



Rudolf Magnus Institute of Neuroscience

Rudolf Magnus Bulletin 38 March 2008

TI Pharma special

The TI-Pharma initiative is an initiative from the government, the pharmaceutical industry in the Netherlands and the Dutch universities. Researchers were invited to write research proposals in several specific areas of medicine. Each proposal should involve academic and pharmaceutical partners and should be a large scale project for four years. Within the Rudolf Magnus Institute six proposals have been awarded with a grant. Since January of this year the projects have officially started and the first researchers are appointed. This issue of the RMI bulletin will give an overview of the projects that have been initiated within the Institute.

Jeroen Pasterkamp, Peter Burbach and Marten Smidt of the department of Neuroscience and Pharmacology have been rewarded with a TI-pharma project on the topic Parkinson and Alzheimer's disease.

Parkinson and Alzheimer disease: from dysregulated human brain targets towards novel therapeutics

The challenging title of this TI-Pharma project reflects the interests and the joint ambition of the nine research groups and two companies that make up this consortium. The Rudolf Magnus Institute of Neuroscience (RMI) participates with one group in this project, consisting of dr. Jeroen Pasterkamp, dr. Marten Smidt, prof. dr. Peter Burbach and three project researchers, all part of the Section Neurodevelopment of the department of Neuroscience and Pharmacology. Two of the three project researchers are in place: the PhD students Asheeta Prasad and Elisa Hoekstra. For the third position we are currently seeking a talented postdoctoral fellow.

The starting point of this project is a unique collection of gene expression profiles of the degenerating human brain generated by Joost Verhaagen and colleagues at the Netherlands Institute of Neuroscience in Amsterdam. Particularly experiments employing post-mortem material of Parkinson's disease patients have revealed that a large set of genes changes upon neuronal degeneration. The work of Verhaagen and co-workers as well as studies by other project members reveals novel markers for disease onset and progression, but also potential therapeutic targets for fighting neurodegeneration.

The overall goal of TI-Pharma project T5-207 is to evaluate and develop these previously identified genes as drug targets for Parkinson's disease. Parkinson's disease is a movement disorder that is characterized by the select degeneration of dopamine neurons. The specific



left to right: (upper row) Bert van der Zwaag, Youri Adolfs, Henk Spierenburg, Asheeta Prasad, Raymond Schellevis, Marten Smidt, Jeroen Pasterkamp, (bottom row) Peter Burbach, Yeping Zhou, Teresa Alves dos Santos, Elisa Hoekstra, Annemarie van der Linden, Anita Hellemons, Lars von Oerthel, Alwin Derijck

contribution of the RMI Section Neurodevelopment is the development of experimental models based on developing and mature dopaminergic systems, in which select candidate genes can be functionally screened and drug targets can be evaluated. This contribution builds on long-standing expertise within the Section Neurodevelopment on dopamine neuron development and maintenance. In the past ten years, the molecular mechanisms that control neuronal identity and subset specification in the dopaminergic systems have been a major research focus within this section (Smidt and Burbach). Recent breakthroughs include the identification of the transcription factor Pitx3 and retinoic acid signalling as central players in dopamine neuron differentiation and survival. Jeroen Pasterkamp has, since his arrival in 2004, complemented the research line of Smidt and Burbach by studying

another crucial aspect of dopamine neuron development; the development of dopaminergic axon pathways. In addition to defining anatomical and cellular features of dopaminergic connections, molecular cues are being identified in the Pasterkamp group that instruct dopamine neurons to form and stabilize axonal connections. In the quest for understanding gene function in dopaminergic systems a significant accumulation of advanced technology and experimental systems has taken place. Today, the section Neurodevelopment exploits novel (conditional) knock-out and knock-in mice approaches to both study gene function and to visualize dopaminergic cell bodies, axon tracts and synapses. In addition, tissue culture approaches, embryonic stem cells, and dopaminergic cell lines are being developed and used to understand the function of specific genes in the development, maintenance and survival of dopaminergic systems. These technologies are invaluable for achieving the aims of this TI Pharma consortium and will help to validate drug targets for Parkinson's disease and other neurodegenerative disorders.

Two project researchers of the RMI TI-Pharma team have started their projects. Within the TI-Pharma consortium, these PhD projects are aimed at developing *in vitro* and *in vivo* tools for studying the above mentioned Parkinson's disease candidate genes. Asheeta Prasad studies the function of Wnts and their receptors in the development of mesostriatal circuitry by combining green fluorescent protein (GFP)-reporter mice and novel tissue culture approaches. Elisa Hoekstra works on the identification of molecular factors that control dopaminergic differentiation and maintenance by searching for target genes of key transcription factors of dopamine development, like Lmx1a and Lmx1b. These projects are embedded in the groups Pasterkamp and Smidt and nourished by other members of the section Neurodevelopment (see picture at the top of the first page).

The second TI-Pharma project within the RMI is a project in which two departments of the Institute collaborate. Louk Vanderschuren of the department of Neuroscience and Pharmacology and Nick Ramsey of the department of Neurology and Neurosurgery are both involved in the TI-Pharma project entitled:

The neurophysiological role of the endocannabinoid system in support of smoking cessation, fighting addiction and treating cognitive decline

This project addresses the role of the brain endocannabinoid system in the modulation of mesocorticolimbic neurotransmission involved in emotion and cognition and the therapeutic opportunities of cannabinoid ligands. To that end, a variety of complementary methodologies are used to study brain functions implicated in psychopathological syndromes with high medical need, such as schizophrenia, ADHD, mood disorders and drug addiction. A particularly strong aspect of this project is its high translational character, since the behavioural models are matched between humans and animals.

The project entails a close collaboration of seven research groups in The Netherlands, two of which are part of the Rudolf Magnus Institute, i.e. the groups of Louk Vanderschuren and Nick Ramsey.

In the group of Louk Vanderschuren, two people, i.e. Viviana Trezza and Ruth Damsteegt, have been hired to investigate the role of the endocannabinoid system in social behaviour, impulsive behaviour and drug addiction. It is well known that the endocannabinoid system plays a key role in reward processes in the brain, and this has mostly been investigated in the context of drug reward. However, the role of the endocannabinoid system in social behaviour in adolescence, which is essential for social and cognitive development, is unknown. Moreover, although the endocannabinoid system has been implicated in drug reward, its role in compulsive patterns of drug seeking, constitute the core of the addiction syndrome, remain elusive. Impulsive behaviour is associated with antisocial behaviour, which is a significant risk factor for addictive behaviour; moreover, prolonged drug use has been shown to cause a breakdown of impulse control in humans and animals. It is therefore well conceivable that changes in endocannabinoid neurotransmission play a key role in impulsive behaviour as well.



left to right Viviana Trezza, Louk Vanderschuren and Ruth Damsteegt

In Nick Ramsey's group, three people are involved in this TI Pharma project, i.e. Gerry Jager, Erika van Hell and Matthijs Bossong. Together, they will perform functional neuroimaging and psychological testing following cannabinoid ligand administration in control and patient human populations. Thus, new paradigms to assess social and impulsive behaviour for use in human fMRI studies will be developed that closely match those used in Louk Vanderschuren's group, as well as those used in Ton Schoffelmeer's group at the VUmc in Amsterdam, who also participate in this project. These tests will be administered to both healthy volunteers, as well as carefully selected patient populations, including drug addiction, impulse control disorders and mood disorders.

The participants will be challenged during the test with either THC or a cannabinoid receptor antagonist, to probe the sensitivity of the endocannabinoid system in these functions in health and disease. Understanding the endocannabinoid system in behavioural, neurochemical and psychological terms in animals and humans will result in the validation of cannabinoid related targets for the treatment of addictive behaviours, impulse control, mood disorders and cognitive dysfunctions. Taken together, this project is expected to increase the drug-ability of the cannabinoid system by providing an empirical basis for new and promising applications of cannabinoid ligands.

The third TI-Pharma project is based at the department of Psychiatry of the Rudolf Magnus Institute with René Kahn as one of the principal investigators.

A translational pharmacogenomics approach to improve drug development strategies for psychiatric disorders

The program makes use of the largest cohort of schizophrenia patients and their siblings assembled to date that has been conducted in the genetic risk and outcome for psychosis (GROUP study) that has been funded by NWO. This study follows longitudinally 1,000 patients and a similar number of their siblings and 1,000 controls to determine genetic risk factors and the influence of the environment on these risk factors, determining outcome in schizophrenia and the conversion from healthy status to the development of schizophrenia. In this study extensive phenotypic characterization and a whole genome analysis funded by the NIMH is taking place. Furthermore, funding from the Wellcome Trust was obtained to do whole genome analysis in the siblings. TI-Pharma is subsidizing the collection of blood for gene expression studies (RNA) in the patients and siblings.



René Kahn

Since the first wave of assessments was three years ago and the second wave is currently starting (2008), the blood will be collected when all patients and siblings will be seen in the reassessment three years after baseline. Therefore, the study that is now being funded by NWO, NIMH, the Wellcome Trust and TI Pharma enables us to examine gene-environment interactions both in DNA as well as on gene expression patterns. In this it forms a unique cohort that has no equal elsewhere in the world.

The last projects to be mentioned are the three projects in which Roger Adan of the department of Neuroscience and Pharmacology is involved. Within these projects Susanne la Fleur and Geert Ramakers, both from the department of Neuroscience and Pharmacology, also play a major role. A short description of the three projects:

How do drugs affect body weight?

The overall goal of this project is to uncover the mechanisms underlying the effect of drug-induced weight alterations and to identify novel targets to develop strategies to treat these obesity-related problems.

The focus of this project is on the antipsychotic Olanzapine. Previous experiments in the department had shown the effect of this drug in counteracting anorectic behaviours. Interestingly, the antiepileptic drug, topiramate, exerts weight loss in obese patients and improves glycemic control. It is prescribed to patients taking Olanzapine to prevent weight gain. The mechanisms via which Olanzapine increases weight gain, and topiramate alleviates olanzapine-induced weight gain are unknown.

This project, in which Roger Adan and Susanne la Fleur participate, brings together experts from the field of CNS pharmacology, diabetes, proteomics and metabolomics from several academic centers and industry. An animal model will be developed to unravel both peripheral and central mechanisms. The model will be validated with data in healthy volunteers. Novel biomarkers and targets for diabetes, dyslipidemia and obesity will be identified and validated. The central mechanisms via which Olanzapine affects feeding, motivation and reward are studied by Esther van der Zwaal (PhD-student), post-doc Sanna Janhunen (starts in April), and two technicians (Judith Hendriks and Rea van Rozen).

The GPCR forum: novel concepts in pharmacology

The overall aim of the 'GPCR forum' is to obtain new vistas in the pharmacological and physiological functioning of G protein-coupled receptors (GPCRs), with special focus on receptor activation, receptor oligomerisation and receptor bioinformatics. The principle investigators in the Department are Roger Adan and Geert Ramakers. Two PhD students, Frank Meye and Edwin Alserda, will work on this project.

The department has a long history of GPCR research. In particular, the melanocortin system has been studied extensively. Melanocortins play a prominent role in the control of energy balance, as shown by the association in the MC4 receptor (MC4R) and obesity.

Previous research showed that Agouti and the Agouti-related peptide (AgRP) are inverse agonists for MC4R, which means that both endogenous agonists (melanocortins like α -MSH) and an endogenous inverse agonist (AgRP) bind to this receptor.

The project is aimed to determine the physiological role of several GPCRs (the melanocortin, the dopamine, the opiate and cannabinoid receptors) in the mesolimbic system, and will focus on the constitutive activity of these receptors (and the effect of inverse agonists) and the interactions between GPCRs (homo- and heteromers). Ultimately, together with the results of the other partners, this will lead to new concepts in GPCR activation and the functioning of these receptors *in vivo*.

CNS drug target validation and therapeutic potential using RNA interference

CNS disorders are complex, poorly understood and debilitating diseases. Therefore the need for new CNS drugs is extremely high. Genetic and functional genomics studies have identified novel drug targets with considerable therapeutic potential which will be validated in this project. RNAi technology is an alternative for selective and rapid validation of novel targets in animals and can also be used in a clinical setting. This project aims to use viral vectors to unravel the role of genes in brain. Earlier work in the department showed that with viral vectors gene expression can be modified locally in rat brains, resulting in behavioural changes (for instance feeding). Moreover, it was shown that with viral vectors (overexpression of shRNA) transgenic rats can be made, with decreased expression of a drug target (in our case the MC4 receptor, which leads to obesity). Rick van Haastert (PhD student) and Maike Brans (biotechnician) work on this project to refine these techniques further, so that it will become feasible to knockdown any (drug target) gene in (rat) brain. In April they will be joined by Olivier van Beekum (postdoc).



Left to right Geert Ramakers, Esther van der Zwaal, Maike Brans, Rick van Haastert, Frank Meye, Judith Hendriks

PhD theses

2008-4

February 28, 2008

Hugo van Oostrom

An attempt to assess animal pain using brain activity

L.J. Hellebrekers, P.J. Stienen
supervisors

Hugo van Oostrom started his PhD in 2004 after he had finished his studies in veterinary sciences. He performed his PhD work in the department of Veterinary sciences and completed his thesis in the section of Behavioural genomics. After his thesis defence Hugo will complete his training as vet.

Evoked potentials recorded from the scalp after noxious stimulation (SEPs) represent neuronal processing of the noxious stimulus in the brain. SEPs recorded from vertex (Vx-SEP) in man show interesting characteristics making them useful to study acute pain and analgesia. Vx-SEPs 1) are sensitive to analgesic intervention, 2) show a positive correlation with the unpleasantness of the noxious stimuli applied to evoke the SEP and 3) are absent or altered in patients suffering from deficits in pain sensation.

In animals, SEPs may also be of interest to study acute pain and analgesia. SEPs recorded in animals are sensitive to analgesic intervention, however, it has not been shown whether these changes correlate with subjective perception of noxious stimuli in animals. In this thesis several experiments are described that show that in animals, there is a similar correlation between the SEP and the unpleasantness of the noxious stimuli that were applied as in humans.

In the experiments of this thesis the recording of SEPs and auditory evoked potentials (AEPs) was used to assess the analgesic and sedative properties of the drug dexmedetomidine in rats and dogs. It was shown that dexmedetomidine causes sedation in the lower dose range and both sedation and analgesia in the higher dose range.

news and other things

Steven Bakker receives VENI grant

Steven Bakker from the department of Psychiatry has been rewarded with a VENI grant of 200.000 euros for his project into the role of fibroblast growth factors (FGFs) in normal brain development and schizophrenia.

A pilot study by the RMI and the Division of Biomedical Genetics recently found evidence for association of two FGF genes with brain volume in healthy individuals, but also with brain volume abnormalities in schizophrenia (Jungerius et al., 2007 and Hoogendoorn et al., submitted). These findings are supported by a large body of literature, which suggests a central role for the FGF system in psychiatric disorders. Bakker will now systematically search human genome databases for genes that interact with, or regulate the expression of FGF genes, using bioinformatics techniques that were recently developed in-house (Franke et al., 2006). He will then study the effects of these genes on volumes of the hippocampus and specific (sub-)cortical regions, in a unique sample of approximately 300 schizophrenia patients and 300 controls with MRI scans. Genes in the FGF network will be studied in detail using a comprehensive set of Single Nucleotide Polymorphisms (SNPs). The powerful combination of a novel bioinformatics approach to map gene networks and the use of brain volumes as measurable endophenotypes could provide new insights into the genetic determinants of normal brain volume and brain volume abnormalities in schizophrenia.

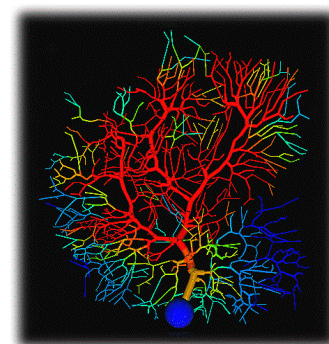
Michael van Es receives *Prinses Beatrix Fonds* award

Michael van Es, of the department of Neurology and the section Neuromuscular diseases, received the Prinses Beatrix Fonds year award for the best research article in the area of neuromuscular diseases of the year 2007. The award is an encouragement award for young promising scientists in the field of neuromuscular diseases. The award of 1,000 euros has been presented to Michael in January. The rewarded article is entitled "PTR2 as a susceptibility gene in sporadic amyotrophic lateral sclerosis: a genome-wide association study" published in *Lancet Neurology* 2007 (6(10): 869-77).

Saskia van der Hel receives Carl Zeiss Poster award

Saskia van der Hel of the department of Neuroscience and Pharmacology has received the Carl Zeiss poster award at the annual meeting of the Dutch Anatomists Association in January. She received the award for the poster entitled: Longitudinal in vivo MR spectroscopy and histochemistry in experimental temporal lobe epilepsy

Authors: W. Saskia van der Hel, Pieter van Eijsden, Ineke W.M. Bos, Robin A. de Graaf, Kevin L. Behar, Jan-Willem Berkelbach van der Sprenkel, Onno van Nieuwenhuizen, Pierre N.E. de Graan, Kees P.J. Braun



agenda and announcements

March 12, 2008 RMI Lecture

Christopher Pierce (Boston University School of Medicine, Boston, USA)

'The intractable problem of treating cocaine addiction: progress and prospects'

12.30-14.00 hr, yellow lecture hall UMC Utrecht

more information: I.j.m.j.vanderschuren@umcutrecht.nl

March 13, 2008 LIBC Lecture

Sarah Durston

'Combining genetics and neuroimaging to investigate cognitive control'

16.30 hr, LUMC, Von Ronnenzaal, K2-052

more information:

<http://www.libc-leiden.nl/colloquia.htm>

March 14, 2008 Helmholtz lecture

David Leopold (NIH, USA)

'What is a neural correlate of perceptual suppression?'

16:00 hr 'Ruppert Building Zaal rood', Utrecht

contact, Veronica Maassen, helmholtz@fss.uu.nl

March 19, 2008 Swammerdam lecture

John O'Doherty, (California Institute of Technology Pasadena, USA)

'Model-based fMRI and its application to reward-learning and decision making'

ONWA Amsterdam

more information: <http://www.onwa.med.vu.nl/swammerdam>

April 2, 2008 CSCA Lecture

Christiaan Keyzers

'Mirror systems and social cognition'

Doelenzaal (UB) Singel 425, Amsterdam

16:00-17:00, afterwards informal drinks

more information: <http://www.csc.a.nl>

April 4, 2008 M-BIC Lecture

Victor Lamme

'Solving the real mystery of consciousness'

13.30 hr UNS40 0673, Maastricht University

more information:

<http://mbic.unimaas.nl/redirect.asp?page=Lectures>

April 10, 2008 LIBC Lecture

Danielle Posthuma

'Intelligence & genes: current status and future directions'

16.30 hr, LUMC, Von Ronnenzaal, K2-052

more information:

<http://www.libc-leiden.nl/colloquia.htm>

April 11, 2008 Helmholtz lecture

Eero Simoncelli (New York University, USA)

'Modeling the visual system'

16:00 hr 'Ruppert Building Zaal rood', Utrecht

contact, Veronica Maassen, helmholtz@fss.uu.nl

April 21-22, 2008 Summer school RMI

Summer school for all PhD students of the Rudolf Magnus Institute of Neuroscience

Location Akersloot, Van der Valk Hotel Akersloot

April 25, 2008 Swammerdam lecture

Frank LaFerla, (University of California, USA)

'Studying and treating Alzheimer's Disease in mice and its translation to humans'

ONWA Amsterdam

more information: <http://www.onwa.med.vu.nl/swammerdam>

May 13-16, 2008 '100 jaar Farmacologie Nederland'

Visit the website for details on the program and registration for the symposia

www.100jaarfarmacologie.nl

13 May 'Publiekssymposium'

10:00- 16:30 Beatrixtheater Utrecht

14 May 'Farmacologie in Utrecht: 100 jaar jong en dynamisch'

14:00-17:15 academiegebouw Utrecht

15 May David de Wied lecture (on invitation)

16 May 'Wetenschappelijk symposium: De farmacologie in Nederland: Heden en toekomst'

10:15-17:00 Beatrixtheater Utrecht

