to the previous and following (control) days and were significantly different from the 24h movement control condition.

Conclusion
We conclude that 12h of sleep deprivation increases switch-costs and that we can use this set-up to study the neurobiological basis of the effects of sleep deprivation on task-switching.

KEY WORDS
Cognition, task-switching, conditional discrimination, sleep deprivation

TELEPHONE-NUMBER: 020-5665495
E-MAIL-ADDRESS: c.leenaars@nin.knaw.nl
The goal of this research was to get a better understanding of the mechanisms in the brain that make us see some things conscious and other things not. It might be that the things we do not see are registered by our brain unconsciously and thereby influencing our behavior. Therefore we wanted to manipulate with medication the brain processes that are involved to see whether consciousness gets less. Therefore a number of substances which influence different neurotransmitters in the brain were used in combination with a manipulation of consciousness. To make sure that the visual stimuli were sometimes seen and sometimes not, the stimuli were presented very briefly and short after a different stimulus, a mask was shown. In this way the first stimulus was 'masked'. To find out to what extend the brain still registered the stimulus, brain activity was recorded using a technique called Elektroencefalografie (EEG). Furthermore, the effect of the medications on detecting two different stimuli (a 'stack' or a 'frame') was also investigated.

**KEY WORDS**
Recurrent processing, EEG, pharmacological intervention

**TELEPHONE-NUMBER:** 020-5256808  
**E-MAIL-ADDRESS:** a.m.vanloon@uva.nl
CHARACTERIZATION OF BREVICAN +/- MICE AS A MODEL FOR RELAPSE VULNERABILITY

AUTHORS
Bart R. Lubbers, Danae Riga, August B. Smit, Taco J. De Vries, Sabine Spijker

DEPARTMENT/INSTITUTE
Department of Molecular and Cellular Neurobiology, Center for Neurogenomics & Cognitive Research, VU University, Amsterdam

ABSTRACT
Drug addiction is a brain disease characterized by drug taking despite negative consequences and high relapse rates. During the last decade, it has become clear that exposure to drugs of abuse leads to long-lasting adaptations in the neuronal circuitry that mediates processing of motivationally relevant stimuli. The molecular mechanisms underlying these neuroadaptations, as well as their exact contribution to relapse to drug seeking, remain largely unresolved. The medial prefrontal cortex (mPFC) is thought to play a pivotal role in relapse to drug seeking during periods of drug abstinence. Previous work in our group has shown downregulation of extracellular matrix (ECM) constituents, brevican and tenascin-R, in rat mPFC after abstinence from heroin self-administration. These proteins are enriched in perineuronal nets (PNNs) surrounding GABAergic interneurons. In addition, inhibition of ECM degradation attenuated relapse rates. To study the implication of reduced ECM levels on vulnerability to relapse, a brevican knock-out mouse was obtained. In order to find a genetical mouse model for the long-term effects of heroin exposure, we investigated mice of all genotypes for reduced ECM expression in synaptosomal preparations of the mPFC and the immunohistochemical integrity of PNNs in the mPFC. First, brevican expression levels of brevican heterozygous (+/-) mice were shown to be reduced to a level similar to brevican levels after abstinence from long-term heroin self-administration. Furthermore, the PNN staining pattern of brevican-null (-/-) mice was found to be diffuse and granular. As yet, the PNN integrity of brevican-/- mice has to be analyzed. This preliminary set of data suggests that brevican-/- mice are a good model to further investigate vulnerability to drug addiction. Pilot experiments using conditioned place preference (CPP) with amphetamine to evaluate differences in reward and drug-primed reinstatement between wildtypes (WT) and brevican-/- mice showed a more robust and significant reinstatement compared with WT littermates. A dose-response curve needs to be made to determine whether reduced levels of brevican are related to differential drug sensitivity.

KEY WORDS
Drug addiction, neuroadaptations, mPFC, amphetamine, extra-cellular matrix, brevican, CPP

TELEPHONE-NUMBER: 020-5982811
E-MAIL-ADDRESS: bart.lubbers@cnrc.vu.nl
TITLE
CANNABIS USE IN PATIENTS WITH A PSYCHOTIC DISORDER AND PATIENTS AT ULTRA HIGH RISK OF PSYCHOSIS: IMPACT ON PSYCHOTIC- AND PRE-PSYCHOTIC SYMPTOMS

AUTHORS
Marise Machielsen, Suzanne van der Sluis, Lieuwe de Haan

DEPARTMENT/INSTITUTE
Zorglijn vroege psychose, Academic Medical Center, Amsterdam

ABSTRACT
Cannabis use disorder (CUD) is a common comorbid diagnosis in patients with a psychotic disorder and is associated with poor outcome. This study aimed to (1) determine prevalence of CUD among patients with a psychotic disorder and patients at Ultra High Risk (UHR) of psychosis, (2) compare patients with a psychotic disorder and comorbid CUD with patients without CUD on psychotic symptomatology, (3) compare patients with CUD and a psychotic disorder with patients without CUD on psychosocial functioning and variables related to the course of the disorder and (4) compare UHR patients with CUD with UHR patients without CUD on pre-psychotic symptomatology. 45% of the patients with a psychotic disorder had a comorbid diagnosis of CUD; in the UHR patients 27% were diagnosed with CUD. Patients with CUD did not differ from patients without CUD on psychotic symptom levels. However, excluding patients with substance use disorder other than CUD resulted in higher scores on positive- and negative symptom levels for patients with cannabis use disorder compared to patients without any substance use disorder. In addition, psychosocial functioning was worse for patients with CUD compared to patients without CUD. Regarding UHR patients, patients with CUD did not differ from patients without CUD on pre-psychotic symptomatology. However a negative correlation was found between the amount of cannabis used recently and scores on the pre-psychotic negative subscale. Concluding, our results suggest a specific adverse effect of cannabis abuse on psychotic symptomatology.

KEY WORDS
Schizophrenia, cannabis use disorder

TELEPHONE-NUMBER: 020-8913674
E-MAIL-ADDRESS: m.w.machielsen@amc.uva.nl
In order to study the genetic background of operant conditioning and the behavioral processes contributing to this type of learning, we carried out behavioral screening of 29 recombinant-inbred (BxD) mouse lines and their progenitor lines C57Bl6/6J and DBA/2J. We aimed at dissecting multiple aspects of operant learning and associated behavioral processes as well as finding correlated quantitative trait loci (QTL’s).

The training consisted of two phases: a reward collection phase, in which the mice learned to collect a sucrose pellet from a food magazine during a trial, and an operant phase, in which the mice had to press a lever in order to collect a food pellet. Furthermore, we studied the initial magazine checking behavior characterized by nose poke entries in the magazine during intertrial interval periods in the beginning of the first session of reward collection training phase.

We found a highly significantly heritable component in both initial magazine checking behavior and operant performance. These traits showed no correlation, however, initial magazine checking behavior correlated with reward collection success, which in turn correlated with operant learning performance, reflecting the subsequent steps of operant learning process. Using the WebQTL interface, QTL mapping revealed a suggestive likelihood ratio score (LRS) peaks for initial magazine checking behaviour (chromosome 2) and operant performance (chromosome 9). These findings indicate that operant conditioning consists of subcomponents that are genetically dissociable and regulated by separate chromosomal areas.

KEY WORDS
QTL mapping, instrumental conditioning, complex trait analysis, behavioural screening, recombinant-inbred mice, BxD
GLUTAMATERGIC NEUROTRANSMISSION IN SCHIZOPHRENIA: WHAT HAPPENS?

Anouk Marsman, Martijn van den Heuvel, Hilleke Hulshoff Pol

Department of Psychiatry, Rudolf Magnus Institute of Neuroscience, University Medical Center, Utrecht

Schizophrenia is a severe chronic psychiatric disease, characterized by hallucinations and delusions. Structural MRI and postmortem studies have established that schizophrenia is a brain disease with tissue loss; however, the origin of the disease is still unknown. Previous studies of our group have suggested a progressive decrease in gray matter volume in patients with schizophrenia, as well as a possible genetic involvement in the development of the disease. Genes involved in schizophrenia may influence glutamate neurotransmission. Glutamatergic dysfunction may have neurotoxic effects and could cause neuroanatomic changes, e.g. gray matter changes. Considering this, we hypothesize that alterations in glutamatergic neurotransmission may be involved in brain changes observed in schizophrenia. With ¹H magnetic resonance spectroscopy (¹H-MRS), in vivo metabolite measurements in the human brain are possible. In our review, we discuss MRS studies reporting on glutamatergic alterations in schizophrenia. These studies suggest that glutamatergic activity is upregulated in early schizophrenia, which might explain the observed brain tissue losses. As the disease progresses, studies suggest a decrease in glutamate neurotransmission. However, it is not clear whether glutamatergic activity normalizes or remains altered in patients with schizophrenia as compared to healthy individuals. Up until now, data concerning glutamatergic neurotransmission in chronic schizophrenia are inconclusive. In upcoming high field ¹H-MRS studies, we will investigate glutamatergic activity during the course of schizophrenia.

KEY WORDS
Schizophrenia, magnetic resonance spectroscopy, glutamate, glutamine

TELEPHONE-NUMBER: 088-7553377
E-MAIL-ADDRESS: a.marsman@umcutrecht.nl
TITLE
MUNC18-1 BINDING TO THE SNARE COMPLEX IS NOT A PREREQUISITE FOR SYNAPTIC VESICLE FUSION

AUTHORS
Marieke Meijer\textsuperscript{1}, Pawel Burkhardt\textsuperscript{2}, Ruud Toonen\textsuperscript{1}, Dirk Fasshauer\textsuperscript{2}, Matthijs Verhage\textsuperscript{1}

DEPARTMENT/INSTITUTE
\textsuperscript{1} Dept. of Functional Genomics, Center for Neurogenomics and Cognitive Research, VU University, Amsterdam
\textsuperscript{2} Research Group Structural Biochemistry, Max Planck Institute for Biophysical Chemistry, Göttingen, Germany.

ABSTRACT
The presynaptic protein Munc18-1 is indispensable for mammalian neuronal exocytosis but its exact role remains heavily debated. The earliest crystal structure shows Munc18-1 embracing Syntaxin1a in its closed conformation, preventing SNARE complex formation and thus inhibiting synaptic transmission. Recently it became clear that Munc18-1 also binds to open Syntaxin, even within assembled SNARE complexes, which could promote or even be required for vesicle fusion. The aim of this study is to clarify the Munc18-1/ Syntaxin1a interaction. We make use of two mutations targeted to disrupt the ability of Munc18-1 to bind SNARE complexes: Munc18(F115E) and Munc18(L130K). ITC measurements show that both mutations indeed disrupt SNARE complex binding and in addition reduce the affinity for monomer Syntaxin1a. Patch clamp recordings from autaptic hippocampal neurons expressing our mutant proteins on a null background reveal a subtle stimulating effect on short-term plasticity while basal transmission is unaffected. Munc18-1 binding to SNARE complexes is thus not a prerequisite for fusion. The mutations might even stimulate release by promoting a complex consisting of Syntaxin1 and SNAP25 which is thought to precede SNARE complex formation.

KEY WORDS
Munc18, Syntaxin, synaptic transmission

TELEPHONE-NUMBER: 020-5987792
E-MAIL-ADDRESS: marieke.meijer@cncr.vu.nl
CHARACTERIZATION OF HUMAN POST MORTEM MICROGLIA ISOLATED BY FACS SORTING

AUTHORS
Jeroen Melief1, Nathalie Koning2, Tessa van der Maaden1, Marco van Eijk3, Jörg Hamann3, Robert Hoek1, Inge Huitinga1

DEPARTMENT/INSTITUTE
1 Netherlands Institute for Neuroscience, Amsterdam
2 VU University medical center, Amsterdam
3 Academic Medical Center, Amsterdam

ABSTRACT
Infiltrating monocyte-derived macrophages play a central role in MS pathogenesis by demyelinating axons. In addition to these macrophages, activated microglia are highly likely to contribute to demyelination, although much is unknown about the exact mechanisms involved in this. Studies of human microglia are exceptional as these cells are difficult to access in a pure and inactivated form. We show a rapid procedure to isolate, sort and culture microglia with high purity from post-mortem human brain tissue. The techniques used include density gradient separation and subsequent fluorescence activated cell sorting (FACS) based on expression of CD11b and CD45 on cells isolated from corpus callosum white matter and choroid plexus. Tissue samples from brain donors with diverse clinical backgrounds were obtained through the Netherlands Brain Bank (www.brainbank.nl). Initially, FACS sorting was severely hampered by the presence of autofluorescence, a common problem in post-mortem human tissue that is caused by apoptotic cells and debris. The addition of a vital dye to the staining protocol enabled us to discard autofluorescence. After this, microglia could be sorted on the basis of their CD11b+CD45dim phenotype, in parallel with the FACS procedure developed by Sedgwick et al. in rat brain. Little is known about the factors involved in microglia activation and differentiation. In vitro stimulation of monocyte-derived macrophages results in polarized cells with different pro- and anti-inflammatory properties. Therefore, studies are now ongoing to investigate whether microglia and other brain-associated myeloid cells polarize in the same manner.

KEY WORDS
Human microglia, Multiple Sclerosis

TELEPHONE-NUMBER: 020-5665508
E-MAIL-ADDRESS: j.melief@nin.knaw.nl
GFAP SPLICE VARIANT EXPRESSION IN ASTROCYTES IS ASSOCIATED WITH AMYLOID PLAQUES IN ALZHEIMER’S DISEASE

AUTHORS
Jinte Middeldorp, Mark Mizee, Jennifer Smit, Jacqueline A. Sluijs, W. Kamphuis, Elly M. Hol

DEPARTMENT/INSTITUTE
Astrocyte Biology & Neurodegeneration, Netherlands Institute for Neuroscience, Amsterdam

ABSTRACT
Glial fibrillary acidic protein (GFAP) is the main intermediate filament protein of astrocytes in the adult brain. The GFAP gene generates multiple isoforms, with GFAPα being the most prevalent. Two out-of-frame splice variants named GFAPΔ164 and GFAPΔexon6 are translated in GFAP proteins with a frameshifted carboxy-terminus that is recognized by a specific antibody called GFAP+1. This antibody stains a specific subset of astrocytes in the human brain and spinal cord.

Alzheimer’s Disease (AD) is the most common form of dementia. One of the main hallmarks of this neurodegenerative disease is the extracellular deposition of the amyloid beta protein (Aβ) that forms plaques in the brain. Astrogliosis, a process characterized by the altered morphology of astrocytes and the increased expression of GFAP is associated with plaque formation in AD.

In this study, we focused on the GFAP+1 isoforms in relation to AD. We discovered that this specific isoform is not expressed during normal brain development and in healthy adults, but only in elderly and AD brains. GFAP+1 positive astrocytes and amyloid plaques were visualized by immunofluorescence in hippocampal cryosections from non-demented controls (Braak stage 0-3) and Alzheimer patients (Braak stage 4-6). Quantitative analysis performed on 10 donors per Braak stage resulted in a significant positive correlation (p<0.01) between the number of GFAP+1 positive cells and the number of amyloid plaques. In addition, GFAP+1 staining was found around plaques in a mouse model of AD, whereas wildtype mouse brains completely lacked GFAP+1 expression.

We hypothesized that Aβ could induce GFAP+1 expression. We are currently testing this hypothesis in vitro, by analyzing the GFAP Δ164/Δexon6 mRNA and protein expression after exposure to Aβ42 oligomers and fibrils in human astrocytes. To further characterize the identity of GFAP+1 astrocytes in Alzheimer and control hippocampi we are performing several co-localization studies with markers for reactive astrocytes, neural progenitors and proliferation.

Future research is required to determine its functional implications in relation to the disease state.

KEY WORDS
GFAP, astrocytes, Alzheimer, splicing

TELEPHONE-NUMBER: 020-5665508
E-MAIL-ADDRESS: j.middeldorp@nin.knaw.nl
Multiple sclerosis (MS) is a chronic demyelinating disease of the central nervous system (CNS), characterized neuropathologically by multiple focal lesions scattered throughout the CNS. Normally, the blood-brain barrier (BBB) prevents the entrance of circulating molecules and immune cells into the CNS by the presence of tight junctions. The BBB also acts as a molecular barrier through the presence of specific endothelial efflux systems (ATP-binding cassette (ABC) transporters). A loss of integrity of the BBB has been described for a variety of neuroinflammatory diseases like MS, but so far data on ABC protein expression and function at the BBB under pathological conditions and the downstream effect on brain homeostasis is lacking. Since ABC transporters are essential in removing detrimental compounds from CNS tissue, loss of expression or function could contribute to the tissue damage observed in MS. We therefore investigated, in various well-characterized post-mortem MS lesions, the expression pattern of different ABC transporters (P-glycoprotein, MRP-1, -2 and BCRP) in various lesion stages. Interestingly, reactive astrocytes and infiltrating myelin-laden macrophages in MS lesions displayed enhanced ABC transporter expression compared to control white matter, which coincided with increased substrate-specific activity in vitro in these cells. Moreover, we observed a striking decreased microvascular expression of P-gp in MS lesions, which correlated with a decreased in vivo P-gp function during experimental autoimmune encephalomyelitis (EAE), an animal model for MS. Loss of P-gp in MS and EAE lesions coincided with the presence of surrounding infiltrated lymphocytes. Our data strongly suggest that CD4+ T cells are the major inflammatory cell type that induce brain endothelial P-gp malfunction by triggering a novel NF-κB signalling pathway through intracellular adhesion molecule-1 (ICAM-1). Together, our data suggest that BBB dysfunction is a critical determinant in MS lesion formation, and alterations in ABC transporter expression and function may contribute to tissue damage by disturbing brain homeostasis during MS pathogenesis.
TOWARDS USING NEUROPILIN-1 RECEPTOR-BODIES AS SCAVENGERS TO NEUTRALISE SEMAPHORIN 3A FUNCTION IN ALS

Elizabeth B. Moloney, Erich Ehlert, Barbara Hobo, Tam Vo, Bas Blits, Joost Verhaagen

Dept. of Neuroregeneration, Netherlands Institute for Neuroscience, KNAW, Amsterdam

Amyotrophic Lateral Sclerosis (ALS) is one of the most common forms of adult-onset neuromuscular disease, with a global incidence rate of 1-2 per 100,000. Progressive degeneration of peripheral motor neurons subsequently leads to atrophy of the skeletal muscle causing muscle weakness as one of many initial symptoms. 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 mice (due to sciatic nerve crush) resulted in increased expression of Semaphorin 3A (sema3A) in terminal Schwann cells (TSC) enwrapping the neuromuscular junction (NMJ) of Type Ib muscle fibres. Sema3A expression in TSCs was also observed in G93A-hSOD1 mice (a mouse model for fALS) upon onset of the disease. Interestingly, type Ib muscle fibers are thought to be the first fibers to degenerate in ALS.

Sema3A is a member of the class III semaphorins; secreted chemorepellant molecules which are expressed during development that play a role in axon guidance. In spinal cord injury increased Sema3A expression is present in the scar tissue, creating a growth inhibitory environment for damaged axons and preventing them from passing the lesion site to reconnect with their target. Sema3A interacts with Neuropilin-1 (nppn-1, a membrane bound receptor) and PlexinA (co-receptor) for signalling. Neuropilin-1 is also a receptor for VEGF-A. Thus Neuropilin-1 can mediate a variety of effects from axonal guidance, to migration and angiogenesis.

We propose that Sema3A plays an important role in the pathogenesis of ALS at the initial stages of the disease. By using a soluble version of nppn-1 to act as a scavenger for sema3A (via viral gene therapy administration), we aim to neutralise the growth inhibitory functions of sema3A at the NMJ. The neuropilin gene was truncated to remove the trans-membrane and cytosolic domains and an IgG Fc fragment (for stability and detection) was fused in their place, creating the neuropilin receptor-body construct. To date, both the Neuropilin-1 and Neuropilin-2 (Sema3F receptor) genes have been cloned (in their truncated forms) into a lenti-viral (LV) vector, and expression analysis from HEK 293T cells confirms that the truncated proteins are produced and secreted by these cells. Preliminary data from a growth cone collapse assay suggests that the Neuropilin-1 receptor-body functions as a Sema3A scavenger by preventing sema3A-associated growth cone collapse of dorsal root ganglion (DRG) neurites in vitro. Once the in vitro results confirm the functionality of neuropilin-bodies to scavenge and neutralize Sema3A function, we plan to move into an in vivo model using the G93A-hSOD1 mouse. Currently in progress is the cloning of the neuropilin-bodies into adeno-associated viral (AAV) vectors, which will then be used to produce AAV6 virus. Literature implicates this serotype as being the best for transduction of muscle cells, in particular skeletal muscle. By infecting ALS mice with this virus, we will determine how efficient this gene therapy approach is in ameliorating disease progression and/or severity with a variety of behavioural and histological analyses. In addition, we are currently preparing expression vectors to generate a mouse which is afflicted with ALS, but also has a conditional knockout of Sema3A in TSCs (using a triple transgenic approach).

Semaphorin 3A, neuropilin-1, amyotrophic lateral sclerosis, motor neuron degeneration, adeno-associated viral vectors

020-5665512
e.moloney@nin.knaw.nl
tRNA SPlicing ENdonuclease MUTATIONS CAUSE PONTOCEREBELLAR HYPOPLASIA

Yasmin Namavar¹, Paul Kasher¹, Birgit S. Budde², Peter G. Barth³, Bwee Tien Poll-The³, Kees Fluitert⁴, Eleonora Aronica⁴, Andrew J. Grierson⁵, Paula van Tijν⁶, Fred van Ruissen¹, Marian Weterman¹, Danica Zivkovic⁶, Peter Nürnberg², Frank Baas¹

Department/Institute
¹ Department of Neurogenetics, Academic Medical Center, University of Amsterdam, Amsterdam
² Cologne Center of Genomics and Institute of Genetics, University of Cologne, Cologne, Germany
³ Division of Pediatric Neurology, Emma Children’s Hospital/ Academic Medical Center, Amsterdam
⁴ Department of Pathology, Academic Medical Center, University of Amsterdam, Amsterdam
⁵ Academic Unit of Neurology, School of Medicine and Biomedical Sciences, University of Sheffield, Sheffield, UK
⁶ Hubrecht Institute, Royal Netherlands Academy of Arts and Sciences & University Medical Centre Utrecht, Utrecht

ABSTRACT
Pontocerebellar hypoplasia (PCH) represents a group of neurodegenerative autosomal recessive disorders with prenatal onset (PCH1-6). Children suffer from severe mental and motor impairments due to atrophy or hypoplasia of the cerebellum, hypoplasia of the ventral pons, microcephaly and variable neocortical atrophy. The disease is progressive and usually patients die before they reach adulthood.

We identified a common mutation in TSEN54 (A307S) in the majority of European PCH2 patients. TSEN54 is one of the four subunits of the tRNA splicing endonuclease (TSEN34, TSEN2 and TSEN15). The TSEN complex is responsible for the splicing of intron containing tRNAs and also plays a role in pre-mRNA 3’end formation. In PCH patients without the A307S mutation, we identified other missense and nonsense mutations in TSEN54, TSEN34 and TSEN2 subunits.

In situ hybridization for TSEN54 using LNA/2OME probes revealed that TSEN54 is highly expressed in neurons of the pons, cerebellar dentate and olivary nuclei.

Northern blot analysis of tRNA-Tyrosine from fibroblasts of 3 patients did not show unspliced products. The molecular mechanism behind pontocerebellar hypoplasia remains unclear. In order to study the mechanisms underlying PCH we performed knock down experiments in zebrafish. Injection of TSEN54 or TSEN 2 morpholino oligonucleotides in zebrafish embryos results in similar neurodevelopmental phenotypes, most prominently affecting brain development.

KEY WORDS
tRNA splicing, pontocerebellar hypoplasia, zebrafish

TELEPHONE-NUMBER: 020-5662518
E-MAIL-ADDRESS: y.namavar@amc.uva.nl
ENDOPLASMIC RETICULUM STRESS IN ALZHEIMER’S DISEASE: EFFECTS ON CELLULAR PROTEOLYSIS

AUTHORS
Diana A.T. Nijholt¹, E.S. van Haastert³, T.R. de Graaf¹, J.M. Rozemuller³, P. Eikelenboom², F. Baas¹, J.J.M. Hoozemans³, W. Scheper¹,²

DEPARTMENT/INSTITUTE
¹ Neurogenetics Laboratory and ² Dept. of Neurology, Academic Medical Center, Amsterdam
³ Dept of Neuropathology, VU University medical center, Amsterdam

ABSTRACT
At the histopathological level Alzheimer’s disease (AD) is characterized by the accumulation of plaques composed of amyloid β (Aβ) and neurofibrillary tangles (NFT’s) composed of hyperphosphorylated tau. Previous studies in our group and others have demonstrated involvement of the unfolded protein response (UPR) in Alzheimer’s disease. Activation of the UPR is observed relatively early in the disease process in neurons that contain little to no tau pathology, suggesting endoplasmic reticulum (ER) stress precedes tangle formation.

The accumulation of Aβ and tau makes AD a protein-misfolding disease and suggests that alterations in the cellular degradation machinery might be involved in disease pathogenesis. Misfolded or aggregated proteins might be degraded via the Ubiquitin Proteasome System (UPS) or via autophagy. Ubiquitin is found to be associated with NFT’s, neuritic plaques and neuropil threads. Data generated in our lab demonstrate ubiquitin positive inclusions in neurons that stain positive for UPR activation, suggesting involvement of the UPS. However, another hallmark of AD, granulovacuolar degeneration (GVD) is thought to be related to autophagy.

In this study we investigate the relation between UPR activation and the different proteolytic machineries. Preliminary data indicates UPR activation decreases proteasome activity but enhances autophagy in a neuronal cell model. Combined, our data suggest autophagy is the preferred route of degradation following UPR activation.

KEY WORDS
Alzheimer’s disease, ER stress, UPS, autophagy

TELEPHONE-NUMBER: 020-5662518
E-MAIL-ADDRESS: a.t.nijholt@amc.uva.nl
THE ROLE OF THE 5-HT₃ RECEPTOR IN THE POSTNATAL DEVELOPMENT OF THE CEREBELLUM

Marlies Oostland, J.A. van Hooft

Center for Neuroscience, SILS, University of Amsterdam, Amsterdam

The serotonin 3 receptor is the only ligand-gated serotonin receptor and is known to be expressed on interneurons in limbic regions such as the cortex, amygdala and hippocampus. Cajal-Retzius cells in layer I of the cerebral cortex express 5-HT₃ receptors and regulate postnatal dendritic maturation of pyramidal neurons in the somatosensory cortex via excretion of the glycoprotein reelin. We recently found that during the first three postnatal weeks, the 5-HT₃ receptor is also expressed abundantly in the cerebellar cortex, except in the Purkinje cell layer. Since the cerebellum continues to develop postnatally, the role of the 5-HT₃ receptor in the postnatal maturation of the cerebellum will be further investigated. Immunostaining with an antibody against reelin will reveal the localisation of the glycoprotein reelin in the cerebellum. Electrophysiology will be used to examine the functional properties of the 5-HT₃ receptor in dissociated cerebellar cells. Furthermore, the effect of a lack of 5-HT₃ receptors on the morphology of Purkinje cells will be examined in 5-HT₃A receptor knockout mice. Together, these results can provide an overview of the role of the 5-HT₃ receptor in the postnatal development of the cerebellum.

5-HT₃ receptor, cerebellum, postnatal development

020-5258258
m.oostland@uva.nl
PROTEASOMAL INHIBITION BY Aβ IN ASTROCYTES IN ALZHEIMER’S DISEASE

Marie Orre, W. Kamphuis, E. Hol

Astrocyte Biology & Neurodegeneration, Netherlands Institute for Neuroscience, Amsterdam

One of the main hallmarks of Alzheimer’s disease (AD) is the accumulation of Amyloid β proteins (Aβ) forming plaques in the brain. Reactive astrocytes characterized by morphological changes and increased expression of cytoskeletal proteins such as GFAP and vimentin are found surrounding these plaques. The transition from a quiescent to an activated astrocyte is named gliosis. The precise trigger for astrogliosis is not fully known; moreover the contribution of astrogliosis to the progress of the AD pathogenesis is not clear. Another main characteristic of AD is accumulation of misfolded proteins within the brain which indicates that the ubiquitin proteasomal system (UPS), a system that controls degradation of misfolded proteins and short lived molecules such as transcription factors might be disrupted in AD. Based on this knowledge we propose that the Aβ deposits interfere with the UPS activity in astrocytes and underlie their activation. We have set out to investigate the UPS function in astrocytes after treatment with Aβ and known proteasomal inhibitors by using a novel fluorescent probe that binds to the active sites of the proteasome and monitors the activity. Furthermore we will study what cellular pathways are altered in the astrocytes after Aβ exposure by using a genomic approach and bioinformatics. Insights in the astrocyte response to Aβ and the involvement of the proteasome might lead to new treatment strategies to counteract the formation of plaques in the AD brain.

Alzheimer’s Disease, astrocytes, Amyloid β, ubiquitin proteasomal system

020-5665508
m.orre@nin.knaw.nl
EFFECTS OF CORTICOSTERONE PULSITILITY ON THE EXCITABILITY (MEPSCS) OF GRANULAR NEURONS OF DENTATE GYRUS

AUTHORS
Natasha Pasricha1,2, Marian Joels1,2, Henk Karst1,2

DEPARTMENT/INSTITUTE
1 SILS-Center for Neuroscience, University of Amsterdam, Amsterdam
2 Department of Neuroscience and Pharmacology, Rudolf Magnus Institute of Neuroscience, University Medical Center Utrecht, Utrecht

ABSTRACT
Following stress, the rodent brain is exposed to elevated levels of corticosterone, catecholamines like noradrenaline and neuropeptides. Up till now it was generally thought that noradrenaline and peptides are the main actors in the initial phase of the stress response. Corticosteroids were thought to be important later on, for normalization of brain activity and consolidation of the event, via a genomic pathway. Recent data from our group, however, show that corticosteroids also exert rapid non-genomic effects on hippocampal cell properties, via mineralocorticoid receptors in the membrane.

To study the corticosteroid effects on hippocampal functioning of mice, we first compared CA1 and DG. From preliminary results, differences in the effect of corticosterone on CA1 and DG were observed. In CA1, e.g. an increase in Ca2+ currents and mEPSCs amplitude had been seen via delayed genomic pathway and an increase in mEPSCs frequency was observed via rapid non-genomic pathway. In the DG, there was no delayed effect on Ca2+ currents. Rapid or delayed effects on mEPSCs were not examined yet. We hypothesize that corticosterone 1) rapidly changes the mEPSC frequency but 2) that there is no delayed effect on mEPSCs in DG. Indeed from this study we can conclude that corticosterone induces a rapid increase in mEPSC frequency. The work on the delayed effect is in progress.

Also, recent studies have shown that circulating corticosterone hormones are secreted in a pulsatile pattern by adrenal glands, showing the ultradian rhythm. The availability of hormones to corticosteroid receptors in pulsatile pattern is of functional significance (in the brain). Therefore, it is important to determine the physiological importance of the rapid corticosteroid effects by studying variations in CA1 and DG mEPSC properties during pulsatile corticosterone release. We propose that the membrane mineralocorticoid receptors are the only means for hippocampal cells to quickly and accurately translate fluctuations in hormone level into changes in electrical activity.

KEY WORDS
Corticosterone, hippocampus, CA1; Dentate Gyrus, mEPSCs, delayed genomic pathway, rapid non-genomic pathway, MRs, ultradian rhythm, pulsatility, circadian rhythm

TELEPHONE-NUMBER: 088-7568877
E-MAIL-ADDRESS: n.pasricha@uva.nl / n.pasricha@umcutrecht.nl
TITLE
REACTIVATION DURING SLEEP OF RECENTLY ACQUIRED MEMORY

AUTHORS
Giovanni Piantoni, Ysbrand D. van der Werf, Eus J.W. van Someren

DEPARTMENT/INSTITUTE
Dept. of Sleep & Cognition, Netherlands Institute for Neuroscience, Amsterdam

ABSTRACT
Previous research has shown that sleep helps to consolidate recently acquired memory. The mechanisms responsible for this memory boost are, however, still unknown. One hypothesis suggests that connections between neurons that have been activated during a task are re-activated during subsequent sleep. Several lines of evidence have lent support to this hypothesis, but, while intracranial recordings in animals have consistently shown reactivation of recently activated neural networks, results in humans have not been consistent.

In this two-day experiment, MEG signal was acquired from twelve participants performing two tasks which are known to engage two very different memory systems: a procedural learning task (in which they were instructed to mirror-trace a trajectory presented on the screen) and a declarative memory task (in which they were asked to learn the association between a name and a face). After each task, participants were invited to take a nap of a duration of 90 minutes, roughly equivalent to the duration of a sleep cycle. Eight participants managed to reach NREM stage 2 sleep during the MEG scanning session and their data were included in the analysis.

The analysis consisted of two steps: (1) identifying regions of interest based on the difference in activation between the two tasks and (2) compare the activity of those regions during the subsequent sleep. Regions of interest were defined as the areas in which the power spectra were maximally different between the two tasks. The spatial distribution of the difference maps was remarkably consistent in the beta band (12-30 Hz). We then compared the activity of the selected regions during NREM stage 2 and preliminary results point to a significant difference in activation for those regions during sleep. This provides evidence for the hypothesis that brain activity during sleep depends at least in part on preceding exposure.

KEY WORDS
Sleep, MEG, memory, reactivation

TELEPHONE-NUMBER: 020-5665492
E-MAIL-ADDRESS: g.piantoni@nin.knaw.nl
NEUROBIOLOGICAL CORRELATES OF DISRUPTIVE BEHAVIOUR DISORDER IN A NORMAL POPULATION; DIFFERENCES BETWEEN BOYS AND GIRLS

Evelien Platje, L.M.C. Jansen, R.R.J.M. Vermeiren, Th.A.H. Doreleijers

Dept. of Child and Adolescent Psychiatry, VU University medical center, Amsterdam

ABSTRACT
An approximated 5% of all adolescents in the general population display such deviant behaviour that they are diagnosed with Disruptive Behaviour Disorder (DBD), incorporating both Oppositional Defiant Disorder and Conduct Disorder. Both include children or adolescents who are hostile, aggressive and display antisocial and delinquent behaviour. The low arousal theories try to explain this behaviour from a neurobiological perspective; hypo-activity of the autonomic nervous system and the hypothalamic-pituitary-adrenal-axis. Decreased levels of ANS and/or HPA activity, are thought to constitute a aversive physiological state. In order to increase this neurobiological activity, these children seek stimulation, which could result in antisocial behaviour (Zuckerman, 1979). Another theory states that these children are fearless, they do not physically feel fear in situations where most people would (Raine, 1993).

In clinical DBD samples these low arousal theories are often, but not consistently, confirmed. In normal population samples, results are even more inconclusive. Normal population studies may be hampered by the low prevalence of disruptive behaviour, which is especially true for girls, who have consequently remained under investigated.
Therefore, in the present population study, both boys and girls from the normal population with a high risk of developing DBD were over-sampled. The study is part of a large longitudinal population cohort study (RADAR), in which 280 boys and 217 girls were first investigated at age 12. DBD was diagnosed using the Diagnostic Interview Schedule for Children (DISC-P). To assess aggressive and delinquent behaviour, both parents filled out the Child Behaviour Check List (CBCL) and the adolescents filled out the Youth Self Report (YSR). Saliva was sampled at awakening, and 30 and 60 minutes later, for determining the Cortisol Awakening Response.

Preliminary results revealed that girls with a DBD diagnosis show the expected low cortisol levels ($F_{(1,125)}=6.995, p=.009$), but boys with DBD do not ($F_{(1,155)}=.838, ns$). This seems to be specifically related to aggressive behaviour in girls ($F_{(1,131)}=10.871, p=.001$) and not to delinquency.

This indicates that disruptive behaviour in girls may be more severe, and stronger associated with neurobiology, than in boys in the normal population. Since particularly aggression, but not delinquency, is related to decreased cortisol levels, disruptive behaviour should be, in relation to basal cortisol, assessed more distinctively. In spite of over-sampling of high-risk youth, the DBD and borderline/clinical groups are small, suggesting that even stronger over-sampling methods may be needed in future research.

KEY WORDS
Disruptive Behaviour Disorders, aggression, delinquency, cortisol, HPA-axis

TELEPHONE-NUMBER: 020-8901351
E-MAIL-ADDRESS: e.platje@debasacle.com
Introduction

Eyes-closed rest EEG/MEG is used for assessing the functional state of the brain in pre-clinical research—typically in terms of disease influences on neuronal oscillation frequency, amplitude, or coherence. However, neuronal oscillations fluctuate considerably in time and amplitude and this is not captured by the classical analyses.

The study of spatial and temporal dimensions of neuronal processing requires different correlation analyses. Coordination of anatomically distributed activity (parallel processing) may be studied by computing correlations between neuronal signals from different brain areas (Cross-correlations). In contrast, coordination of brain activity over time (serial processing) may be studied by computing auto-correlations in neuronal signals within a single brain area (Auto-correlations). See Figure.

Methods

The MATLAB-based "Neurophysiological Biomarker Toolbox" (or NBT) provides algorithms for characterizing the temporal structure of ongoing oscillations.

Results

Using the NBT, we have observed a prominent decrease in auto-correlations in early-stage Alzheimer's disease (Montez & Poil et al., PNAS, 2009). See Figure.

Conclusions

The temporal structure of ongoing oscillations may be important for brain function and its quantitative analysis provides an important insight into the functional organization of the brain in pre-clinical research using resting-state EEG/MEG. We invite collaborators to use our NBT toolbox (Linkenkaer-Hansen et al., 2007).

KEY WORDS

Ongoing oscillations, MEG, EEG, biomarkers, Alzheimer’s disease

TELEPHONE-NUMBER: 020-5982833
E-MAIL-ADDRESS: simonshlomo.poil@cncr.vu.nl
WEBPAGE: http://www.poil.dk/s
THE ROLE OF ATTENTION IN FIGURE-GROUND SEGREGATION IN V1 AND V4

Jasper Poort, Aurel Wannig, Pieter R. Roelfsema

Vision and Cognition, Netherlands Institute for Neuroscience, Amsterdam

ABSTRACT
The visual cortex segregates the scene into figures and background. A correlate of this segregation is observed in primary visual cortex (V1), where neurons have an enhanced response when their receptive field (RF) is on a figure compared to when it is on the background, an effect known as Figure-Ground Modulation (FGM). FGM starts early after the presentation of the stimulus if the RF is on the edge of a figure (edge modulation), and it appears later when the RF is on the centre of the figure (centre modulation) as if the FGM gradually fills in the figure starting from the edges. Edge modulation presumably reflects local computations within area V1, while centre modulation has been hypothesized to reflect the feedback from higher visual areas. If so, centre modulation might depend on visual attention as attentional effects are generally believed to originate from feedback while edge modulation might be less dependent on attention. To investigate the role of attention in FGM, we here recorded multi-unit activity from V1 and area V4 of macaque monkeys. We used two tasks with identical visual stimulation, where figures were either relevant or completely irrelevant, and presented them so that the RF was on the centre, the edge of the figure, or on the background.

The FGM during the initial response epoch (before 150ms) did not depend on the relevance of the figure, but after 150ms attention started to influence the responses. When attention was directed away from the figure, FGM in V1 and V4 was substantially weaker than when the figure was attended. Moreover, the involvement of attention in FGM differed between centre and edge. If the figure was attended, FGM in the centre of the figure became as strong as FGM at the edge. If the figure was not attended, however, centre modulation was much weaker while edge modulation was only slightly reduced. The latency of the edge modulation in area V1 and V4 was similar and earlier than the V1 centre modulation, in accordance with higher visual areas acting as the source for V1 centre modulation.

In conclusion, these results show that selective attention and figure-ground segregation interact. Edge modulation is largely independent of attention, whereas centre modulation is greatly reduced in the absence of attention as if the edge comes for free but filling in of the centre of the figure only occurs if the figure is relevant.

KEY WORDS
Visual cortex, attention, figure-ground segregation

TELEPHONE-NUMBER: 020-5664841
E-MAIL-ADDRESS: j.poort@nin.knaw.nl
**TITLE**
NICOTINE RAPIDLY DESENSITIZES NICOTINIC RECEPTORS IN THE PREFRONTAL CORTEX AFFECTING ENDOGENOUS MODULATION OF THE NEURONAL NETWORK BY ACETYLCHOLINE

**AUTHORS**
Rogier B. Poorthuis, Huibert D. Mansvelder

**DEPARTMENT/INSTITUTE**
Integrative Neurophysiology, CNCR, Neuroscience Campus Amsterdam, VU University, Amsterdam

**ABSTRACT**
Nicotine has a stimulating effect on cognitive functioning, especially on attention and working memory related tasks. A key structure in attention-related tasks is the prefrontal cortex (PFC). Nicotinic acetylcholine receptors are highly expressed in this area, suggesting that part of nicotine's actions occur directly at the level of the PFC circuitry. In layer V of the PFC it was found that nicotine activates receptors on both thalamic glutamateric inputs to pyramidal neurons and on local GABAergic interneurons. Besides receptor activation on the short-term, nicotine is also able to desensitize receptors on a longer timescale (minutes). Hereby it interacts with ongoing endogenous cholinergic signalling, which has been shown to be involved in cognitive operations during attention tasks. Currently it is unknown whether nicotine desensitizes nicotinic receptors in the prefrontal cortex. In this study we investigate how layer V and layer II-III of the prefrontal cortex are modulated by acetylcholine and how concentrations of nicotine seen during smoking change the responsiveness to acetylcholine. We found that bath application of acetylcholine enhances glutamatergic signals onto layer V pyramidal neurons. In layer II-III, however, glutamatergic inputs to pyramidal neurons are not affected by nicotinic receptor activation. This data indicate that information carried through glutamatergic projections coming from other brain areas is not enhanced by nicotinic receptors in layer II/III. In addition, acetylcholine enhanced inhibitory signalling onto pyramidal neurons in both layer II/III and layer V. Nicotine, in concentrations seen by smokers (300 nM), transiently increased inhibitory signalling in LV, but not in layer II/III. Prolonged exposure to nicotine decreased inhibitory currents again indicating that nicotinic receptors desensitize. Indeed, acetylcholine was not able to enhance GABAergic signalling anymore in both layers after prolonged exposure to nicotine. Simultaneously nicotine enhances glutamatergic signalling onto LV pyramidal neurons transiently whereas it desensitizes nicotinic receptors and acetylcholine is not able to enhance excitative signalling anymore. In addition, we investigated which interneurons show nicotinic currents upon pressure application of acetylcholine. Nicotinic receptors (nAChR) were found to be present on fast-spiking, low-threshold and regular-spiking interneurons in both layer II/III and LV. We are currently investigating how fast these receptors on single cells desensitize.

Together these data point to a situation in which nicotine during smoking desensitizes nicotinic receptors in the prefrontal cortex within minutes. Given the fact that nAChR can be continuously activated by endogenous acetylcholine, nAChR desensitization can affect ongoing neuronal activity just as nAChR activation. By doing so, it could affect ongoing behaviour in which acetylcholine signalling plays an important role.

**KEY WORDS**
Nicotine, acetylcholine, PFC, desensitization, neuronal networks

**TELEPHONE-NUMBER:** 020-5987099
**E-MAIL-ADDRESS:** rogier.poorthuis@cnrc.vu.nl
THE TRIPLE REUPTAKE INHIBITOR DOV216,303, A PUTATIVE NEW ANTIDEPRESSANT, DECREASES ICSS THRESHOLDS WITHOUT PRODUCING WITHDRAWAL EFFECTS

Jolanda Prins\textsuperscript{1,2}, P.J. Kenny\textsuperscript{3}, I. Doomernik\textsuperscript{1,2}, B. Olivier\textsuperscript{1,2}, S.M. Korte\textsuperscript{1,2}

\textsuperscript{1} Dept. Psychopharmacology, Utrecht Institute for Pharmaceutical Sciences, Utrecht Univ., Utrecht, \textsuperscript{2} Rudolf Magnus Institute of Neuroscience, Utrecht Univ., Utrecht, \textsuperscript{3} Dept. Molecular Therapeutics, The Scripps Research Institute, Jupiter, Florida, USA

ABSTRACT

Selective serotonin reuptake inhibitors (SSRIs) are the most frequently prescribed antidepressant agents, and are relatively safe to use. Nevertheless, SSRIs can induce adverse side effects and have a long therapeutic lag-time. New promising antidepressants are triple reuptake inhibitors (TRIs), which not only enhance serotonin and norepinephrine neurotransmission, but significantly, also increase brain dopamine levels. By impacting dopamine transmission, TRIs are believed to have faster therapeutic onset than SSRIs, and may be particularly useful for the treatment of anhedonia (loss of pleasure and reward sensitivity), one of the core symptoms of depression. Importantly, the increased dopamine transmission induced by TRIs raises concerns related to their abuse potential and possible reinforcing effects. In this study, we directly assessed the reward-related effects of the TRI, DOV216,303. Specifically, we compared the effects of DOV216,303 and amphetamine, and their withdrawal, on intracranial self stimulation (ICSS) thresholds in rats. ICSS is considered a direct measure of brain reward function. Bipolar stimulating electrodes were implanted into the lateral hypothalamus of rats, and animals were trained in a discrete-trial current-threshold ICSS procedure. After stable ICSS reward thresholds were established, animals received one injection per day of DOV216,303 (20 mg/kg) or amphetamine (5 mg/kg) for four consecutive days. ICSS thresholds were assessed 3, 6, and 23 hours after injection and daily for ten days after the last injection. Both DOV216,303 and amphetamine decreased ICSS thresholds 3 hours after injection, suggesting a drug-induced potentiation of brain reward function. In contrast, whereas withdrawal from amphetamine induced significant elevations of ICSS thresholds 23 hours after each injection, threshold elevations were not observed at any time-point after DOV216,303 administration. These data suggest that DOV216,303 can transiently activate brain reward systems, but under present conditions does not induced long-term compensatory adaptations in reward circuitries similar to those induced by drugs of abuse like amphetamine.

KEY WORDS

Triple reuptake inhibitors, intracranial self stimulation, depression, anhedonia, reward

TELEPHONE-NUMBER: 030-2537382
E-MAIL-ADDRESS: j.prins1@uu.nl
**TITLE**

EXPRESSION OF STRESS-RELATED GENES IN THE HUMAN PREFRONTAL CORTEX IN MOOD DISORDERS

**AUTHORS**

Xin-Rui Qi¹ ², Jiang-Ning Zhou², Willem Kamphuis¹, Qian Wang³, Paul J.Lucassen³, Dick F. Swaab¹

**DEPARTMENT/INSTITUTE**

¹ Netherlands Institute for Neuroscience, Amsterdam
² Hefei National Laboratory for Physical Sciences at Microscale and Department of Neurobiology and Biophysics, Life Science School, University of Science and Technology of China, Hefei, Anhui, PR China
³ Center for Neuroscience, Swammerdam Institute of Life Science, University of Amsterdam, Amsterdam

**ABSTRACT**

Accumulating preclinical and clinical evidence indicates that the hypothalamic stress axis, i.e. the hypothalamo-pituitary-adrenal (HPA) axis, is hyperactive in a subset of depressed patients and represents an important common pathway in the pathogenesis of depression. There is a strong interaction between the HPA-axis and the prefrontal cortex (PFC) in stress regulation and depression. Also lesions in the PFC lead to an activated HPA-axis and depressive symptoms. Functional imaging studies have shown a multitude of alterations in the PFC of depressed patients. In an animal experimental model for depression we previously found an increased expression in stress-related mediators in the PFC. Here, we tested the hypothesis that a similar increase in stress-related molecules also occurs in the PFC of depressed patients in snap frozen human postmortem brain samples from the Netherlands Brain Bank. From cryostat sections we isolated grey matter from (i) the superior gyrus of the PFC (SPFC) of 14 subjects with mood disorders and 14 matched controls and from (ii) the anterior cingulate cortex (ACC) of 12 subjects with mood disorders and 12 matched controls. Both major depression (MD) and bipolar disorder (BD) patients were studied. MRNA expression levels of 20 genes were determined by quantitative real-time PCR and expression of several stress-related genes was found to be altered in both the SPFC and ACC of patients with mood disorders. Furthermore an imbalance of both GRα/MR and ER2/AR was found in both regions of the mood disorder groups. Some of the findings are now confirmed on the protein level. In conclusion, our results provide evidence for the dysregulations of stress-related molecules in the prefrontal cortex in mood disorders.

**Acknowledgement**

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**KEY WORDS**

Prefrontal cortex, anterior cingulate cortex, depression, post-mortem brain tissue

**TELEPHONE-NUMBER:** 020-5665505

**E-MAIL-ADDRESS:** x.qi@nin.knaw.nl
TITLE
ANTI-EPILEPTIC DRUGS BIND TO \(\alpha\)-SUBUNITS OF VOLTAGE-GATED NA\(^+\) CHANNELS WITH DIFFERENT BINDING KINETICS

AUTHORS
Xin Qiao, Guangchun Sun, Taco Werkman, Jeffrey Clare, Wytse Wadman

DEPARTMENT/INSTITUTE
Center for Neuroscience, Swammerdam Institute for Life Sciences, University of Amsterdam, Amsterdam

ABSTRACT
Voltage-gated Na\(^+\) channels play an important role in the generation and propagation of action potential in excitable cells like neurons. Voltage-gated Na\(^+\) channels are composed of a pore-forming alpha-subunit and auxiliary beta-subunits, and four different types of alpha-subunits (Na\(_{\text{V}1.1}\), Na\(_{\text{V}1.2}\) and Na\(_{\text{V}1.3}\) and Na\(_{\text{V}1.6}\)) comprise the human brain Na\(^+\) channels. Studies show that these four types of alpha-subunits have different expression patterns and functional roles in the central nervous system. Na\(_{\text{V}1.1}\) has been shown to be primarily expressed in inhibitory interneurons, which might explain why severe myoclonic epilepsy in infancy is associated with loss-of-function mutations in human SCN1A gene encoding the Na\(_{\text{V}1.1}\)subunit.

Several anti-epileptic drugs (AEDs) such as carbamazepine (CBZ), phenytoin (DPH), lamotrigine (LTG) block voltage-gated Na\(^+\) channels in a use-dependent manner. Pharmacoresistance is a common phenomenon in epilepsy patients where during time AEDs can lose their therapeutic efficacy. To explore whether different expression patterns and functional roles of the four brain alpha-subunits may play a role in pharmacoresistance, we compare the interaction of AEDs with these Na\(^+\) channel alpha-subunits.

We use HEK293 cell lines which stably express the four human Na\(^+\) alpha-subunits as preparation and the whole-cell voltage clamp technique to record voltage-gated Na\(^+\) currents. We determine AED effects on steady-state inactivation and removal of inactivation properties of the subunits and the binding and unbinding rates of the AEDs to the alpha-subunits. Our results show that the binding rates of LTG to Na\(_{\text{V}1.1}\) are faster than to the other subunits (Binding rate constants of LTG: Na\(_{\text{V}1.1}\): ~30 mM\(^{-1}\)s\(^{-1}\); Na\(_{\text{V}1.2}\): ~20 mM\(^{-1}\)s\(^{-1}\); Nav1.3: ~23 mM\(^{-1}\)s\(^{-1}\); Nav1.6: ~13 mM\(^{-1}\)s\(^{-1}\)). With relatively more Na\(_{\text{V}1.1}\) alpha-subunits being expressed in inhibitory interneurons, AED efficacy may be different in the interneurons (as compared to that of principal neurons). Treatment with AEDs may therefore have important consequences for network excitability, especially when altered expressions patterns occur in epileptic tissues. In addition, these differences in AED binding kinetics and shifting expression patterns could play a role in the development of pharmacoresistance.

KEY WORDS
Epilepsy, pharmacoresistance, Na\(^+\) channels, anti-epileptic drugs

TELEPHONE-NUMBER: 020-5257639
E-MAIL-ADDRESS: x.qiao@uva.nl
Aversive emotional learning has been studied extensively in rodents using Pavlovian Fear Conditioning. Although synaptic plasticity in the form of Long Term Potentiation (LTP) is known to be a critical molecular mechanism underlying the acquisition of fear memory, the role of glutamate receptor mediated synaptic plasticity in the extinction of learned fear remains unclear.

In this study, we aimed to assess the role of AMPA receptor mediated plasticity after memory retrieval in the dorsal hippocampus-dependent learning paradigm of contextual fear conditioning in the C57Bl/6J inbred mouse strain. During the memory retention test, performed 24 h after training, the animal not only retrieves the original aversive association of Conditioned Stimulus (CS) – Unconditioned Stimulus (US) that elicits fear response, but at the same time acquires a new associative memory of CS – no US. This new non-aversive association is thereafter consolidated into an extinction memory.

Synaptic hippocampal protein expression analysis by mass spectrometry and immunoblotting, 1 and 4 h after the memory retention test showed temporal down-regulation of glutamate receptor (AMPARs) subunits and AMPAR-interactors at the synapse. Furthermore, reduced AMPA currents measured at glutamatergic hippocampal synapses after the retrieval test support the hypothesis that reduction in expression levels at the synaptic membrane was due to endocytosis of the AMPARs from the membrane. Dorsohippocampal pretest injection of a synthetic peptide derived from the GluR2 carboxyl tail that blocks the regulated AP2-dependent AMPAR endocytosis inhibited the expression of extinction memory in subsequent retention tests, without affecting the the fear memory of the initial memory test.

These findings indicate that expression of new extinction learning may be attributed to stimulated endocytosis of postsynaptic AMPARs. Future experiments will be aimed at identifying downstream effects of this form of synaptic plasticity during the expression of the extinction memory trace.
MRI CORRELATES OF COGNITIVE DECLINE IN PATIENTS WITH TYPE 2 DIABETES

Yael D. Reijmer\textsuperscript{a}, Jeroen H.J.M. de Bresser\textsuperscript{b}, Esther van den Berg\textsuperscript{a}, Cynthia Jongen\textsuperscript{b}, Max A. Viergever\textsuperscript{b}, Roy P.C. Kessels\textsuperscript{c}, L. Jaap Kappelle\textsuperscript{a}, Geert Jan Biessels\textsuperscript{a} (on behalf of the Utrecht Diabetic Encephalopathy Study group)

\textsuperscript{a} Department of Neurology, University Medical Center Utrecht, Utrecht
\textsuperscript{b} Image Sciences Institute, University Medical Center Utrecht, Utrecht
\textsuperscript{c} Donders Institute for Brain, Cognition and Behaviour, Radboud University Nijmegen, Nijmegen

ABSTRACT

Background
Type 2 diabetes (DM2) is associated with an increased risk of cognitive dysfunction and dementia. In a previous cross-sectional study we demonstrated cognitive decrements and brain abnormalities in DM2 patients compared to controls. Patients and controls were re-examined after 4 years to study changes over time. The present sub study focuses on brain MRI abnormalities underlying cognitive decline in patients with DM2.

Methods
Cognitive functioning was assessed twice in 54 DM2 patients without dementia, with a 4-year interval. 1.5T MRI scans were obtained at both time-points. Automated volumetric measurements of subcortical structures, cortical gray-matter, lateral ventricles, white-matter lesions and cerebrospinal fluid volume were performed and expressed as a percentage of intracranial volume. Cognitive assessment consisted of 11 neuropsychological tasks, covering 5 cognitive domains. Only the 3 cognitive domains which showed a group difference at baseline were used for the present analyses (i.e. memory, information processing speed, attention and executive functioning). A regression based index, using the control group as a reference, was calculated to assess changes in cognitive test performance over time, adjusted for age, estimated IQ and sex. Changes in brain volume in DM2 patients with the 15\% greatest decline in cognitive performance on any of the three domains (n=22) were compared to the other DM2 patients (n=32).

Results
There were no significant differences in any of the baseline parameters between patients that attended (n=54) and patients that did not attend follow up (n=44). DM2 patients within the 15\% of greatest decline on any of the three domains showed a significant increase in white matter lesion volume (mean difference±S.E.: 0.12\%±0.05; p<0.05) and ventricular volume (0.21\%±0.06; p=0.001) over the 4-year period compared to DM2 patients without this reduction in cognitive performance. The groups did not differ on changes in subcortical structures (0.01\%±0.20) or cortical gray-matter volumes (-0.34\%±0.29).

Conclusion
These results indicate that white matter lesions and subcortical atrophy in DM2 may underlie the cognitive decline observed in a subgroup of DM2 patients.

KEY WORDS
Type 2 diabetes, cognitive decline, atrophy, white matter lesions

TELEPHONE-NUMBER: 088-7557969
E-MAIL-ADDRESS: y.d.reijmer@umcutrecht.nl
TITLE
MLC1 IS INVOLVED IN BRAIN WATER HOMEOSTASIS AND IS ASSOCIATED WITH CHLORIDE CHANNEL ACTIVITY

AUTHORS
Margreet C. Ridder1, Ilja Boor1, Johannes C. Lodder2, Nienke L. Postma1, Gert C. Scheper1, Huibert D. Mansvelder2, Marjo S. van der Knaap1

DEPARTMENT/INSTITUTE
1 Department of Pediatrics / Child Neurology, VU University medical center, Neuroscience Campus Amsterdam, Amsterdam
2 Department of Integrative Neurophysiology, Neuroscience Campus Amsterdam, Center for Neurogenomics and Cognitive Research (CNCR), VU University, Amsterdam

ABSTRACT
Megalencephalic leukoencephalopathy with subcortical cysts (MLC) is a progressive cerebral white matter disease with onset in childhood, caused by mutations in the MLC1 gene. MLC1 is a plasma-membrane protein of unknown function, which is mainly expressed in astrocytic endfeet near the blood-brain and cerebrospinal fluid-brain barriers. The function of MLC1 still remains unknown. The increased white matter water content in MLC1 patients and the expression of the protein in astrocytic endfeet near brain-fluid barriers suggest a role for MLC1 in maintaining brain water and ion homeostasis. We used whole cell patch-clamp electrophysiology to test the hypothesis that MLC1 is associated with ion channel function. Overexpression of MLC1 causes increased chloride currents in SF9's cells after hypotonic stimulation. In cultured cortical rat astrocytes reduced levels of MLC1 (by RNAi knockdown) decreases whole-cell chloride currents, whereas the delayed rectifier K+ current is not affected by knockdown of MLC1. Our findings imply that a defect in chloride channel activity in astrocytes affecting volume regulation underlies the white matter edema in MLC patients.

KEY WORDS
MLC1, astrocytes, dystrophin-glycoprotein complex, electron microscopy, leukodystrophy, Whole-cell patch clamp

TELEPHONE-NUMBER: 020-5982833
E-MAIL-ADDRESS: mc.ridder@vumc.nl
ALTERNATIVE SPLICING OF EPSILON-SARCglyCAN IN MYOCLONUS-DYSTONIA

Katja Ritz1, Barbera van Schaik2, Ted Bradley1, Marja E. Jakobs1, Eleonora Aronica3, Marina A. Tijssen4, Frank Baas1

1 Neurogenetic Laboratory, Academic Medical Center, University of Amsterdam, Amsterdam
2 Bioinformatic Laboratory, Academic Medical Center, University of Amsterdam, Amsterdam
3 Department of (Neuro)Pathology, Academic Medical Center, University of Amsterdam, Amsterdam
4 Department of Neurology, Academic Medical Center, University of Amsterdam, Amsterdam

Myoclonus-Dystonia (M-D) is an autosomal-dominant inherited movement disorder characterized by jerky movements and dystonic features. A major gene locus maps to human chromosome 7q21-31, the epsilon-sarcoglycan gene (SGCE), which encodes a transmembrane protein that is widely expressed in several tissues including the brain. The function of SGCE and the pathophysiology of the disease remain still unknown. Recently, new brain-specific exons coding for a different C-terminal of the protein have been identified in mouse and human suggesting a gain of function for those transcripts in the brain. To further investigate the qualitative and quantitative distribution of these newly identified isoforms massive parallel sequencing (454, FLX, Roche) and isoform-specific in-situ hybridizations (ISH) were performed. Preliminary data indicates that a brain-specific isoform is highly expressed in regions involved in movement control. ISH data of the brain-specific exon shows a high expression in neurons. In addition, we identified new SGCE isoforms. Their role still needs to be investigated.

Alternative splicing, epsilon-sarcoglycan, Myoclonus-Dystonia, deep sequencing

TELEPHONE-NUMBER: 020-5662518
E-MAIL-ADDRESS: k.a.ritz@amc.uva.nl
ABSTRACT
Several studies have shown that the amount of fatty acid in the blood can influence cognitive development and ability. It has been previously reported that common intronic variants of the FADS1 and FADS2 (Fatty Acid Desaturase 1 and 2) genes are associated with fatty acid level and composition in phospholipids in serum. Recently two independent groups found association of FADS3 and FADS2 with cognitive ability. It’s noteworthy that besides the increasing interest in fatty acid metabolism, none of the groups have looked at the direct correlation between expression of these genes in the brain and cognitive ability. Therefore to address this correlation we carried out a linear regression of 3 genes implicated in fatty acid pathway (FADS1, FADS2 and FADS3) using a publicly available dataset of 193 neuropathologically normal human brain genotypes and expression data. Of the 11 tested SNPs, we found significant correlation between 6 variants and the expression of FADS3 and FADS1 (p-values between $10^{-3}$ and $10^{-6}$). Among these correlated SNPs, 4 of them were in high $r^2$ with variants previously associated with intelligence. Interestingly we also observed a correlation between an intronic variant in FADS2 and the variance of expression of FADS3 gene (p-value $10^{-3}$).
To further test the interaction between the SNPs previously associated with cognition and brain expression (this study); we conducted an epitasis test using a publicly available data of 633 individuals participating in the International Multi-centre ADHD Genetics (IMAGE). Among the 12 tested SNPs, we observed one interaction that showed significant p-value after multiple test correction. These preliminary results suggest that fatty acid metabolism genes variants could be involved in the cognitive ability.

KEY WORDS
Fatty acid, intelligence, expression
IDENTIFICATION OF NOVEL GLIAL PROTEINS WITH THE CAPACITY TO STIMULATE THE EXTENSION OF NEURITES IN VITRO

Kasper C.D. Roet 1, Nitish Fagoe 1, Elske F. Franssen 1, Anke H.W. Essing 1, Ronald van Kesteren 2, Guus Smit 2, Freddy M. de Bree 1, Joost Verhaagen 1

Department/Institute
1 Department of Neuroregeneration, Netherlands Institute for Neuroscience, Amsterdam
2 Center for Neurogenomics and Cognitive Research, Neuroscience Campus Amsterdam, VU University, Amsterdam

Abstract

Being part of the central nervous system, the olfactory system is unique in its capacity to regenerate after injury. The presence of olfactory ensheathing glia (OEG) is crucial for this process. We hypothesize that there are several currently unidentified proteins expressed by OEG that promote and direct outgrowth of injured neurons. To test this hypothesis, gene expression analysis of OEG in culture and of OEG in the regenerating olfactory nervous system was conducted using an Agilent microarray containing 22K probes representing 14689 genes. By combining the expression data with a gene ontology analysis and literature study we selected 96 genes that are potentially involved in cell adhesion, matrix formation and cell migration. These genes are potential axon outgrowth-promoting genes. All 96 genes have been functionally validated by siRNA-mediated knockdown studies in medium-throughput in vitro bioassays. Eleven genes were identified that significantly decrease neurite outgrowth and three genes that increase neurite outgrowth of E15 dorsal root ganglion neurons (DRG) after knockdown in OEG. To determine whether over-expression of the former eleven genes would result in increased neuronal outgrowth we generated lentiviral over-expression vectors. One gene, scavenger receptor class II B (Scarb2), significantly increased average total neurite length of DRG neurons on primary skin fibroblasts with 70%, almost as much as the 78% increase observed when neurons were cultured in the presence of NGF. Two genes, neuroserpin and S100 calcium binding protein A9 (S100A9) increased the total number of neurites significantly with respectively 134% and 108%. For the other eight genes no significant effects were observed. We have shown for the first time that increasing the expression of Scarb2 or S100A9 leads to enhanced neuronal outgrowth. Therefore we conclude that OEG express proteins that have the potential to enhance regeneration and that might be used for future therapeutic purposes.

Keywords
Regeneration, OEG, fibroblasts, outgrowth, siRNA, lentivirus, over-expression
SENSING THE FUTURE: SKIN TEMPERATURE PREDICTS LAPSES IN VIGILANCE

Nico Romeijn, Ysbrand D. van der Werf, E.J.W. van Someren

Dept. Sleep & Cognition, Netherlands Institute for Neuroscience, Dept. of Clinical Neurophysiology, Neurology and Medical Psychology, VU University medical center, Amsterdam

The preoptic area and anterior hypothalamus (POAH) contain neurons that are essentially involved in both sleep- and thermo-regulation. In this area, and several other brain areas involved in sleep regulation, neuronal activity can be modulated by mild warming and cooling of the skin. Previous studies in humans showed that sleep onset latency and performance on a sustained vigilance task are sensitive to mild skin temperature manipulation\(^1,2,3\). So far little is known about the predictive properties of unmanipulated skin temperature for lapses in vigilance. We performed a validation of the predictive value of spontaneous fluctuations in skin temperature for the risk of lapses and slow reactions in a sustained vigilance task. Eight healthy participants (5 males, 22-47 years of age) underwent vigilance assessment for two days, in 4 task sessions per day. The four consecutive task sessions started at 09.00 with two hour intervals. During each session, participants were asked to perform a psychomotor vigilance task for 20 minutes while sitting in a dimly lit room. Skin temperature was monitored at several locations throughout the task, whereafter temperature in relation to response speed and lapses were analyzed. Especially proximal skin temperature measured subclavicularly was able to predict lapses in vigilance. These findings are consistent with earlier studies which showed that manipulation of proximal skin temperature is able to influence both sleep onset latency as well as vigilance, thereby strengthening the arguments for the hypothesis that skin temperature is a causal element in the sleep/wake cycle\(^1\).

References
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KEY WORDS
Skin temperature, vigilance, attention

TELEPHONE-NUMBER: 020-5665500
E-MAIL-ADDRESS: n.romeijn@nin.knaw.nl
A STUDY ON THE PHAGOCYTIC PROPERTIES OF OLFACTORY ENSHHEETING GLIA CELLS. IS THE OLFACTORY ENSHHEETING GLIA CELL A PROFESSIONAL PHAGOCYTE?

AUTHORS
Rubén Saavedra, Kasper Roet, Anke Essing, Jairo Peña, Freddy de Bree, Joost Verhaagen

DEPARTMENT/INSTITUTE
Netherlands Institute for Neuroscience, Amsterdam

ABSTRACT
The primary olfactory nervous system regenerates successfully after neuronal damage. Regenerating olfactory axons grow out over long distances and achieve highly accurate targeting inside the central nervous system. Axonal outgrowth from new neurons in the olfactory epithelium (OE) is supported by olfactory ensheathing glia (OEG), which engulf outgrowing axons all along the olfactory nerve and are the main cell type in the olfactory nerve layer (ONL).

A microarray study was performed to reveal the molecular mechanisms involved in axonal outgrowth in the olfactory system after a lesion. This study indicated the coordinated upregulation of Complement factors, Fc receptors and the Vav1-Rac2-Wasp pathway in the olfactory nerve layer. The upregulation of these genes point to the activation of a phagocytic response in the olfactory nerve layer during the process of axon ingrowth. Simultaneous to the upregulation of genes involved in phagocytosis, genes that constitute the cholesterol biosynthesis pathway were downregulated. This may be due to the increased uptake of debris-derived cholesterol by phagocytosis. The rapid removal of axonal debris from the ONL may be a process that critically contributes to the unprecedented repair potential of the primary olfactory system.

The ONL consists of multiple cell types, including specialized phagocytic cells like macrophages. The aim of this project is to determine whether OEG have phagocytic properties and are responsible (possible together with macrophages) for the clearance of debris after a lesion in the olfactory system. First, the in vivo protein expression of a number of selected target genes was validated by immunohistochemical staining of the olfactory bulb, including the ONL, after a lesion of the OE, followed by colocalizations with specific markers. Second, an in vitro model for phagocytosis was established with the aim to examine the functional activity of these genes in OEG. In this in vitro assay, purified primary OEG cultures were exposed to neuronal debris and qPCR was performed to quantitatively measure the differential expression of these genes compared to a basal (control) expression, after exposure to neuronal debris in vitro. Preliminary results indicate that Vav-1, Rac-2 and Wasp are all induced in a pure primary OEG culture within 24 hours after addition of neuronal debris. Currently we are investigating a number of additional genes implicated in the phagocytosis pathway, including Btk, Blnk and Hck.

KEY WORDS
Olfactory nervous system, Olfactory Ensheathing Glia (OEG), phagocytosis, immunohistochemistry, immunofluorescence, microarray, qPCR

TELEPHONE-NUMBER: 020-5665512
E-MAIL-ADDRESS: r.saavedra@nin.knaw.nl
TITLE
VOLUNTARY MOTOR PREPARATION IN TICS AND PSYCHOGENIC MOVEMENT DISORDERS

AUTHORS
Sandra M.A. van der Salm, A.F. van Rootselaar, J.H.T.M. van Koelman, M.A.J. Tijssen

DEPARTMENT/INSTITUTE
Department of Neurology and Clinical Neurophysiology of the Academic Medical Center, University of Amsterdam, Amsterdam

ABSTRACT
Background
In movement disorders practice, it is difficult to determine the origin of jerky movements. These movements are often associated with psychiatric co-morbidity and range from psychogenic jerks to tics and genetically inherited myoclonic jerks. The purpose of this study is to aid in the differentiation of jerky movements, with a focus on psychogenic jerks and tics in Gilles de la Tourette syndrome (GTS). By using combined electro-encephalography with surface electromyography (EEG-EMG) we investigate whether Bereitschaftspotential (BP) precedes voluntary jerks. The BP is presumed to differentiate between 'voluntary' movements (tics and psychogenic jerks), in which the patient can exert control over the movement, and involuntary jerks (myoclonus).
We hypothesize to find a BP before spontaneous motor tics, though more brief and less distinct (lower amplitude) as the BP prior to psychogenic jerks.

Preliminary findings
Nine patients with GTS and eleven patients with psychogenic movement disorder were studied. EEG-EMG recordings were preformed during spontaneous jerks/tics and self-paced wrist extension. Prior to spontaneous jerks, five out of eleven psychogenic patients and two out of nine GTS patients showed a BP, albeit of shorter duration (500 ms prior to jerk onset). A BP was found prior to self-paced movements three psychogenic and eight GTS patients.

Conclusions
Spontaneous motor tics can be preceded by a BP, but the psychogenic group showed more apparent potentials, both in amplitude and duration. The absence of a BP in some patients may be attributed to automatic execution of the movement.

KEY WORDS
Gilles de la Tourette syndrome, psychogenic movement disorders, bereitschaftspotential

TELEPHONE-NUMBER: 020-5663614
E-MAIL-ADDRESS: s.m.vandersalm@amc.uva.nl
The endocannabinoid (eCB) system is involved in the regulation of behavioural and physiological processes. Studies to the role of this system in the hippocampus have demonstrated that cannabinoid modulation is primarily achieved through attenuation of GABAergic and glutamatergic synaptic transmission. The prefrontal cortex (PFC) is involved in cognition and impulse control and cannabinoid modulation in this area could have important functional consequences. The whole-cell voltage clamp technique was used to investigate the effects of the CB agonist WIN-552122 and the antagonist (SR141716) on spontaneous excitatory (sEPSC’s) and inhibitory postsynaptic currents (sIPSC’s) measured in layer 2/3 pyramidal neurons. WIN-52122 at concentrations of 1 and 5 6M reduced the frequency of sIPSC’s and sEPSC’s. This effect could be reversed by SR141716 (at 1 and 5 6M). Immunostaining for CB1R showed that CB1R receptors are present in layer 2/3 and also in layer 5 of the PFC.

**KEY WORDS**
Endocannabinoid system, prefrontal cortex, excitatory and inhibitory synaptic transmission

**TELEPHONE-NUMBER:** 020-5256742
**E-MAIL-ADDRESS:** q.schaafsma-zhao@uva.nl
Development of a Rat Model to Study the Emotional Component of Pain Using the Somatosensory-Evoked Potential

Manon W.H. Schaap¹, J.J. Uilenreef¹, A. Doornenbal¹, J.G. van 't Klooster², S.S. Arndt², L.J. Hellebrekers¹

University Utrecht, Faculty of Veterinary Medicine, Department of Clinical Sciences of Companion Animals, Division of Anesthesiology & Neurophysiology, Utrecht

University Utrecht, Faculty of Veterinary Medicine, Department of Animals in Science & Society, Division of Laboratory Animal Science, Utrecht

According to the International Association for the Study of Pain, pain is a multidimensional experience, consisting of a sensory and emotional dimension. To date, knowledge about the recognition and effective treatment of animal pain is limited, negatively influencing pain alleviation in animals. In order to develop objective and valid parameters to quantify animal pain, detailed knowledge of pain physiology is needed, including the neurobiology of the emotional and sensory experience of pain.

The somatosensory evoked potential (SEP), a time- and stimulus-locked fragment of the electroencephalogram, represents the processing of noxious stimuli and is recently implemented in a model developed by us to quantify and differentiate between the emotional and sensory component of animal pain. From previous research it is concluded that SEPs recorded from the vertex represent the emotional dimension, whereas SEPs recorded from the primary somatosensory cortex represent the sensory dimension.

It is well established that pain is inextricably bound to negative emotions and that emotions by themselves can both inhibit and enhance pain. However, the nature of the interactions between pain and the emotional state are currently not firmly established. For example, some behavioural studies report that fear conditioning enhances animal pain, whereas others report that fear conditioning inhibits animal pain.

In order to further investigate the interaction between pain and the emotional state on a behavioural, neurobiological and neurophysiologic level, we are currently modifying and validating our SEP-model in the awake, freely moving rat. These modifications enable the measurement of behaviour and manipulation of the animal’s emotional state while measuring SEPs simultaneously. Once validated, our modified SEP-model, which incorporates fear conditioning, can be used as a method to shed light on the interaction between pain and other emotions. This knowledge will ultimately contribute to the development of valid parameters to quantify pain perception in animals, which will lead to the development of more adequate pain treatment strategies.

Somatosensory-evoked potential, pain, emotion

TELEPHONE-NUMBER: 030-2533828
E-MAIL-ADDRESS: m.schaap@uu.nl
TITLE
THE EFFECTS OF PUBERTY SUPPRESSION AND THE CONSECUTIVE ADDITION OF CROSSEX SEX HORMONES ON BRAIN ACTIVATION DURING MENTAL ROTATION IN TRANSSEXUAL ADOLESCENTS: AN FMRI STUDY

AUTHORS

DEPARTMENT/INSTITUTE
Pediatric Endocrinology, VU University medical center, Neuroscience Campus Amsterdam, Amsterdam

ABSTRACT
The VUmc gender team for children and adolescents assesses early-onset transsexuals on a regular basis. Little is known about aetiological aspects of transsexualism. Sex differences in cognition, gender identity and sexual orientation may all reflect sex-related neuroanatomical differences in the human brain. In transsexual people a reversal of the sex difference in the Bed Nucleus of the Stria Terminalis has been observed.

We hypothesize that in early-onset transsexualism the brain develops to some extent in the direction of the desired sex. The present study focuses on brain activation and performance during a cognitive task known to reflect sex differences. Mental rotation tasks appear to produce the most robust sex differences among all neuropsychological tests. Mental rotation is a complex cognitive skill depending on the manipulation of mental representations. Neuroimaging studies have consistently reported activation of the parietal cortex during performance on mental rotation tasks.

We present data from an fMRI study on mental rotation using an adapted version of the 3D mental rotation test of Vandenberg and Kuse. Colored 3D drawings were presented pair wise in different orientation. In half of the trials the 3D shapes were congruent, the remaining were incongruent. A single 3D figure with an arrow beneath was presented as control image.

We included 14 male-to-female adolescents (mean age 15.3 years) before the start of their hormone treatment and 12 (mean age 19.03 years) after at least one year of estrogen treatment, and 15 female-to-male adolescents (mean age 16.2 years) before the start of the testosterone treatment and 20 (mean age 19.2 years) after at least one year of treatment. In addition 38 female controls (15 mean age 15.6 and 23 mean age 19.0 year for both group representatives resp.) and 31 male controls (14 mean age 15.9 and 17 mean age 19.2 years resp.) were included in this study.

Magnetic resonance imaging (MRI) included a coronal 3D gradient-echo T2-weighted sequence (matrix 256 x 256, voxel size 3 x 3 x 3 mm, 35 sections) for functional MRI and was performed on a Philips Intera 3.0 Tesla with a standard 8-channel head coil.

Analysis of response accuracy revealed expected but non-significant differences between control males and females in advantage of the males. The transsexual adolescents showed performance in accordance with the desired sex, these differences also didn’t reach significance. Activation data during mental rotation will be shown.

KEY WORDS
fMRI, mental rotation, transsexual adolescents

TELEPHONE-NUMBER: 020-4441035
E-MAIL-ADDRESS: s.schagen@vumc.nl
ABSTRACT

Background
The prevalence of substance abuse in patients with schizophrenia is about 50%. Substances that are often used are nicotine, alcohol, cannabis, cocaine and amphetamines. Comorbid substance abuse is associated with unfavourable outcome. There are some indications that clozapine has a favourable effect on substance abuse in schizophrenia. These possible benefits should be weighed against the risk of adverse effects.

If this study proves that clozapine is effective in reducing substance abuse of patients with schizophrenia, clozapine should get a more prominent place in the treatment protocol of patients with substance abuse and schizophrenia.

Aim
To investigate if there is a difference in effectiveness and costs of clozapine treatment compared to olanzapine treatment in the reduction of substance abuse in patients with schizophrenia and related psychotic disorders.

Methods
In a multicenter double blind trial clozapine and olanzapine will be randomly allocated to patients with schizophrenia (or a related disorder) and substance abuse. Subsequently at baseline, after 4 weeks, 12 weeks and 6 months substance abuse, medical and non-medical costs, other psychopathology and quality of life will be evaluated by means of blood and urine samples and questionnaires.

Results
Our research protocol has been approved by the ethical commission; we expect to start including patients in September 2009.

Conclusion
This study will address a major clinical problem in the treatment of schizophrenia. Co-morbid substance abuse of patients with schizophrenia is associated with overall poorer outcome and has devastating effects in many patients. Clozapine may decrease substance abuse and improve symptomatic outcome. Our aim is to investigate this hypothesis by means of a multicenter doubleblind randomized clinical trial.

KEY WORDS
Schizophrenia, substance abuse, clozapine
During the development of substance dependence, drug-associated stimuli become increasingly relevant to the substance user. In addition to the role of motivational cues, deficits in cognitive functioning play a key role in addiction. In particular, cognitive functions that involve behavioural control, and also control over behaviour when confronted with motivationally relevant drug cues, appear to be crucial for the development and course of addictive disorders. We will study the neural substrates of these two processes and their interaction. The interrelation between poor impulse control, motivational relevance of drug cues, and vulnerability to relapse predicts that improving cognitive performance may represent a promising new approach in the treatment of addiction. We therefore present a pharmacological challenge study with (1) a cognitive enhancer (studied in humans with cocaine dependence: modafinil), and (2) an agent influencing the motivational relevance of drug! cues in animals (N-acetylcysteine). Thirty alcohol dependent patients, thirty cocaine dependent patients and thirty healthy controls will be tested using neurocognitive tasks on impulsivity and motivational drug cues in an fMRI study both before and after acute administration with N-acetylcysteine, modafinil, or placebo. Pilot data will be presented on the acute effects of N-acetylcysteine on brain metabolism during cognitive and motivational tasks in cocaine dependent males.
Cyclin-dependent kinase 5 (Cdk5) and its activators p35 and p39 are mainly found in the nervous system and are thought to be involved in many neuronal processes such as neuronal development, synaptic transmission and neurodegeneration. Here we focus on the role of Cdk5 on synaptic transmission through phosphorylation of the presynaptic protein Munc18-1 using a panel of Munc18-1 mutations: non-phosphorylatable Munc18(T574A) and phospho-mimicking Munc18(T574D). To analyze synaptic transmission and plasticity on a cellular level we use autaptic cultures from munc18-1 null mutant mice that are rescued with either wild-type (WT) Munc18, Munc18(T574A) or Munc18(T574D). We show that Cdk5 phosphorylation is an important determinant of (pre) synaptic efficacy by either modulation of release probability or readily releasable pool size.
TITLE
SLEEP AND DAYTIME FUNCTIONING IN CHILDHOOD – A META-ANALYSIS

AUTHORS
Rebecca G. Schutte¹, K. van der Heijden², M.H. van IJzendoorn², H.T. Swaab-Barneveld², E.J.W. van Someren¹

DEPARTMENT/INSTITUTE
¹ Netherlands Institute for Neuroscience, Amsterdam
² University of Leiden, Leiden

ABSTRACT
Introduction
Past research has shown that normal adult sleep is vital for daytime functioning, with sleep deprivation resulting in significantly disrupted daytime performance (Pilcher and Huffcutt, 1996). However, less is known about the consequences of sleep loss in childhood. The recent years have seen an increased interest in childhood sleep and its relationship to daytime function. However, as yet results appear inconclusive, and there remains a paucity of empirical studies. It therefore seems timely to aggregate all previous findings by Meta-Analysis to determine the status of, and gaps in, our current knowledge. This information will be invaluable for future research on the relationship between sleep and daytime functioning in childhood.

Methods
All past research containing an objective measure of sleep, a measure of daytime functioning and a childhood participant group was sought after. Peer-reviewed literature was searched through online databases, and the subsequent back-tracing of their reference lists. Grey literature was searched through conference proceedings, theses databases and contacts with the invisible college. All relevant articles were scored blindly by two raters, and statistical results were imported into CMA software where analyses were performed.

Results & Discussion
Analyses are currently being performed; therefore results are not yet available. However, these very new outcomes will be presented and discussed at the ONWAR annual meeting.

References

KEY WORDS
Sleep, cognition, behaviour, childhood, meta-analysis

TELEPHONE-NUMBER: 020-5665492
E-MAIL-ADDRESS: r.schutte@nin.knaw.nl
Tissue transglutaminase [tTG / TG2] is a bifunctional enzyme, acting as a G-protein in addition to its transamidation activity. The transamidation active site is exposed only when Ca\(^{2+}\) concentrations are elevated beyond physiological norms. A specific, potent tracer may therefore provide a proxy for Ca\(^{2+}\) concentration and tTG transamidation activity (which has been implicated in both beneficial and pathological processes).

A group of peptidomimetic inhibitors containing the reactive ‘DON’ glutamine-analogue have been found to be excellent inhibitors of tTG. Radiolabelling of such species is challenging due to the necessity of incorporating the DON group in the final reaction step and the desire to apply the radiolabel as late as possible, so as to maximise the available activity. [C-11]diazomethane has been successfully synthesised for this purpose but with sub-optimal reliability and yields. Attempts to produce it at the radionuclide centre have so far met with limited success and optimisation is ongoing.

Following demonstrations of satisfactory biodistribution/kinetics of tracer(s) in healthy rats; investigations in rat disease models of inflammation, such as cr-EAE and/or LPS-induced inflammation will be conducted; tTG is known to be highly-active in inflammation processes.

Given difficulties with the \(^{11}C\) peptide labelling and the biodistribution of certain tTG inhibitors [especially in situations where the BBB remains intact]; alternatives may need to be sought. Different ligand classes and/or fluorinated analogues of inhibitory compounds labelled with longer-lived \(^{18}F\), for instance.

**KEY WORDS**
Transglutaminase, PET, neurodegeneration, imaging, radiochemistry, transamidation, Carbon-11, radiolabelling

**TELEPHONE-NUMBER:** 020-4449704
**E-MAIL-ADDRESS:** l.setchell@vumc.nl
HISTAMINE PRODUCTION IN PARKINSON’S DISEASE BRAIN: RELATIONSHIP WITH LEWY BODIES AND GENDER

AUTHORS
Ling Shan1*, Chun-Qing Liu2*, Rawien Balesar1, Joop J. Van Heerikhuize1, Dick F. Swaab1, Ai-Min Bao2,1

DEPARTMENT/INSTITUTE
1 Laboratory for Neuropsychiatric Disorders, Netherlands Institute for Neuroscience, Amsterdam
2 Department of Neurobiology, Institute of Neuroscience, Zhejiang University School of Medicine, Hangzhou, China
* Authors contributed equally to this work

ABSTRACT
The hypothalamic Tubero-mamillary Nucleus (TMN) is the exclusive source of neuronal histamine. Previous studies have reported conflicting results on the nature of alterations in the TMN in Parkinson’s disease (PD). On the one hand it was claimed that the abundant presence of Lewy bodies (LBs) and Lewy neurites (LNs) in the TMN of PD patients would result in a strong degeneration of the TMN. On the other hand, some experimental data have indicated the presence of an activation of the TMN that was presumed to accelerate the degeneration of the substantia nigra in PD. We aimed to clarify this controversy by quantitative in situ hybridization for the mRNA of the rate limiting enzyme of histamine production, histidine decarboxylase (HDC), in postmortem human brain tissue from PD patients (early, preclinical PD, stages n = 6 late PD stages, n = 9) and well-matched controls (n = 15). No significant alteration in HDC-mRNA expression was found in male PD patients, either in early-stage PD or in late-stage PD. However, HDC-mRNA expression of the pooled early- and late-stage PD female PD patients was significantly lower than that of the female controls (P = 0.035). Such a difference was not present in the pooled male PD patients versus their controls. There was no significant difference of HDC-mRNA in the TMN between male subjects and female subjects in the control group. However, a significant sex difference was found; for both the early-stage PD group and the late-stage PD group the HDC-mRNA levels in the females were lower than the levels in males (P = 0.050). The pooled female PD patients showed significantly lower HDC-mRNA expression than the pooled male PD patients (P = 0.004), and this expression was also lower than that of the pooled female controls (P = 0.035). Such a sex difference was not present in controls. The amount of typical PD lesions, i.e. the LBs and LNs, as stained by α-synuclein, was significantly increased in the TMN in the late-stage PD patients (P < 0.0001) but not in the early-stage PD patients. There was no correlation between HDC-mRNA expression and the amount of LBs and LNs, or between HDC-mRNA expression and PD duration. Concluding, histamine production in the TMN remains unaffected in PD males, and is diminished in PD females. The presence of LBs and LNs does not seem to affect HDC-mRNA expression.

KEY WORDS
Parkinson disease, histamine, hypothalamus, Lewy bodies, sex difference

TELEPHONE-NUMBER: 020-5665514
E-MAIL-ADDRESS: l.shan@nin.knaw.nl
SYNAPTIC PROPERTIES, DYNAMICS AND CONNECTIVITY IN THE FMR1-KO MOUSE: A MODEL FOR FRAGILE X MENTAL RETARDATION

AUTHORS
Guilherme Silva¹, C.D. Holmgren¹,², R.M. Meredith¹, H.D. Mansvelder¹

DEPARTMENT/INSTITUTE
¹ Department of Integrative Neurophysiology, CNCR, VU University, Amsterdam
² INMED, Marseille, France

ABSTRACT
Development of cognitive function requires the formation and refinement of synaptic networks in the brain during early postnatal life. In many forms of mental retardation, including inherited Fragile X syndrome, there is an increase in the density of postsynaptic spines and filopodia on neuronal dendrites along with a longer and more immature spine phenotype in both cortical and hippocampal brain regions. The effects of these morphological changes on information processing at pre- and postsynaptic levels are not fully known.

Using the Fmr1-KO mouse, a model for Fragile X syndrome, we are investigating both pre- and postsynaptic parameters of excitatory postsynaptic currents at specific synaptic inputs in hippocampal and prefrontal cortex networks. Multiple whole-cell patch-clamp recordings are made from identified cell types in living brain slices from 2 week old Fmr1-KO and wildtype mice. Synaptic activity protocols are tested to measure paired-pulse ratios, short-term plasticity and neuronal connectivity. During this period of synaptogenesis, we see significant but smaller changes in paired-pulse ratios and short-term synaptic plasticity at Schaffer collateral inputs to CA1 pyramidal neurons in Fmr1-KO mice, which may arise from alterations in presynaptic protein levels in the hippocampus (see Klemmer et al., Sfn abstract 2009). We are investigating whether similar changes in presynaptic dynamics are found in the prefrontal cortex and furthermore, whether alterations in postsynaptic kinetics and synaptic properties are also present in these networks.

These data aim to investigate subtle alterations in kinetic and dynamic properties of synaptic transmission in hippocampal and cortical networks of the Fmr1-KO mouse model, whose changes have significant implications for synaptic information processing in the brain during mental retardation.

KEY WORDS
FragX, synaptic connectivity, multi-patch paired recordings

TELEPHONE-NUMBER: 06-81901857
E-MAIL-ADDRESS: guilherme.silva@falw.vu.nl
TITLE
THE HIJACKED BRAIN; AN FMRI STUDY ON THE NEURAL CORRELATES OF HABIT FORMATION IN ALCOHOL DEPENDENCY

AUTHORS
Zsuzsika Sjoerds1,2, Dick J. Veltman1,2, Wim van den Brink2, Sanne de Wit3, Brenda W.J.H. Penninx1

DEPARTMENT/INSTITUTE
1. Dept. Psychiatry, VU University medical center, Amsterdam
2. Dept. Psychiatry, Amsterdam Institute of Addiction Research, Academic Medical Center, Amsterdam
3. and VU University medical center Center for the study of adaptive control in brain and behavior (Acacia), Department of Psychology, University of Amsterdam, Amsterdam

ABSTRACT
Alcohol dependence (AD) has a negative impact on both individual and public health. The DSM-IV classified 1-year prevalence in the general adult Dutch population of alcohol dependence is 4% (1). Neurophysiological mechanisms have been proposed to underlie the development of AD: it is characterized by dysregulation in the brain of the mesolimbic dopaminergic system, including ventral tegmental area, ventral- and dorsal striatum and the orbitofrontal cortex (2). From a functional perspective, it has been postulated that this dysregulation is characterized by increased responsiveness to stimuli which have become associated with the drug through Pavlovian conditioning. This has been described as the ‘hijacking’ of the neural circuitry involved in processing reward stimuli (3). Also, it has been proposed that in chronic dependence, loss of behavioral control becomes more prominent, implying that drug use is no longer voluntary, but habitual or even compulsory (4). Animal literature suggests that this transition is reflected in abnormal dorsal striatal activity, coupled with deficient prefrontal function, but more confirmative research in humans is required. The present study provides a unique opportunity to do so. Within the longitudinal NESDA-consortium (Netherlands Study for Depression and Anxiety) an fMRI-study will be performed in 40 alcohol dependants with varying chronicity and severity, and 40 non-alcoholic controls matched according to age, sex, education and presence of depression or anxiety disorder. Processing of emotional and motivational information as well as cognitive control will be examined and compared between groups using three different functional tasks in the MRI scanner. We expect that more chronic AD is characterized by a prevalence of maladaptive habit formation and deficiencies mainly present in cognitive control, while in less chronic AD mostly deficiencies in reward processes and motivation will be visible.

References

KEY WORDS
Addiction, alcohol, habit formation, goal-directed behavior, cognitive control, functional MRI, striatum, orbitofrontal cortex

TELEPHONE-NUMBER: 020-4449635
E-MAIL-ADDRESS: z.sjoerds@vumc.nl
Fluoxetine is a selective serotonin reuptake inhibitor (SSRI) antidepressant and is certified for use during pregnancy. However, evidence is accumulating that fluoxetine causes cardiological and neurological abnormalities in infants who were exposed to fluoxetine in utero. In a recent study it was shown that these clinical manifestations can be reproduced in mice, where prenatal fluoxetine exposure causes cardiomyopathy and a higher vulnerability to depressive and anxiety related behavior later in life.

Here we show that prenatal fluoxetine exposure in mice induces life-long changes in dendritic morphology of cortical layer 2/3 pyramidal neurons, which is accompanied by an increase in reelin levels. More importantly, we show that the effects of fluoxetine on the maturation of the cortex are absent in serotonin 5-HT3 receptor knockout mice, and can be rescued in vitro by pharmacological block of the 5-HT3 receptor. These findings highlight a novel adverse side-effect of fluoxetine during pregnancy, and indicate a key role of serotonergic signaling via 5-HT3 receptors.

KEY WORDS
SSRI, cortex, neurodevelopment, dendritic complexity, reelin, 5-HT3 receptor

TELEPHONE-NUMBER: 020-5258369
E-MAIL-ADDRESS: l.a.rigter@uva.nl
Critical period (CP) plasticity of the mouse visual cortex occurs around week 4-5 of development. When one eye is closed during the CP, the cortex becomes more responsive to the open eye relative to the closed eye. This ocular dominance (OD) shift in responsiveness is absent in the pre- and post-critical period. Timing of the CP is regulated by the maturation of the GABAergic inhibitory system which acts by parvalbumin (PV) positive perisomatic innervation onto postsynaptic α1 subunit containing GABA\textsubscript{A} receptors.

Previous literature, involving the use of benzodiazepines, suggested the α1 subunit of GABA\textsubscript{A}R to be essential in the CP plasticity of the visual cortex.

With in vivo intrinsic optical imaging in a knock-out line for α1, we measured ocular dominance plasticity and acuity in the cortex to see if α1 was necessary to open the critical period. We show that α1 subunit is not essential for CP plasticity by inducing a reduced OD shift after monocular deprivation in KO mice, compared to WT mice. PV positive inhibitory innervation of layer II-III pyramidal cells is still present in α1 KO mice, but whether the quantity of perisomatic puncta is different compared to WT mice is currently under investigation. We are focusing on elucidating whether the change in CP plasticity is caused by a difference in timing of the CP, or by a functional change of the GABA\textsubscript{A} receptor.

**KEY WORDS**
Critical period, ocular dominance plasticity, inhibitory, GABA\textsubscript{A} receptor, α1
INNATE AND ADAPTIVE IMMUNITY IN AMYOTROPHIC LATERAL SCLEROSIS (ALS): EVIDENCE OF COMPLEMENT ACTIVATION


Neurogenetics Laboratory and Departments of Neuro-Pathology and Neurology, Academic Medical Center, University of Amsterdam, Amsterdam

Background
Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disorder characterized by loss of motor neurons and gliosis in motor cortex (MCx) and spinal cord (SC). Although the etiology of ALS is still unclear, recent evidence suggests a role for the immune system in the disease process.

Objective
To analyze the presence and distribution of inflammatory cells, such as microglia/macrophages and T lymphocytes, as well as components of the complement system in sporadic and familial ALS cases.

Methods
We investigated the distribution of cellular components of innate and adaptive immunity using immunohistochemistry on SC and MCx sections of both sporadic (n = 16; sALS) and familial (n = 4; with frontotemporal dementia; FTD-ALS) ALS cases. We analyzed the expression and cellular distribution of complement components as well. Quantification was performed for all stainings.

Results
In all ALS cases a prominent presence of microglial cells expressing class II-antigens (HLA-DR) and CD68-positive macrophages was found in both SC (ventral horn and corticospinal tracts) and MCx. We also observed perivascular and parenchymal T-lymphocytes (CD3+; with a predominance of CD8+ T-cytotoxic/suppressor cells) and the presence of dendritic cells (DCs; DC-SIGN+). Quantitative analysis showed a significantly higher number of HLA-DR+, CD68+, CD3+, CD8+ cells and DCs in ALS SC and MCx compared to control tissues. The number of microglia/macrophages and T-lymphocytes was higher in long term sALS patients as compared to sALS patients with rapid ALS progression. In contrast DCs were more prominently observed in patients who had a more rapid progression. Several components of the complement cascade (C1q, C3c, C3d and MAC) were observed in active microglia and reactive astrocytes in SC and MCx of ALS patients.

Conclusions
Our findings demonstrate a persistent activation of immune/inflammatory responses in ALS, including the activation of the complement system. Understanding the role of complement activation in motor neuron degeneration in ALS may be of great importance in the development of new therapeutic strategies.

KEY WORDS
ALS, neuro-immunity, complement

TELEPHONE-NUMBER: 020-5665889
E-MAIL-ADDRESS: m.sta@amc.uva.nl
MRI PATTERN RECOGNITION IN HYPOMYELINATING DISORDERS

AUTHORS
Marjan E. Steenweg, A. Vanderver, S. Blaser, F. Barkhof, N. Wolf, M.S. van der Knaap

DEPARTMENT/INSTITUTE
1 Department of Child Neurology and 2 Department of Radiology, VU University medical center, Amsterdam
3 Department of Neurology, Children’s National Medical Center, Washington DC, USA
4 Department of Neuroradiology, Hospital for Sick Children, Toronto, Canada

ABSTRACT
Aim
The aim of this study was to determine whether MRI pattern recognition can play a role in distinguishing between different hypomyelinating disorders. This would greatly facilitate the diagnostic progress. In addition, MRI pattern recognition might help to define novel disorders among the hypomyelinating disorders of unknown origin.

Material and methods
MRIs of 128 patients with defined hypomyelinating disorders, including Pelizaeus-Merzbacher disease (PMD), Pelizaeus-Merzbacher-like disease (PMDLD), hypomyelination with congenital cataract (HCC), hypomyelination with hypogonadotrophic hypogonadism and hypodontia (4H-syndrome), hypomyelination with atrophy of the basal ganglia and cerebellum (HABC), Fucosidosis, Salla disease, and GM1 and GM2 gangliosidosis, were retrospectively reviewed according to a previously established scoring list and grouped according to their pattern of abnormalities. The investigators were blinded for the diagnosis.

Results
Most patients were grouped with other patients with the same disease, although some diseases were divided into two categories. Mixing of different diseases within the same group rarely occurred. Twenty-eight patients could not be assigned to one of these groups. In general, MR images of HABC patients showed a variable degree of hypomyelination, early disappearance of the putamen and early atrophy of the cerebellum. Fucosidosis, a T2 hypointensity of the globus pallidus was seen, while GM1 and GM2 gangliosidosis displayed a T2 hyperintensity of the putamen and caudate nucleus. In PMD, supratentorial myelination was often arrested at an early age with homogeneous hypomyelination without any spatial variation. There was no significant early cerebellar atrophy. PMDLD displayed a prominent T2 hyperintensity of the pons or part of the pons, i.e. the pyramidal tract at the level of the pons. Patients with HCC had hypomyelination with additionally more prominent signal abnormalities in the deep white matter. In 4H-syndrome, cerebellar atrophy was severe and often early. Salla disease did not display a homogeneous pattern.

Conclusion
Features on MRI can give useful hints on the disease causing the hypomyelination (figure 3). It is important, however, to note that hypomyelinating disorders of unknown origin were not included in this study.

References

KEY WORDS
MRI, childhood white matter disorder, hypomyelination, pattern recognition

TELEPHONE-NUMBER: 020-4441035
E-MAIL-ADDRESS: me.steenweg@vumc.nl
REDUCED PARIETAL P300 PRECEDING A FIRST PSYCHOTIC EPISODE

Mirjam J. van Tricht1, 2, D.H. Nieman1, J.H.T.M. Koelman2, H.E. Becker1, J.N. van der Meer2, L.J. Bour2, D. Linszen1

1 Department of Psychiatry and 2 Department of Neurology and Clinical Neurophysiology, Academic Medical Center, University of Amsterdam, Amsterdam

ABSTRACT

P300 abnormalities relate to changes in information processing and have been suggested to be one of the most reliable biological markers of schizophrenia. We sought to investigate whether P300 abnormalities are also helpful in predicting transition to a first psychotic episode in our group of patients at Ultra High Risk (UHR) for developing psychosis. P300 amplitudes and latencies were assessed and compared in 61 UHR patients, of whom 17 patients made a transition to psychosis (UHR+T), and 28 age and intelligence matched healthy controls. All subjects were tested at baseline and followed for three years. The relationship between P300 parameters, social functioning and psychiatric symptoms was investigated in all UHR subjects. The UHR+T patients showed smaller P300 amplitudes at Pz and Cz at baseline, compared to healthy controls. Moreover, in comparison to UHR patients who did not make a transition to psychosis, UHR+T patients showed P300 decrements at Pz. Therefore, smaller Pz P300 amplitudes at baseline appear to be helpful in predicting a subsequent transition to psychosis. Pz P300 amplitudes were related to measures of social functioning and psychiatric symptoms.

KEY WORDS

Ultra high risk patients, psychosis, P300, social functioning, psychiatric symptoms

TELEPHONE-NUMBER: 020-5668417
E-MAIL-ADDRESS: m.j.vantricht@amc.uva.nl
AGING IN THE CENTRAL NERVOUS SYSTEM OF DNAGE MOUSE MODELS

AUTHORS
Marlene J. Végh1, M.C. de Waard2, I. van der Pluijm2, G. Zondag2, M.J.M. Sassen1, R.Y. Ridwan2, R. Brandt3, A.B. Smit1, R.E. van Kesteren1

DEPARTMENT/INSTITUTE
1 Dept. of Molecular and Cellular Neurobiology, Center for Neurogenomics and Cognitive Research, Neuroscience Campus Amsterdam, VU University, Amsterdam
2 DNage B.V., Leiden
3 Department of Genetics, Erasmus MC, Rotterdam

ABSTRACT
DNA is constantly attacked by a variety of genotoxic agents, which are either exogenous, present in the environment, or endogenous, as a byproduct of natural metabolism. Since the presence of damage in our genome can interrupt vital cellular processes like replication and transcription, several pathways for DNA repair exist. More and more evidence directs towards a role of DNA damage accumulation in the pathology of the aging central nervous system. Furthermore, it has repeatedly been hypothesized that accumulation of DNA damage, or the inability of neurons to appropriately handle DNA damage, may serve as an initiation point in the etiology of neurodegenerative diseases.

Recent studies of patients with Alzheimer’s disease, Parkinson’s disease, amyotrophic lateral sclerosis, Friedreich’s ataxia, xerodermia pigmentosa and Huntington’s disease suggest that oxidative stress and neuronal damage are common features of these diseases. In addition, defects in genes involved in the maintenance of DNA, such as NER, give rise to human inherited progeroid disorders. Human segmental progeroid syndromes are monogenic diseases accelerating some, but not all features found in normal aging. This suggests that failure in DNA repair mechanisms underlie at least some aging phenotypes or aging-related pathologies and this again points to the fact that DNA integrity and DNA damage plays an important role in the aging process.

Using existing Ercc1 transgenic mice that have impaired DNA repair capabilities and as a result display premature aging, we study cellular and molecular mechanisms underlying aging and premature aging of the nervous system. We hypothesize that neuronal viability, outgrowth, synapse formation and connectivity are early-affected neuronal processes underlying DNA damage-induced aging. In vitro primary neuron culture experiments are used to study the effects of defective DNA repair pathways on neuron viability. In addition, immunohistochemistry is used to study morphological changes in brains of prematurely aging mice. Furthermore, synapse proteomics is being performed on the DNAge mouse models. Proteomics analysis of Ercc1∆/− have been performed to study the in vivo molecular composition of synapses and to detect changes in synapse composition and function. Normalization and cluster analyses have been performed, and these result in clearly differentially regulated groups of proteins. These results are now being validated using western blot and interesting targets are being chosen to further investigate.

KEY WORDS
Aging, DNA damage, synapse, proteomics, primary cultures

TELEPHONE-NUMBER: 020-5987122
E-MAIL-ADRESS: marlene.vegh@cnrc.vu.nl
RESEQUENCING PCLO AS A CANDIDATE GENE FOR MAJOR DEPRESSIVE DISORDER

AUTHORS
Eva C. Verbeek\textsuperscript{1}, Marianna R. Bevova\textsuperscript{1}, Zoltan Bochdanovits\textsuperscript{1}, Witte J.G. Hoogendijk\textsuperscript{2}, Peter Heutink\textsuperscript{1}

DEPARTMENT/INSTITUTE
\textsuperscript{1}Department of Medical Genomics, VU University medical center, Amsterdam; \textsuperscript{2}Department of Psychiatry, VU University medical center, Amsterdam

ABSTRACT
Major depressive disorder (MDD) is a common complex trait characterized by prolonged dysphoria, changes in appetite and sleeping behaviour, feelings of worthlessness and additional cognitive symptoms. Besides being a burden for patients and their relatives, MDD is also one of the leading causes of disability in western civilization, with enormous consequences for the economy and healthcare.

In a 2008 genome wide association study (GWAS) for MDD, Sullivan et al. found a significant single nucleotide polymorphism (SNP) in the piccolo gene (pclo). Pclo codes for a protein that is localized in the presynaptic area.

We plan to resequence pclo in 50 control samples to look at all normal variants, find possible new risk factors for MDD, refine patterns of inheritance and to find further evidence that the SNP found by Sullivan et al. is truly the causal SNP. The variations that we want to find are SNPs, Copy Number Variations and Insertions/Deletions.

We hybridize genomic DNA to NimbleGen Sequence Capture arrays to capture the DNA of interest. Hybridized DNA is eluted using NaOH and then amplified with ligation mediated PCR. After amplification we compare the enrichment of our target DNA to genomic DNA for several control loci. After having sufficient enrichment (>100x), indexes are ligated to the DNA for identification. Samples are then sequenced using an Illumina GAIIL-machine. Sequence data is then aligned back to the reference genome and variants are detected by CLC Genomics Workbench software.

KEY WORDS
Major depressive disorder, high throughput sequencing, piccolo, single nucleotide polymorphism, complex trait

TELEPHONE-NUMBER: 020-5982832
E-MAIL-ADDRESS: e.verbeek@vumc.nl

AUTHORS
Joost Verbeek, A.D. Windhorst, J. Eriksson, A.A. Lammertsma, G. Luurtsema

DEPARTMENT/INSTITUTE
Department of Nuclear Medicine & PET Research, VU University medical center, Amsterdam

ABSTRACT

Objectives
Using [11C]verapamil and positron emission tomography, it is possible to measure P-glycoprotein activity. In the optimal tracer kinetic model, however, it is assumed that the main radioactive metabolite of [11C]verapamil, [11C]D617, has similar pharmacokinetics as [11C]verapamil itself. To assess whether this assumption is correct, the pharmacokinetic properties of [11C]D617 need to be evaluated.

The aim of this study is to develop the synthesis of [11C]D617 and to study its biodistribution in rats.

Methods

The precursor for labelling D617 with C-11, compound 5, was synthesized in 4 steps and subsequently reacted with [11C]methyl triflate to give [11C]D617 (scheme 1). Purification was achieved using preparative HPLC and the isolated product was reformulated with solid phase extraction in 10% ethanol and 90% 7.09 mM NaH2PO4 in 0.9% saline.

The biodistribution of [11C]D617 was determined in male Wistar rats (N=4) at 5, 15, 30 and 60 min after injection. Tissues of interest were dissected, counted for radioactivity and weighed. The biodistribution was also assessed at 30 min following injection of [11C]D617 in a group of rats (N=4), pre-treated with 15 mg/kg tariquidar (PgP inhibitor) in order to determine whether [11C]D617 is a PgP substrate.

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, [11C]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.

Brain uptake of [11C]D617 was very low (<0.1% ID/g) and homogeneous. Peripheral biodistribution showed high clearance via liver and urinary tract. Pre-treatment with tariquidar did not alter the distribution of [11C]D617, nor its uptake in the brain.

Conclusion

[11C]D617 was synthesized with good yield and SA. Brain uptake was low and could not be enhanced by pre-treatment with tariquidar, indicating that [11C]D617 is not a PgP substrate in this rat model. Whether metabolism of [11C]D617 is causing this effect is currently under investigation.

Acknowledgment

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Reference

KEY WORDS
[11C], Verapamil; D617; blood brain barrier; P-glycoprotein

TELEPHONE-NUMBER: 020-4445989
E-MAIL-ADDRESS: jverbeek@rnc.vu.nl
TISSUE TRANSGLUTAMINASE (TTG) AND NEURODEGENERATION: ADDED VALUE OF A CELL BASED ASSAY FOR SELECTION OF EFFECTIVE INHIBITORS IN PRECLINICAL DEVELOPMENT

AUTHORS

DEPARTMENT/INSTITUTE
Department of Anatomy and Neurosciences, Neuroscience Campus Amsterdam, VU University medical center, Amsterdam

ABSTRACT
TTG-mediated protein aggregation is implicated in a number of neurodegenerative diseases, in particular Alzheimer’s- and Parkinson’s disease. As a consequence, there has been increasing pharmaceutical interest for development of TTG inhibitors. Available compounds are generally based on reactive groups, such as tetra methyl thioimidazolium, 6-diazo-5-oxo norleucine or 3-halo-4,5-dihydroxyazole, which bind irreversibly to the catalytic site of TTG. The pharmacological profile of these compounds, however, is established principally in vitro and their effectiveness in a cellular environment, where they have to act in vivo, often remains undetermined. In an attempt to set-up a cellular assay optimized for early identification of effective TTG inhibitors prior to further testing in more dedicated in vitro and in vivo models of neurodegeneration, we compared the in vitro and in situ performance of several of the above described classes of TTG inhibitors. In vitro TTG activity was measured fluorospectrometrically via incorporation of monodansylcadaverine into donor substrate Z-GLN-GLY-CAD-DNS. In situ TTG activity was measured via Ca2+ ionophore- induced incorporation of 5-biotinamidopentylamine into proteins using SH-SY5Y neuroblastoma cells. While most compounds displayed excellent, submicromolar, in vitro TTG inhibition, this performance was substantially reduced, or even absent, in situ. Our results indicate that in vitro screening of TTG inhibitors does not reliably predict their performance in a relevant (patho)physiological environment. We therefore suggest that in situ TTG inhibitor profiling provides valuable, additional, information about TTG inhibitor efficacy, which helps to select suitable compounds prior to testing in more complex and time-consuming (cell- and animal) neuroprotection models.

KEY WORDS
Tissue transglutaminase, protein aggregation

TELEPHONE-NUMBER: 020-4448096
E-MAIL-ADDRESS: r.verhaar@vumc.nl
TITLE
EVALUATION OF RILUZOLE TREATMENT IN A MARMOSE T MPTP MODEL FOR EARLY PARKINSON’S DISEASE

AUTHORS
Peternella Suzanne Verhavea,b, Roland M. van der Berga, Marjan. J. Jongsmac, Raymond A.P. Vanwerscha, José C. Visb, Herman P.M. van Heldenb, August B. Smitb, Ingrid H.C.H.M. Philippensd

DEPARTMENT/INSTITUTE
a. Chemical Security, TNO Defence, Security and Safety, Rijswijk
b. Department of Molecular and Cellular Neurobiology, Center for Neurogenomics and Cognitive Research, Neuroscience Campus Amsterdam, VU University, Amsterdam
c. Sleepvision, Berg en Dal
d. Department of Immunobiology, Division Neuropathology, Biomedical Primate Research Centre, Rijswijk

ABSTRACT
One of the major problems in the treatment of neurodegeneration is the fact that diagnosis of neurodegenerative diseases takes place at a late stage in the disease. For Parkinson’s Disease (PD) this means that more than 60% of the dopaminergic neurons in the substantia nigra (SN) has already degenerated at the moment of diagnosis. Gaining more insight in the early phase of neurodegeneration, to improve neuroprotective treatment strategies, is therefore extremely challenging. We developed an interval MPTP marmoset model to mimic this preclinical phase of PD. In this model the impact of a neuroprotective Riluzole treatment, at the start of neurodegeneration, was evaluated using several clinically relevant motor related behaviors, sleep parameters and post-mortem biochemical and histological markers of neurodegeneration.

Three groups (each n = 6) of marmoset monkeys were used: 1) an MPTP-group receiving a total dose of 7 mg/kg of MPTP, given in four injections over two weeks, to induce a PD like state; 2) a Riluzole-group receiving Riluzole twice daily (orally 10 mg/kg) starting one week before MPTP and up to one week after the last MPTP injection; 3) a vehicle-group receiving saline instead of MPTP and Riluzole. The animals’ home cage behavior was scored daily and their nocturnal sleep EEG/EMG, locomotor activity, hand-eye coordination, jumping behavior and axial turning were recorded and tested once a week. Dopamine levels in the striatum and the number of functional dopaminergic neurons in the SN were measured post-mortem at three weeks after the MPTP administration.

This PD induction protocol induced neurodegeneration of the dopaminergic neurons in the SN below the diagnosis threshold of 60%. This MPTP dose affected all behavioral parameters. Riluzole significantly improved the MPTP disturbed home cage behavior, hand-eye coordination and turning ability. In contrast, Riluzole did not prevent the MPTP-induced impairments in locomotor activity and jumping ability. However, Riluzole significantly prevented the impact of MPTP on sleep architecture. On a cellular level both the number of the dopaminergic neurons and dopamine levels in the striatum were significantly less affected in the Riluzole-treated parkinsonian marmosets.

Our data show that the 50% degeneration of the dopaminergic neurons slightly affected some of the motor behaviors, which mimics PD at the time of early diagnosis. Riluzole treatment was able to counteract these MPTP-induced deterioration of movement-related behavior, sleep-EEG changes, and dopaminergic cell death in the SN. Therefore Riluzole seems to be a promising neuroprotective compound for moderate dopaminergic cell death in the early phase of PD that protects as well on cellular as for PD clinically relevant behavioral aspects.

KEY WORDS
Parkinson’s disease, sleep, MPTP, marmoset, motor behavior

TELEPHONE-NUMBER: 015-2843047
E-MAIL-ADDRESS: nelleke.verhave@tno.nl
The formation of precise synaptic connectivity depends both on molecular mechanisms as well as neuronal activity, such as sensory experience. Monocular deprivation (MD) by suturing one of the eyes is an established model for experience-dependent plasticity. During the critical period, MD induces neurons in the primary visual cortex (V1) to shift their responsiveness towards the open eye, a so-called ocular dominance shift. Most likely, altering visual input will result in synaptic remodelling.

We are interested in the effects of MD on the dynamics of inhibitory synapses. To investigate this we have set up chronic 2-photon imaging in vivo in young mice that express both a cytoplasmic and inhibitory synapse marker. Previous studies that have used this technique have looked at spine dynamics but until now it was not possible to link this to evoked visual activity and ocular dominance at the individual cell level. To address this we will make use of mice that express a fluorescent genetic marker for calcium activity in the entire cell for chronic imaging.

This is the first time that neuronal activity can be chronically measured at the individual cell level and moreover, can be correlated to changes in cell morphology as a result of altered visual input. In both mice, the DNA is introduced in E16 embryos by means of in utero electroporation.

**KEY WORDS**

Ocular dominance plasticity, structural plasticity, calcium imaging, chronic in vivo 2-photon imaging

**TELEPHONE-NUMBER:** 020-5664529
**E-MAIL-ADDRESS:** d.van.versendaal@nin.knaw.nl
FEED-FORWARD AND FEED-BACK CONNECTIONS TARGET DIFFERENT GLUTAMATE RECEPTOR CONFIGURATIONS IN MACAQUE PRIMARY VISUAL CORTEX

AUTHORS
Roxana Voitcu, Floris G. Wouterlood

DEPARTMENT/INSTITUTE
Netherlands Institute for Neuroscience, Amsterdam, and Dept. of Anatomy and Neurosciences, VU University medical center, Amsterdam

ABSTRACT
We are interested in the distribution of Glutamate receptor subunits on various neuronal populations, as function of cortical layer location in primary visual cortex V1. To this purpose, we focused on AMPA (GluR1 to GluR4) and NMDA (NMDAR1, NR2A to NR2D) receptor subunits and Parvalbumin (interneurons) and CaMKIIalpha (principal cells), as markers. We fluorescently tagged the targeted proteins, in V1 slices from 2 macaque monkey specimens. Subsequently, we imaged the slices using a Zeiss LSM 510 confocal microscope. We applied a deconvolution algorithm on the scanned images and then obtained 3-dimensional reconstructions of the cell surfaces and receptor distributions. We then calculated the co-localization probability of two markers for all marker pairs.

KEY WORDS
Glutamate receptors, confocal microscopy, deconvolution, 3D reconstruction, co-localization, V1

TELEPHONE-NUMBER: 020-5664293
E-MAIL-ADDRESS: r.voitcu@nin.knaw.nl
AMPHETAMINE INDUCED STRIATAL DOPAMINE RELEASE IN TOURETTE’S SYNDROME USING POSITRON EMISSION TOMOGRAPHY

Froukje E. de Vries1,2, M. Figee3, T.F. van der Doef1, R. Boellaard1, D.C. Cath2,4, A.A. Lammertsma1, A.J.L.M. van Balkom2, D.J. Veltman2,3, D.A.J. Denys3, B.N.M. van Berckel1

1 Dept. of Nuclear Medicine & PET Research, VU University medical center, Amsterdam
2 Dept. of Psychiatry, VU University medical center, Amsterdam
3 Dept. of Psychiatry, Academic Medical Center, Amsterdam
4 Dept. of Clinical Psychology, Utrecht University, Utrecht

ABSTRACT

Objective

Tourette’s Syndrome (TS) is a neuropsychiatric disorder, characterized by motor and vocal tics, often accompanied by obsessive-compulsive symptoms and attention deficits. Abnormalities in dopamine transmission in the corticostriatal circuits are thought to be involved in the pathophysiology of TS. The goal of this study was to assess amphetamine induced dopamine release in the striatum of TS patients and its relation to tic severity.

Methods

To test the hypothesis that TS is associated with excessive dopamine release (DArel) in the striatum, we used positron emission scanning (PET) and the D2-receptor radioligand [11C]raclopride. DArel is indirectly measured using the reduction of [11C]raclopride binding potential (BP) following the administration of amphetamine. Twelve medication free patients with TS and twelve matched healthy control subjects were included in the study. Two [11C]raclopride steady state PET scans were performed: one at baseline, the second scan after intravenous administration of amphetamine (0.3 mg/kg). Regions of interest (ROI) in the striatum were delineated on a co-registered structural MRI scan. DVR, equal to BP+1, was derived from the ratio of activity concentrations between ROI and cerebellum, which was used as reference region. DArel was defined as the percentage change in BP (ΔBP) between the two scans. Baseline BP and ΔBP for each ROI was compared between groups. A voxel based analysis using the parametric BP images was carried out using Statistical Parametric Mapping.

Results

ROI analysis showed that a significantly larger ΔBP was present in the posterior caudate of TS patients compared to healthy controls (p= 0.029), while no differences in baseline BP were observed. Increased tic severity after amphetamine administration was related to DArel in the ventral striatum and dorsal putamen. SPM analysis revealed a greater change in BP in healthy controls than in TS (in bilateral putamen and left ventral caudate) together with a higher baseline BP in controls. Increased tic severity was related to DArel in the left ventral striatum.

Conclusion

ROI and SPM analysis show different results, possibly due to a mismatch between ROI definition and the regions identified by SPM. These differences need to be further explored. However, both analyses reveal that increase of tic severity is related to DArel in the ventral striatum, possibly indicating a postsynaptic hypersensitivity to dopamine.

KEY WORDS
Tourette’s Syndrome, dopamine, PET, striatum, amphetamine

TELEPHONE-NUMBER: 020-7885666
E-MAIL-ADDRESS: f.devries@ggzingeest.nl
Glucocorticoid Receptor Expression in the Human Hippocampus; Changes With Age

Qian Wang, J. Wouda, L. Seress, J. Van Heerikhuize, DF Swaab, M. Joels, P. J. Lucassen

SILS-Center for Neuroscience, University of Amsterdam, Amsterdam
Central Electron Microscopic Laboratory, University of Pécs, Hungary
Netherlands Institute for Neuroscience, Amsterdam

Abstract

The glucocorticoid receptor (GR) is involved in feedback regulation of the stress system. In rodent brain, abundant GR expression is found in key regions of the hypothalamo-pituitary-adrenal (HPA) axis and the hippocampus. In contrast, in rhesus monkey, a relative absence of GR has been described in the main neuronal layers of the hippocampal formation, whereas astroglia prominently expressed GR (Sanchez et al., JNS 2000).

As distribution of GR protein is still obscure in human brain, we studied GR distribution in the human hippocampus and explored whether any changes occur with age. GR distribution was studied immunohistochemically in 30 samples of postmortem hippocampal tissue of young, middle aged and old control subjects, ranging in age from 1 to 98 years, which were obtained through the Netherlands Brain Bank. We used polyclonal GR antisera directed against the N-terminus of the classic human GRα protein and against a part of the transcription modulation (TR) domain of rat GR. Extensive validation studies were done to exclude an influence of fixation time and postmortem delay.

Abundant GR-immunoreactivity was found throughout the pyramidal and granule layers of the human hippocampus, with a predominantly nuclear localization. Intense nuclear staining was present in the dentate gyrus (DG) and cornu ammonis (CA) areas including the CA3 subregion although the latter region displayed a more sparse distribution. Colocalization with the astrocyte marker GFAP confirmed that GR was almost absent from glial cells and GR was mainly confined to the neuronal population. In conclusion, in contrast to previous studies in primate, prominent nuclear GR expression is present in neurons of the human DG and CA subregions, indicating that the hippocampus can be an important target for GR-mediated effects in human.

Acknowledgement

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Key Words

Glucocorticoid receptor, human brain, immunocytochemistry

Telephone-Number: 020-5667741
Email-Address: q.wang@uva.nl
FUNCTIONAL CHARACTERIZATION OF TARGET GENES INVOLVED IN ALZHEIMER’S DISEASE DEVELOPMENT AND PROGRESSION

AUTHOR(S)
Kerstin T.S. Wirz¹, K. Bossers¹, J.A. Korecka¹, R. Zwart², W. Scheper², E. Blaas³, R.E. van Kesteren³, A.B. Smit³, D.F. Swaab¹, J. Verhaagen¹

DEPARTMENT/INSTITUTE
¹ Netherlands Institute for Neuroscience, Amsterdam, ² Neurogenetics Laboratory, Academic Medical Center, University of Amsterdam and ³ Center for Neurogenomics and Cognitive Research, Neuroscience Campus Amsterdam, VU University, Amsterdam

ABSTRACT
Alzheimer’s disease (AD) is the most prevalent form of dementia, but the biological processes behind the development and progression of sporadic AD remain poorly understood. We have previously generated genome-wide gene expression profiles from the human medial frontal gyrus of all seven Braak stages, and identified gene expression alterations that occur just before the onset of AD pathology in this brain region. Among the upregulated transcripts we find eight genes that are involved in the generation and degradation of Aβ, the main component of senile plaques. To understand the significance of these and other dysregulated genes, we are currently engaged in two lines of research: 1) Using immunohistochemistry we localize protein expression of target genes in human AD patients, and 2) we apply functional screening assays in two cellular models. In SK-N-SH neuroblastoma cells that overexpress human APP the influence of gene knockdown and overexpression on Aβ42 to Aβ40 ratios, cell morphology and survival is investigated. In SH-SY5Y neuroblastoma cells we use Aβ-induced toxicity to study effects of gene knockdown and overexpression on cell survival, cell integrity, and mitochondrial activity on a medium throughput scale.

KEY WORDS
Alzheimer’s disease, Aβ, cell culture, gene knockdown, gene overexpression, human tissue, immunohistochemistry, SH-SY5Y, SK-N-SH

TELEPHONE-NUMBER: 020-5665512
E-MAIL-ADDRESS: k.wirz@nin.knaw.nl
AN IMPORTANT ROLE FOR MESOLIMBIC MU-OPIOID RECEPTORS IN AMPHETAMINE-INDUCED INHIBITORY CONTROL DEFICITS

Joost Wiskerke, Dustin Schetters, Inge van Es, Yvar van Mourik, Bjørnar R.O. den Hollander, Anton N.M. Schoffelmeer, Tommy Pattij

Department of Anatomy and Neurosciences, Neuroscience Campus Amsterdam, VU University medical center, Amsterdam

It is well known that addictive substances, particularly psychostimulants such as amphetamine, acutely increase impulsive behaviour in both animals and humans. This phenomenon is known to depend critically on enhanced mesolimbic dopamine transmission, but the exact neuronal mechanisms remain largely unknown. Especially for amphetamine, it is important to unravel how it induces impulsivity, since it is not only a widely-abused recreational drug but also a regularly prescribed pharmacotherapy for Attention Deficit Hyperactivity Disorder (ADHD). Therefore, we studied the effects of amphetamine in two rat models of impulsivity, the 5-choice serial reaction time task (5-CSRTT) and the delayed reward task (DRT), which provide measures of respectively inhibitory control and impulsive choice. We specifically tested the role of the endogenous opioid system in amphetamine-induced impulsivity as there is ample evidence indicating an important role for this neurotransmitter system in behavioural and neurochemical effects of amphetamine. Results showed that amphetamine-induced decrements in inhibitory control could dose-dependently be attenuated by pre-treatment with the opioid receptor antagonist naloxone, but not with selective delta- or kappa-opioid receptor antagonists, indicating involvement of particularly mu-opioid receptors. In contrast, naloxone did not affect amphetamine-induced decreases in impulsive choice. Naloxone also completely prevented inhibitory control deficits induced by GBR-12909, a selective dopamine transporter inhibitor, suggesting an important role for a ‘mu-opioid-dopamine’ interaction in amphetamine-induced impulsivity. Subsequently performed intracranial infusion experiments indicated involvement of at least mu-receptors in the nucleus accumbens shell and to a lesser extent the VTA, but not the nucleus accumbens core in amphetamine-induced impulsivity. Together, these results indicate an important role for mesolimbic mu-opioid receptors in amphetamine-induced decrements in inhibitory control.

Impulsivity, amphetamine, endogenous opioid system, 5-choice serial reaction time task, delayed reward task

020-4448097
j.wiskerke@vumc.nl
TITLE
EMOTION REGULATION IN OBSESSIVE-COMPULSIVE DISORDER PATIENTS AND HEALTHY CONTROLS: PRELIMINARY DATA

AUTHORS
Stella de Wit, O.A. van den Heuvel, Y.D. van der Werf, K. Verhoef, D. Veltman

DEPARTMENT/INSTITUTE
Department of Psychiatry, VU University medical center, Amsterdam

ABSTRACT
Introduction: Obsessive-compulsive disorder (OCD) is a psychiatric disorder characterised by obsessions (recurrent and persistent intrusive thoughts) and compulsions (repetitive behaviour aimed at reducing the distress caused by obsessions or a dreaded event). In several symptom provocation studies it was shown that patients with OCD have a heightened response in ventral ‘emotional’ brain areas to disease-relevant stimuli in comparison to healthy controls (HCs). Furthermore, on multiple executive tasks OCD patients show a deficit compared to HCs, associated with the attenuated recruitment of dorsal brain regions associated with executive function and cognitive control (e.g. left dorsal lateral prefrontal cortex (DLPFC), Van den Heuvel et al., 2005).

An influential model of the neurobiology of emotion perception and regulation postulates that a ventral system (including the amygdala) mediates the identification of an emotional stimulus and the production of an affective state, whereas a dorsal system (including the DLPFC) is concerned with the subsequent regulation of that affective state (Phillips et al., 2003). Indeed, activation of dorsal prefrontal areas has been shown to be associated with successful regulation of negative affect in HCs (e.g. Phan et al., 2005).

These findings led to the hypothesis that the heightened emotional response to disease-specific stimuli in OCD patients is due to a deficit in emotion regulation (ER). We speculate this is caused by a failure of emotional control by the dorsal system, and more specifically by the DLPFC. Furthermore, we hypothesise that through the modulation of the dorsal system, with on the one hand excitatory high-frequent repetitive transcranial magnetic stimulation (rTMS) of the DLPFC in OCD patients, and on the other hand inhibitory low-frequent rTMS in controls, we will be able to respectively, temporarily increase ER capabilities in patients and decrease ER function in HCs.

Methods: To assess emotion regulation in OCD patients as compared to HCs, an emotion-regulation task (ERT) with OCD-specific, general anxiety-inducing, and neutral visual stimuli was devised. Pictures were viewed in either an ‘attend’ or ‘regulate’ condition during functional magnetic resonance imaging (fMRI). Subjects scored each picture during the task on a visual distress scale. A behavioural pilot in 5 medicated OCD patients from an outpatient clinic was performed to assess the use of OCD specific regulation instructions. In an ongoing study 40 unmedicated OCD patients and 40 HCs will perform the ERT before and after rTMS (real versus sham) during fMRI.

Results: Pilot data and preliminary fMRI data will be presented.

Conclusions: Pilot data show that our paradigm is suitable to study emotion regulation in OCD.

Future directions: The effect of rTMS on brain areas associated with emotion processing and regulation will be studied in OCD patients and healthy controls. Furthermore, physiological measures of emotional arousal (i.e. pupil diameter and heart rate) will be correlated with both the self-report visual distress scores and the fMRI data. Also, the paradigm enables us to study the specific neural correlates of the different symptom dimensions in OCD. In addition, endophenotype fMRI analysis in both patients and their first degree relatives allows us to gain further insight into the genetic background of altered brain function in OCD.

KEY WORDS
Obsessive-compulsive disorder, emotion regulation, functional magnetic resonance imaging, transcranial magnetic stimulation

TELEPHONE-NUMBER: 020-4449635
E-MAIL-ADDRESS: st.dewit@vumc.nl
MITOCHONDRIAL ALTERATIONS IN MS BRAINS

Maarten E. Witte¹, W.H. Gerritsen¹, H.E. de Vries², J. Drexhage², P. van der Valk¹, J. van Horssen¹²

 Departments of Pathology and ²Molecular Cell Biology & Immunology, VU University medical center, Amsterdam

ABSTRACT
Mitochondria are the energy producing organelles in eukaryotic cells and play important roles in various cellular processes, such as apoptosis, calcium handling and fatty acid metabolism. Recent studies demonstrate that mitochondrial dysfunction and subsequent free radical production are involved in multiple sclerosis (MS) lesion development and progression. Previously, we demonstrated an increase in mitochondrial density in MS lesions, more specifically in reactive astrocytes and demyelinated axons. This increase in mitochondria coincided with increased mitochondrial oxidative stress, indicating increased mitochondrial reactive oxygen species (ROS) production. To protect themselves against ROS-mediated damage, mitochondria contain a specific set of antioxidant enzymes. Expression and activity of these mitochondrial enzymes are upregulated upon exposure to free radicals; however their involvement in MS pathogenesis is unknown so far. Hence, we investigated the expression of mitochondrial antioxidant enzymes, including peroxiredoxin III (Prx3) and thioredoxin II (Tnx2) in MS brain tissue. Immunohistochemical analysis revealed an increase in both Prx3 and Tnx2 expression in various types of white matter MS lesions. Currently, double immunofluorescence stainings are performed to identify the cellular localization of Prx3 and Tnx2. In future, we will further delineate the putative protective properties of Prx3 and Tnx2 using in vitro models of neuroinflammation and demyelination.

Besides increased free radical production, mitochondrial dysfunction in MS could have many other deleterious consequences, e.g. impaired energy production, altered calcium handling and decreased fatty acid metabolism, all of which could be detrimental to the cell or axon. To investigate the involvement of various mitochondrial processes in MS pathogenesis, we performed microarray analysis using a customized human mitochondria-focused cDNA microarray. This chip contains a selection of 12 genes encoded by mitochondrial DNA and over 1100 nuclear-encoded mitochondria-related genes. We collected chronic inactive lesions and surrounding normal appearing white matter from 6 MS patients and white matter samples from 6 age-matched controls for the isolation of mRNA. Currently we are analyzing the microarray data to identify potential targets for future research.

KEY WORDS
Multiple Sclerosis, mitochondria, oxidative stress, antioxidants

TELEPHONE-NUMBER: 020-4444096
E-MAIL-ADDRESS: m.witte@vumc.nl
DIFFERENTIAL OLIVO-CEREBELLAR CORTICAL CONTROL OF REBOUND ACTIVITY IN THE CEREBELLAR NUCLEI

Laurens Witter\textsuperscript{a,*}, Freek E. Hoebeek\textsuperscript{a*}, Chris I. De Zeeuw\textsuperscript{a,\textdagger}, Tom J.H. Ruigrok\textsuperscript{a}

\textsuperscript{a} Neuroscience Dept., Erasmus MC, Rotterdam and \textsuperscript{b} Netherlands Institute for Neuroscience, Amsterdam

\* These authors contributed equally
\textdagger To whom correspondence should be addressed

ABSTRACT
The output of the cerebellar cortex is controlled by two main inputs, i.e. the climbing fiber and mossy fiber – parallel fiber pathway, and activations of these inputs elicit characteristic effects in its Purkinje cells, i.e. the so-called complex spikes and simple spikes. The target neurons of the Purkinje cells in the cerebellar nuclei (CN) show rebound firing, which has been implicated in the processing and storage of motor coordination signals. Yet, it is not known to what extent rebound phenomena depend on different modes of Purkinje cell activation. Here we investigated in awake and anaesthetized rodents whether electrical train stimuli mimicking complex spike activities show a rebound that differs from single stimuli. We show that train stimuli to the cerebellar cortex and direct activation of the inferior olive both result in long-lasting increases of the firing frequencies of up to 250 ms, whereas single pulse stimuli to the cerebellar cortex elicit well timed, but short-lasting (5.1 ± 0.5 ms), rebound activities in the CN neurons. Whole cell patch clamp recordings of CN neurons in vivo confirmed the rebound nature of both types of responses and showed the occurrence of rebound firing during spontaneous activity. We conclude that the rebound phenomenon offers a rich and powerful mechanism for CN neurons, which should allow them to differentially process the climbing fiber and mossy fiber inputs in a physiologically operating cerebellum.

KEY WORDS
Cerebellum, in-vivo, electrophysiology, patch clamp, rebound, mouse, rat, awake, anaesthetized

TELEPHONE-NUMBER: 020-5668404
E-MAIL-ADDRESS: l.witter@nin.knaw.nl
FIGURE-GROUND SIGNALS IN EARLY AND OBJECT SPECIFIC VISUAL AREAS: A COMBINED FMRI, EEG AND TMS STUDY

AUTHORS
Martijn E. Wokke¹, H. Steven Scholte¹, Victor A.F. Lamme¹,²

DEPARTMENT/INSTITUTE
¹ Cognitive Neuroscience Group, Psychology, University of Amsterdam, Amsterdam
² Netherlands Ophthalmic Research Institute, Amsterdam

ABSTRACT
Two processes can be discriminated when distinguishing a figure from its background: boundary detection and surface segregation. We studied the neural correlates of these two processes using texture and motion defined stimuli that differentiate between surface segregation and edge detection.

In two ROI’s (LO [lateral occipital] and V5), BOLD-MRI was measured to establish whether figure-ground signals can be found within these regions. Data analysis showed that both LO and V5 seem to be involved in figure-ground segregation.

Currently we combine rTMS, EEG and the above-described stimuli to investigate the role of the “dorsal” and “ventral” route in relation to figure-ground segregation. Results indicate that rTMS alters figure-ground related processes differentially depending on whether LO or V5 is stimulated. Preliminary rTMS/EEG results suggest separate causal relationships between LO and MT and recurrent figure-ground signals in occipital areas.

KEY WORDS
Figure-ground segregation, V5/MT, lateral occipital complex

TELEPHONE-NUMBER: 020-5256804
E-MAIL-ADDRESS: martijnwokke@gmail.com
LONG-TERM EFFECTS OF CHRONIC AND BINGE-LIKE ALCOHOL EXPOSURE DURING ADOLESCENCE ON MOTIVATIONAL AND COGNITIVE BEHAVIOR

AUTHORS
Jelte A. Wouda, T. Pattij, A.N.M. Schoffelmeer, A.B. Smit, T.J. de Vries

DEPARTMENT/INSTITUTE
Dept Anatomy and Neurosciences, Neuroscience Campus Amsterdam, VU University medical center, Amsterdam

ABSTRACT
Adolescence is a transition phase that can be characterized by increased risk taking, increased novelty seeking and intensified peer relationships in both humans and rats. These behavioral changes are accompanied by maturation of prefrontal cortex and hippocampus, extensive synaptic pruning throughout the brain, and changes in several neurotransmitter and receptor systems towards adult levels.

Adolescence is also often the age of onset of alcohol use. In the last two decades an alarming trend is seen of increased alcohol intake among youth in this unique developmental period. There is, however, hardly any information on how alcohol affects these changing neurological systems and whether this will result in permanent changes in adult behavior. The present study was designed to investigate the effects of adolescent alcohol exposure on disturbances in the motivational and cognitive (attention, impulsivity) domain in later life. To that end, we compared the effects of chronic and binge-like alcohol exposure during adolescence on operant alcohol self-administration (SA) and five-choice serial reaction time task (5CSRTT) performance.

Animals that received chronic treatment were allowed to drink a 10% alcohol /0.2% saccharin in a two bottle free choice paradigm from postnatal day (PND) 34-43 (peri-adolescents) or PND 60-69 (post-adolescents). Ethanol intake was between 3-6 g/kg/day. At adult age, 91 days after the last treatment day, rats were either trained to self administer a 10% alcohol solution (EXP1) or on 5CSRTT performance (EXP2).

Exp1: All groups acquired SA at the same pace and no difference was found in performance during between session progressive ratio or extinction training. A cue-induced relapse test revealed a borderline significant reduction of reinstatement in alcohol treated peri-adolescent group compared to water treated animals.

Exp2: No treatment effects were found on baseline performance in the 5CSRTT. However, a reduced correct response latency in post-adolescent treated animals compared to peri-adolescent animals was observed.

A history of chronic alcohol exposure during adolescence had no effect on acquisition, motivation, extinction and reinstatement of operant self administration of adult Wistar rats or on performance in 5CSRTT. The age related effect on response latency might be attributed to differences in neurobiology at time of treatment. Adolescent binge-like alcohol exposure has been reported to have more detrimental behavioural effects than chronic exposure. Therefore, effects of adolescent binge-like (5x 2.5g/kg, ip) exposure on SA and 5CSRTT performance are currently being investigated.

KEY WORDS
Alcohol exposure, cognitive behavior

TELEPHONE-NUMBER: 020-4445677
E-MAIL-ADDRESS: j.wouda@vumc.nl
ANALYSIS PLAN FOR THE ASSOCIATION OF THE PCLO SNP WITH FUNCTIONAL NEUROIMAGING DATA

AUTHORS
Saskia Woudstra1,2,3,6, D.J. Veltman1, Z. Bochdanovits2, N.A.J. van der Wee3,4, F.G. Zitman3, A. Aleman5, M.J. van Tol1,3,4, B.W.J.H. Penninx1,2,3, W.J.G. Hoogendijk1,6

DEPARTMENT/INSTITUTE
(1) Department of Psychiatry, VU University medical center, Amsterdam (2) Department of Medical Genomics, VU University medical center, Amsterdam (3) Department of Psychiatry, Leiden University Medical Center, Leiden (4) Leiden Institute for Brain and Cognition, Leiden University (5) BCN Neuroimaging Center, Rijksuniversiteit Groningen (6) Neuroscience Campus Amsterdam

ABSTRACT
Expanding the insight of the disease pathology of major depressive disorder (MDD) has recently been extended with a genome-wide association study (GWAS) on MDD, conducted by Sullivan et al (2009). The data for this GWAS came from the large NESDA cohort (Netherlands Study of Depression and Anxiety), which is a multi-site naturalistic study to gain insight in the long-term course and consequences of depression and anxiety disorders (Penninx et al., 2008). The top 25 of the most strongly associated SNPs (single nucleotide polymorphism) included 11 SNPs within the piccolo (PCLO) gene, which protein product localizes to the cytomatrix of the presynaptic active zone and is important in monoaminergic neurotransmission in the brain. One SNP in particular was of interest, a non-synonymous coding SNP in the C2-domain. A reanalysis of the PCLO replication study argued that the data favor this SNP to be a causal risk factor for MDD (Bochdanovits et al., 2009).

In the NESDA study, next to genotype data, MRI data was collected as well. It is known that executive functioning is different in depressed patients compared to healthy controls (Elliott et al., 1997). Therefore, the differences in brain activity may serve as an endophenotype for depression. One of the tasks performed by subjects to examine this was the Tower of London task, while the subjects were scanned.

Given that brain activity may serve as an endophenotype for depression, we are interested in the role of the PCLO SNP in this endophenotype. This particular analysis is based on the findings of the GWAS on MDD. I will show how we will analyse the association of PCLO with brain activity, taking into account several aspects of association studies, such as the power of an association study. In addition, I will present a model for the possible role of PCLO in altered brain activity.

Abbreviations
MDD Major Depressive Disorder - GWAS Genome Wide Association Study - NESDA Netherlands Study of Depression and Anxiety - SNP Single Nucleotide Polymorphism - PCLO Piccolo - MRI Magnetic Resonance Imaging

References

KEY WORDS
Depression, GWAS, imaging genetics, NESDA, methods

TELEPHONE-NUMBER: 020-4449635
E-MAIL-ADDRESS: s.woudstra@vumc.nl
DETERMINATION OF SUBSTRAIN DIFFERENCES BETWEEN C57BL/6J AND C57BL/6N MICE IN BEHAVIORAL FLEXIBILITY USING TWO SPATIAL LEARNING TASKS

AUTHORS
Jiun Youn, Janneke van der Laan, Matthijs Verhage, Oliver Stiedl

DEPARTMENT/INSTITUTE
Dept. of Functional Genomics, Behavioral and Cognitive Neuroscience Group, CNCR, VU University, Amsterdam

ABSTRACT
In our stressful modern life, the prevalence of emotional disorders such as posttraumatic disorder (PTSD) and depression is increasing rapidly. However, it remains as an intriguing fact that most of us emerge from even extremely stressful episodes nearly unscathed. Studying individual differences in stress response can provide us an opportunity to find protective mechanisms against emotional dysregulation, and this in turn can offer possibilities for the development of novel therapeutic approaches. We have studied individual difference in two commonly used C57BL/6 substrains of mice. C57BL/6N (6N) mice showed much more pronounced impairment in fear extinction compared to C57BL/6J (6J) in spite of their nearly identical genetic background. In the present study, we investigated if such a difference in emotional perseveration has its cognitive equivalent in spatial memory tests. For that purpose, we tested the two substrains in Barnes maze and holeboard test. The animals were trained to locate an escape hole (Barnes maze) or a set of baited holes (holeboard). Importantly, the location of the goal was changed after the animals learned the task to study if any difference in cognitive perseverance could be observed between the groups. In the Barnes maze no difference was observed in the latency to locate the escape hole, and both substrains showed similar performances during reversal learning. The holeboard test turned out to be very demanding for both substrains with profound differences in performance. This finding indicates that spatial learning impairments may be observed only when mice are subjected to an increased task complexity. While 66% of the 6N mice failed to learn the task within the given time, 75% of the 6J mice showed successful performance. Since only an insufficient number of 6N mice acquired this task, reversal learning could not be tested. In addition, a strong tendency of increasing thigmotaxic behavior was observed in 6N mice in both tests, suggesting an elevated anxiety level. Interestingly, anxiety-like behavior did not differ between the two substrains in anxiety tests of single exposure (dark-light box). Presumably, the cognitive impairment or enhanced anxiety arises only with higher levels of stress such as repeated handling procedure is involved with 6N mice being more vulnerable to its effect. It is also possible that opposing (negative and positive) motivations used as reinforcers in the two behavior tests led to an attentional bias affecting the performance differences in the two strains. Our further studies will focus on delineating the interaction between emotion and cognition for spatial memory performance in mouse strains using a refined experimental approach, and then to investigate the influence of environmental stressors on individual performance differences, while trying to get more insight into potential genetic contributions based on single nucleotide polymorphisms (SNPs) between 6J and 6N mice.

KEY WORDS
Strain difference, posttraumatic stress disorder, PTSD, C57BL/6J, C57BL/6N, flexibility, barnes maze, holeboard

TELEPHONE-NUMBER: 020-5987089
E-MAIL-ADDRESS: jiun.youn@cnocr.vu.nl
TWO FORMS OF FEEDBACK INHIBITION DETERMINE THE DYNAMICAL STATE OF A SMALL HIPPOCAMPAL NETWORK

Fleur Zeldenrust, Wytse J. Wadman

SILS-Center for Neuroscience, University of Amsterdam, Amsterdam

Pyramidal cells in the hippocampus are part of a small neuronal network that performs computations on external input. The network consists of principal cells and various forms of feedback inhibition. Experimental evidence indicates at least two functionally distinct inhibitory feedback loops in the CA3 area of the hippocampus: 1) a loop in which O-LM interneurons project to the distal dendrites of pyramidal cells with synapses that have slow kinetics 2) a loop in which basket interneurons project to the somata of pyramidal cells with synapses that have fast kinetics. There is an interconnection between the two loops in the form of O-LM to basket interneuron inhibition and the configuration is further complicated by the presence of distinct propagation delays and short-term facilitation and depression of certain synapses in the two basic loops. We investigated the consequences of various configurations of the circuit and modulations of the components of inhibition for the computation that the network can perform on its input.

Gaussian noise was used as the input to the dendrite of the pyramidal cell and evoked two types of events: spikes or bursts. The Event-Triggered Average (ETA) and the Event-Triggered Covariance (ETC) were determined and the inter-event-intervals between spikes and bursts were analyzed. The ETA and ETC on the pyramidal cell show that this model behaves in first approximation as an activity integrator: with sufficient positive input bursts as well as spikes are evoked. Which of the two is determined by the input just after the (first) spike: positive input results in a burst; negative input results in a spike. Stronger feedback inhibition, in the slow as well as in the fast loop, increases the event-rate of the pyramidal cell. For a single input and large propagation delays the interaction between the two feedback loops is not of great importance. The consequences of the presence of the slow and/or fast feedback inhibitory loop, with or without facilitation and depression, were analyzed in relation to synapse strength. Facilitation and depression are most relevant when their recovery time constant is of the same order as the mean inter-event interval. Short-term depression can stop activity in the fast loop after several fast spikes and can switch the network to a different state, thus functioning as a kind of ‘brake’ on the fast inhibitory feedback loop. Thus inhibition and the details of the micro-circuit organization play an important role in the information processing of the small neuronal circuit.

KEY WORDS
Hippocampus, CA-3, inhibition, feedback loop, covariance analysis, Pinsky and Rinzel pyramidal cell model

TELEPHONE-NUMBER: 020-5257630
E-MAIL-ADDRESS: f.zeldenrust@uva.nl
TITLE
AN IMBALANCE OF GABA AND GLUTAMATE IN ALZHEIMER DISEASE BUT NOT IN DEPRESSION IN THE SUPERIOR GYRUS OF THE PREFRONTAL CORTEX AND ANTERIOR CINGULATED CORTEX

AUTHORS
Juan Zhao1,2, A.-M. Bao1,3, W.J.G. Hoogendijk4, X-R Qi1, W. Kamphuis1, S. Luchetti1, J.-S. Lou2, D.F. Swaab1

DEPARTMENT/INSTITUTE
1 Netherlands Institute for Neuroscience, Amsterdam
2 Department of Pharmacology, Basic Medicine School, Tianjin Medical University, Tianjin, China
3 Department of Neurobiology, Zhejiang University School of Medicine, Hangzhou, Zhejiang, PR China
4 Department of Psychiatry and CNCR NCA, VU University medical center, Amsterdam

ABSTRACT
Depression is, with a lifetime prevalence of 12-20%, a common disease with serious consequences, and is also occur in 20-50% of the Alzheimer disease (AD) patients. We found that the neurobiological basis of major depressive disorder (MDD), bipolar disorder (BD) and depression in AD partly overlaps in terms of brain systems and neurotransmitters involved. One of the brain structures that is presumed to be involved in the pathogenesis of these 3 types of depression mentioned above is the prefrontal cortex (PFC). Lesions in the PFC have been reported to cause depression. There is equivocal evidence in the literature for a glutamate/gamma-aminobutyric acid (GABA) imbalance in the PFC in depression. Such an imbalance is presumed to stimulate the hypothalamo-pituitary-adrenal (HPA)-axis and so to contribute to the signs and symptoms of depression. In order to test the hypothesis that an imbalance of the two principle neurotransmitters, glutamate and GABA, may be a basis for mood disorder, either in MD, BD or depressed AD, we obtained postmortem brain material from the Netherlands Brain Bank. We determined by qPCR, in the isolated gray matter of the anterior cingulate cortex (ACC) and/or the superior gyrus (SG) of the PFC, the expression of mRNA of enzymes, transporters and receptors of the glutamate and GABA pathways in 15 depressive disorder patients matched with 22 controls, and 16 AD patients matched with 8 non-demented controls. We found significantly (p<0.001) decreased GABRA1, GABRA4, GABRG2, GABBR2, GAD1, GAD2, EAAT4 and SNAT1 mRNA in the SG-PFC of AD patients compared with the controls. In MDD and BD we found a significantly decreased GABRB2 and PSD95 mRNA in the ACC compared with the controls while there was no significant difference in the SG-PFC in the 3 types of depression compared with the controls. These findings show that MD and BD show the region specific alterations. In addition, there are GABA/glutamate and glutamate/glutamine imbalance in the PFC in AD, but not in mood disorder and depressed AD. These imbalance may also play a role in the activation of the HPA-axis in AD but not in mood disorder. Moreover, there is a mild activation of markers for astrocytes in AD, but not in depression.

Acknowledgement
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KEY WORDS
Depression, Alzheimer Disease, glutamate, gamma-aminobutyric acid, prefrontal cortex, postmortem human material

TELEPHONE-NUMBER: 020-5665505
E-MAIL-ADDRESS: z.juan@nin.knaw.nl
A NON-ENZYMATIC ROLE OF CAMKIIβ IN HIPPOCAMPAL SYNAPTIC PLASTICITY

Nils Zuiderveen Borgesius, Geeske van Woerden, Ype Elgersma

Department of Neuroscience, Erasmus University Medical Center, Rotterdam

CaMKII plays an essential role in hippocampal synaptic plasticity, learning and memory. However, almost all research has focused on CaMKIIα and little is known about the role of CaMKIIβ. Here we show that the CaMKIIβ knock-out mouse has severely impaired fear conditioning and hippocampal LTP. Remarkably, the effect is very similar to the CaMKIIα knock-out, despite the fact that CaMKIIα is three times more abundant. Interestingly, preventing CaMKIIβ activation by mutating the Ca2+/Calmodulin binding domain (A303R) has little effect. This suggests that the CaMKIIβ protein has a non-enzymatic role in hippocampal learning and LTP.

Learning, LTP, CaMKII, hippocampus, plasticity, structural

06-14775776
c.zuiderveenborgesius@erasmusmc.nl
Cannabinoid Receptors, CB1 and CB2, Expression During Human Cortical Development and in Epileptogenic Developmental Pathologies


Department of Pathology, Academic Medical Center, University of Amsterdam, Amsterdam
Service Histologie-Embryologie-Cytogénétique, Groupe Hospitalier Necker Enfants-Malades, Paris, France
Department of Neurosurgery, VU University medical center, Amsterdam
Departments of Pathology and Neurosurgery, Rudolf Magnus Institute for Neuroscience, University Medical Center Utrecht, Utrecht
Stichting Epilepsie Instellingen Nederland, Heemstede

ABSTRACT

Recent data support the involvement of the endocannabinoid (eCB) signaling in brain development, suggesting a role for cannabinoid receptors (CBR) during early corticogenesis. In addition, compelling evidence suggests that CBRs play a key role in pathological conditions associated with unbalanced neuronal excitability and inflammation. In the present study we explored, using immunocytochemistry, the cellular expression and distribution of cannabinoid receptors 1 and 2 (CB1 and CB2) during prenatal human cortical development, as well as in focal malformations of cortical development (MCD) associated with intractable epilepsy (focal cortical dysplasia; cortical tubers in patients with the tuberous sclerosis complex and ganglioglioma).

Strong CB1 immunoreactivity (IR) was detected in the cortical plate (CP) in developing human brain from the earliest stages tested (GW 9) and it persisted throughout prenatal development. In contrast, CB2 IR was only transiently present in the CP (GW 9 and 13). Both CBR were undetectable in neural progenitor cells located in the ventricular zone (VZ). Only CB1 was expressed in the subventricular zone (SVZ; GW 13) and in Cajal-Retzius cells in the molecular zone of the developing neocortex. CB2 was detected in microglia/macrophages and in endothelial cells during development. In MCD, prominent CB1 expression was demonstrated in dysplastic neurons. Both CBR were detected in balloon/giant cells, but CB2 appeared to be more frequently expressed than CB1 in these cell types. Reactive astrocytes were mainly stained with CB1, whereas cells of the microglia/macrophage lineage were stained with CB2.

These findings confirm the early expression pattern of CBR in the developing human brain, suggesting a function for CB1 in the early stages of corticogenesis. The expression patterns in MCD suggest a role of CBR as mediators of the eCB signaling and as potential pharmacological targets to modulate neuronal and glial cell function in epileptogenic developmental pathologies.

KEY WORDS
Cannabinoid receptors, immunocytochemistry, malformations of cortical development

TELEPHONE-NUMBER: 020-6564369
E-MAIL-ADDRESS: e.zurolo@amc.uva.nl
<table>
<thead>
<tr>
<th>name</th>
<th>school</th>
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<tbody>
<tr>
<td>Rana Al Hussainy</td>
<td>ONWAR</td>
<td><a href="mailto:r.alhussainy@vumc.nl">r.alhussainy@vumc.nl</a></td>
<td>020-4449704</td>
<td>O</td>
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</tr>
<tr>
<td>Henrique Alves</td>
<td>ONWAR</td>
<td><a href="mailto:h.alves@nin.knaw.nl">h.alves@nin.knaw.nl</a></td>
<td>020-5664687</td>
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</tr>
<tr>
<td>Tara Arbab</td>
<td>ONWAR</td>
<td><a href="mailto:t.arbab@uva.nl">t.arbab@uva.nl</a></td>
<td>020-5258372</td>
<td>P</td>
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</tr>
<tr>
<td>Jorrit van Asselt</td>
<td>ONWAR</td>
<td><a href="mailto:j.van.asselt@nin.knaw.nl">j.van.asselt@nin.knaw.nl</a></td>
<td>020-5664422</td>
<td>BP</td>
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</tr>
<tr>
<td>Ingrid Bakker</td>
<td>ONWAR</td>
<td><a href="mailto:imc.bakker@vumc.nl">imc.bakker@vumc.nl</a></td>
<td>020-5982832</td>
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<td></td>
</tr>
<tr>
<td>Paolo Bazzigaluppi</td>
<td>ONWAR</td>
<td><a href="mailto:p.bazzigaluppi@erasmusmc.nl">p.bazzigaluppi@erasmusmc.nl</a></td>
<td>06-33702028</td>
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</tr>
<tr>
<td>Laura van Berge</td>
<td>ONWAR</td>
<td><a href="mailto:l.vanberge@vumc.nl">l.vanberge@vumc.nl</a></td>
<td>020-5982832</td>
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<tr>
<td>Simone van den Berge</td>
<td>ONWAR</td>
<td><a href="mailto:s.van.den.berge@nin.knaw.nl">s.van.den.berge@nin.knaw.nl</a></td>
<td>020-5665508</td>
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<tr>
<td>Ofir Betsalei</td>
<td>ONWAR</td>
<td><a href="mailto:o.betsalei@vumc.nl">o.betsalei@vumc.nl</a></td>
<td>020-4442418</td>
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<tr>
<td>Bernard Bloem</td>
<td>ONWAR</td>
<td><a href="mailto:bernardbloem@gmail.com">bernardbloem@gmail.com</a></td>
<td>06-41325590</td>
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<tr>
<td>Oswald Bloemen</td>
<td>ONWAR</td>
<td><a href="mailto:o.j.n.bloemen@amc.nl">o.j.n.bloemen@amc.nl</a></td>
<td>06-24148589</td>
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<tr>
<td>Pieter van Bokhoven</td>
<td>ONWAR</td>
<td><a href="mailto:pieter.van.bokhoven@cnacr.vu.nl">pieter.van.bokhoven@cnacr.vu.nl</a></td>
<td>06-41880326</td>
<td>BP</td>
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<tr>
<td>Hetty Boleij</td>
<td>RMI</td>
<td><a href="mailto:hboleij@uu.nl">hboleij@uu.nl</a></td>
<td>020-2534149</td>
<td>P</td>
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<tr>
<td>Femke den Boon</td>
<td>ONWAR</td>
<td><a href="mailto:femke.denboon@uva.nl">femke.denboon@uva.nl</a></td>
<td>020-5257642</td>
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<tr>
<td>Jeroen Bos</td>
<td>ONWAR</td>
<td><a href="mailto:j.j.bos@science.uva.nl">j.j.bos@science.uva.nl</a></td>
<td>06-33752567</td>
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<tr>
<td>Rhea van de Bospoort</td>
<td>ONWAR</td>
<td><a href="mailto:rhea.van.de.bospoort@cnacr.vu.nl">rhea.van.de.bospoort@cnacr.vu.nl</a></td>
<td>06-18171434</td>
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<td>Marco Bottelier</td>
<td>ONWAR</td>
<td><a href="mailto:bottelier@tiscali.nl">bottelier@tiscali.nl</a></td>
<td>06-24891190</td>
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<td>Zimbo Boudewijnjs</td>
<td>ONWAR</td>
<td><a href="mailto:zimbo.boudewijnjs@cnacr.vu.nl">zimbo.boudewijnjs@cnacr.vu.nl</a></td>
<td>020-5987099</td>
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<tr>
<td>Anouk den Braber</td>
<td>ONWAR</td>
<td><a href="mailto:a.den.braber@psy.vu.nl">a.den.braber@psy.vu.nl</a></td>
<td>020-5982723</td>
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<tr>
<td>Nienke Broos-Boersma</td>
<td>ONWAR</td>
<td><a href="mailto:n.broos@vumc.nl">n.broos@vumc.nl</a></td>
<td>020-4445677</td>
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<tr>
<td>Caroline Bruinisma</td>
<td>ONWAR</td>
<td><a href="mailto:c.bruinisma@erasmusmc.nl">c.bruinisma@erasmusmc.nl</a></td>
<td>06-23879313</td>
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<tr>
<td>Hans Butler</td>
<td>ONWAR</td>
<td><a href="mailto:hjc.butter@vumc.nl">hjc.butter@vumc.nl</a></td>
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<td>Sarah Burke</td>
<td>ONWAR</td>
<td><a href="mailto:s.burke@vumc.nl">s.burke@vumc.nl</a></td>
<td>06-16408740</td>
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<tr>
<td>Henrique Cabral</td>
<td>RMI</td>
<td><a href="mailto:h.deoliveiracabral@uva.nl">h.deoliveiracabral@uva.nl</a></td>
<td>020-5257637</td>
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<tr>
<td>Hayriye Cagnan</td>
<td>ONWAR</td>
<td><a href="mailto:hayriye.cagnan@philips.com">hayriye.cagnan@philips.com</a></td>
<td>040-2749181</td>
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<tr>
<td>Nutila Camargo</td>
<td>ONWAR</td>
<td><a href="mailto:nutila.camargo@cnacr.vu.nl">nutila.camargo@cnacr.vu.nl</a></td>
<td>020-5987122</td>
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<tr>
<td>Anna Carrano</td>
<td>ONWAR</td>
<td><a href="mailto:a.carrano@vumc.nl">a.carrano@vumc.nl</a></td>
<td>020-4444032</td>
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<tr>
<td>Ning Chen</td>
<td>ONWAR</td>
<td><a href="mailto:ning.chen@falw.vu.nl">ning.chen@falw.vu.nl</a></td>
<td>06-42868336</td>
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<tr>
<td>Tony Cisouw</td>
<td>ONWAR</td>
<td><a href="mailto:tony.cisouw@cnacr.vu.nl">tony.cisouw@cnacr.vu.nl</a></td>
<td>020-5986929</td>
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<tr>
<td>Jochem Cornelis</td>
<td>ONWAR</td>
<td><a href="mailto:jochem.cornelis@cnacr.vu.nl">jochem.cornelis@cnacr.vu.nl</a></td>
<td>06-44778487</td>
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<tr>
<td>Sandra Cornelisse</td>
<td>ONWAR</td>
<td><a href="mailto:s.cornelisse@uva.nl">s.cornelisse@uva.nl</a></td>
<td>06-53123688</td>
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<tr>
<td>Janna Cousijn</td>
<td>ONWAR</td>
<td><a href="mailto:j.cousijn@gmail.com">j.cousijn@gmail.com</a></td>
<td>020-5256729</td>
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<tr>
<td>Cleo Cruenelle</td>
<td>ONWAR</td>
<td><a href="mailto:c.l.cruenelle@amc.uva.nl">c.l.cruenelle@amc.uva.nl</a></td>
<td>06-14805200</td>
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<tr>
<td>Julia Dawitz</td>
<td>ONWAR</td>
<td><a href="mailto:julia.dawitz@cnacr.vu.nl">julia.dawitz@cnacr.vu.nl</a></td>
<td>06-16562895</td>
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<tr>
<td>Rocio Díez Arazola</td>
<td>ONWAR</td>
<td><a href="mailto:rocio.diezarazola@gmail.com">rocio.diezarazola@gmail.com</a></td>
<td>06-16395240</td>
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<tr>
<td>Addy van Dijk</td>
<td>ONWAR</td>
<td><a href="mailto:a.vandijk@amc.uva.nl">a.vandijk@amc.uva.nl</a></td>
<td>06-43908206</td>
<td>P</td>
<td>56</td>
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</tr>
<tr>
<td>Sarai van Dijk</td>
<td>RMI</td>
<td><a href="mailto:s.c.vandijk@umcutrecht.nl">s.c.vandijk@umcutrecht.nl</a></td>
<td>088-7559192</td>
<td>A</td>
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</tr>
<tr>
<td>Tessa Douma</td>
<td>RMI</td>
<td><a href="mailto:t.douma@uu.nl">t.douma@uu.nl</a></td>
<td>030-2533038</td>
<td>P</td>
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<tr>
<td>Hyung Elfrink</td>
<td>ONWAR</td>
<td><a href="mailto:H.L.Elfrink@amc.uva.nl">H.L.Elfrink@amc.uva.nl</a></td>
<td>020-5662518</td>
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<tr>
<td>Catherine van Engen</td>
<td>ONWAR</td>
<td><a href="mailto:c.e.vanengen@amc.uva.nl">c.e.vanengen@amc.uva.nl</a></td>
<td>020-5666039</td>
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<tr>
<td>Nitish Fagoe</td>
<td>ONWAR</td>
<td><a href="mailto:h.fagoe@nin.knaw.nl">h.fagoe@nin.knaw.nl</a></td>
<td>06-43532513</td>
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<tr>
<td>Ping Gao</td>
<td>ONWAR</td>
<td><a href="mailto:p.gao@vumc.nl">p.gao@vumc.nl</a></td>
<td>06-43832148</td>
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<td>Lieve Geerts</td>
<td>ONWAR</td>
<td><a href="mailto:lieke.geerts@cnacr.vu.nl">lieke.geerts@cnacr.vu.nl</a></td>
<td>06-39723737</td>
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<td>Hans-Rüdiger Geis</td>
<td>ONWAR</td>
<td><a href="mailto:h.geis@erasmusmc.nl">h.geis@erasmusmc.nl</a></td>
<td>010-7044526</td>
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<td>Asiya Giniatullina</td>
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<td><a href="mailto:asiya.giniatullina@cnacr.vu.nl">asiya.giniatullina@cnacr.vu.nl</a></td>
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<td>Andrea Goudriaan</td>
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<td>Ewout Groen</td>
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<td>Torben Hager</td>
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<td>Felisa van Hasselt</td>
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<td><a href="mailto:f.n.vanhasselt@uva.nl">f.n.vanhasselt@uva.nl</a></td>
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<td>Sasja Heetveld</td>
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<td>Enika van Hell</td>
<td>RMI</td>
<td><a href="mailto:h.l.vanhell@umcutrecht.nl">h.l.vanhell@umcutrecht.nl</a></td>
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<td>ONWAR</td>
<td><a href="mailto:s.hoynig@nin.knaw.nl">s.hoynig@nin.knaw.nl</a></td>
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<td>Willem Huijbers</td>
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<td><a href="mailto:w.huijbers@uva.nl">w.huijbers@uva.nl</a></td>
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<td>Sheena Isenia</td>
<td>ONWAR</td>
<td><a href="mailto:s.isenia@erasmusmc.nl">s.isenia@erasmusmc.nl</a></td>
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<td>Sarah Janssen</td>
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<td><a href="mailto:s.janssen@nin.knaw.nl">s.janssen@nin.knaw.nl</a></td>
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<td>Arthur de Jong</td>
<td>ONWAR</td>
<td><a href="mailto:arthur.de.jong@cnclr.vu.nl">arthur.de.jong@cnclr.vu.nl</a></td>
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<td>Regina Kanski</td>
<td>ONWAR</td>
<td><a href="mailto:r.kanski@nin.knaw.nl">r.kanski@nin.knaw.nl</a></td>
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<td>Timo van Kerkoerle</td>
<td>ONWAR</td>
<td><a href="mailto:t.van.kerkoerle@nin.knaw.nl">t.van.kerkoerle@nin.knaw.nl</a></td>
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<td>Marianne Klanker</td>
<td>ONWAR</td>
<td><a href="mailto:m.klanker@nin.knaw.nl">m.klanker@nin.knaw.nl</a></td>
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<tr>
<td>Pieter Klein</td>
<td>ONWAR</td>
<td><a href="mailto:p.klein@rcn.vu.nl">p.klein@rcn.vu.nl</a></td>
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<td>Patricia Klemmer</td>
<td>ONWAR</td>
<td><a href="mailto:p.klemmer@cnclr.vu.nl">p.klemmer@cnclr.vu.nl</a></td>
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<td>Anne Klomp</td>
<td>ONWAR</td>
<td><a href="mailto:a.klomp@cmc.uva.nl">a.klomp@cmc.uva.nl</a></td>
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<td>Evert-Jan Kooi</td>
<td>ONWAR</td>
<td><a href="mailto:e.kooi@vumc.nl">e.kooi@vumc.nl</a></td>
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<td>Joanna Kerecka</td>
<td>ONWAR</td>
<td><a href="mailto:j.kerecka@nin.knaw.nl">j.kerecka@nin.knaw.nl</a></td>
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<td>Ozlem Korucuoglu</td>
<td>ONWAR</td>
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<td>Marijn Kuijpers</td>
<td>ONWAR</td>
<td><a href="mailto:m.kuijpers.1@erasmusmc.nl">m.kuijpers.1@erasmusmc.nl</a></td>
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<td>Cathalijn Leenaars</td>
<td>ONWAR</td>
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<td>Esther Lips</td>
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<td>Anouk van Loon</td>
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<td>ONWAR</td>
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<td>ONWAR</td>
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<td>ONWAR</td>
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<td>ONWAR</td>
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<td>ONWAR</td>
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<td>Evelien Platje</td>
<td>ONWAR</td>
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<td>Simon-Shlomo Poll</td>
<td>ONWAR</td>
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<td>ONWAR</td>
<td><a href="mailto:j.poort@nin.knaw.nl">j.poort@nin.knaw.nl</a></td>
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<td><a href="mailto:rogier.poorthuis@cnocr.vu.nl">rogier.poorthuis@cnocr.vu.nl</a></td>
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<tr>
<td>Jolanda Prins</td>
<td>RMI</td>
<td><a href="mailto:j.prins1@uu.nl">j.prins1@uu.nl</a></td>
<td>030-2537362</td>
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<td>Xin-ni Qi</td>
<td>ONWAR</td>
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<td>Xin Qiao</td>
<td>ONWAR</td>
<td><a href="mailto:x.qiao@uva.nl">x.qiao@uva.nl</a></td>
<td>020-5257639</td>
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<tr>
<td>Priyanka Rao</td>
<td>ONWAR</td>
<td><a href="mailto:priyanka.rao@cnocr.vu.nl">priyanka.rao@cnocr.vu.nl</a></td>
<td>020-5987122</td>
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<tr>
<td>Yael Reijmer</td>
<td>RMI</td>
<td><a href="mailto:y.d.reijmer@umcutrecht.nl">y.d.reijmer@umcutrecht.nl</a></td>
<td>088-7557969</td>
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<td>Margreet Ridder</td>
<td>ONWAR</td>
<td><a href="mailto:mc.ridder@vumc.nl">mc.ridder@vumc.nl</a></td>
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<tr>
<td>Danai Riga</td>
<td>ONWAR</td>
<td><a href="mailto:alloplasma@yahoo.com">alloplasma@yahoo.com</a></td>
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<tr>
<td>Katja Ritz</td>
<td>ONWAR</td>
<td><a href="mailto:k.a.ritz@amc.uva.nl">k.a.ritz@amc.uva.nl</a></td>
<td>020-5662518</td>
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<tr>
<td>Thais Rizzi</td>
<td>ONWAR</td>
<td><a href="mailto:ts.rizzi@psy.vu.nl">ts.rizzi@psy.vu.nl</a></td>
<td>020-5982832</td>
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<tr>
<td>Kasper Roet</td>
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<td>ONWAR</td>
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<td>Esther van der Zwaal</td>
<td>RMI</td>
<td><a href="mailto:e.m.vanderzwaal@umcutrecht.nl">e.m.vanderzwaal@umcutrecht.nl</a></td>
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<td>RMI</td>
<td><a href="mailto:j.p.h.burbach@umcutrecht.nl">j.p.h.burbach@umcutrecht.nl</a></td>
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<td>Elly Hol</td>
<td>ONWAR</td>
<td><a href="mailto:e.hol@nin.knaw.nl">e.hol@nin.knaw.nl</a></td>
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<td>Martien Kas</td>
<td>RMI</td>
<td><a href="mailto:m.j.h.kas@umcutrecht.nl">m.j.h.kas@umcutrecht.nl</a></td>
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<td>Jeroen Pasterkamp</td>
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<td><a href="mailto:r.j.pasterkamp@umcutrecht.nl">r.j.pasterkamp@umcutrecht.nl</a></td>
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<td>ONWAR</td>
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<td>SWL</td>
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<td>Dick Veltman</td>
<td>ONWAR</td>
<td><a href="mailto:dj.veltm@vumc.nl">dj.veltm@vumc.nl</a></td>
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<td>ONWAR</td>
<td><a href="mailto:matthijs.verhage@cncr.vu.nl">matthijs.verhage@cncr.vu.nl</a></td>
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<td>ONWAR</td>
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