Rudolf Magnus
Institute of Neuroscience

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interview

Fast, Faster, Marike

Many neuronal diseases can theoretically be resolved via targeted gene therapy. Marike Broekman demonstrated that Adeno-Associated Virus (AAV) is a save vehicle to transfer genes to the mouse brain. She was able to cure mice suffering from GM1-gangliosidosis, a lysosomal storage disease, by injecting an AAV vector encoding for the missing enzyme into the ventricles of neonatal mice. The results are highly relevant for further development of gene therapy in human diseases of the central nervous system. Another remarkable fact is that she is still a medical student …

The sixth year in the training of medical students at the UMC Utrecht leaves room for students to do research of their own choosing. Broekman, who is highly interested in neuronal stem cell technology and gene therapy of the central nervous system, decided to go to the lab of Xandra Breakefield and Miguel Sena-Esteves at Massachusetts General Hospital. In about 18 months, starting September 2004, she was able to first-author five articles and to co-author another two articles; sufficient for an upcoming graduation to PhD.

Presently, AAV technology is at the centre of attention in gene therapy. Broekman: ”AAV is a small non-pathogenic, single stranded DNA virus, which can be used to introduce genes directly into neurons, both *in vitro* and *in vivo*. As the transgene that is introduced does not integrate into the host’s genome, there is no risk of disturbing normal gene expression, such as the accidental activation of an oncogene. Stable expression of transgenes has been demonstrated and AAV can be used in many species such as mouse, rat, cat, monkeys, and humans. We were able to demonstrate that different (novel) AAV serotypes were able to deliver transgenes to the mouse brain. Upon injection of a construct with green fluorescent protein as marker into the cerebral ventricles of neonatal mice, we observed that all serotypes transduced a large number of neurons in the brain. Interestingly, some serotypes were more efficient than others; AAV8 is by far the most superior serotype for the delivery of genes to the CNS.”

Broekman demonstrated that it was possible to cure mice from chronic disease. She injected AAV containing a β-galactosidase gene construct in the brain of mice with the lysosomal storage disease GM1-gangliosidase. Using both adult and neonatal mice, that were deficient for β-galactosidase, she was able to elicit stable β-galactosidase expression in the brain neurones of these mice, demonstrating the feasibility of gene therapy. Broekman is optimistic with regard to the possibilities of gene therapy in humans: “In humans safety trials have begun with AAV, to explore the feasibility of AAV as agents for gene therapy. A phase I trial in Canavan Disease showed so far that the AAV infusion in the human brain was safe.”

When asked about her success, Broekman explains, ”I was probably at the right moment in the right place. I was given complete confidence by my supervisors to plan and do my own experiments. And I needed to learn a lot in a very short while. Yet, I feel that it helped that I had a certain unprejudiced view towards this field of research, which enabled me to ask questions and open up discussion that may not have occurred to the minds of established researchers.”

Marike Broekman (December 21, 1978) studied Law (2004) and Medicine (MD, expected 2006). The work as described was performed in the Department of Neurology and Neuroscience Programme, Massachusetts General Hospital and Harvard Medical School, Charlestown, MA, USA. Presently she works as coassistant at the Department of Neurosurgery of the UMC Utrecht. She will receive her PhD for the work that she performed in Charlestown later this year.

2006-8
April 6, 2006

Y. Benita

In silico strategies for proteomics closure. Prediction of small human genes and analysis of high-throughput protein synthesis

D.J.A. Crommelin, B. Olivier
supervisors

2006-9
Socialising among horses

April 19, 2006

Machteld C. Van Dierendonck

The importance of social relationships in horses

B. Colenbrander, B.M. Spruijt, M.B.H. Schilder
supervisors

Horses are social animals, which rely on survival strategies centred on the formation of cohesive social bonds within their herd. Machteld Van Dierendonck performed ethological studies on groups of captive and free-living horses. She found that horses actively influence their own social network. She established that physical interaction such as play and mutual grooming among horses were very important for the social wellbeing in horses, and that deprivation of these essential behaviours may easily lead to stress in captive animals.

Many problems in the husbandry of horses are due to the fact that the limits of their (social) adaptive abilities are exceeded. Thirty percent of horses in the western world suffer from abnormal behaviour related to various less optimal husbandry practices. Field studies in (semi)natural environments provide an excellent way to study the social life of horses, and may provide clues to define the social needs of domestic horses. Van Dierendonck investigated social structure, social strategies and social interactions in established groups of horses. She investigated how horses build a social network and whether individual horses use interventions to influence their social network. She also investigated familiarity as a social variable, and determined the importance of positive social interactions as an ethological need in domestic horses. 'Ethological needs' are behaviours which are so important to the animal that the impossibilities to execute such behaviours will cause chronic stress.

From her extensive studies Van Dierendonck concluded that social life of domestic horses is dominated by preferred bonds which can exist for years and are established and maintained by mutual grooming, play, proximity and dominance interactions. Dominance relationships appeared primarily related to social experience. Individuals regulate their social network by means of interventions in interactions between other members of the herd. Van Dierendonck argues that the execution of affiliative behaviours may be rewarding in itself and that the execution of this behaviour can also be classified as an 'ethological need'. She found that grooming and play satisfy the criteria as 'ethological needs'.

Van Dierendonck; "All horses need physical social contact, and horses which lack appropriate social learning experiences during ontogeny, may be hampered in their social functioning later in life. The performance of social interactions is a necessity for domestic horses which notion should be reflected in modern husbandry and management systems."

Fighting Przewalski horses (courtesy Machteld Van Dierendonck)

Machteld van Dierendonck (March 10, 1961, Amsterdam). Secondary education, 1980 (Montessori Lyceum, Amsterdam), Biology (Amsterdam University), 1987. She held multiple positions in the ethological study and conservation of wild and captive horse populations in Mongolia, Sweden, Italy, Iceland, and the Netherlands, during which she gathered the data on which this thesis was presented. Presently she works as teacher, researcher, and consultant on the (clinical) ethology of horses.

2006-10
April 25, 2006

A.J.M. Van Wijck

Postherpetic neuralgia

C.J. Kalkman, T.J.M. Verheij, K.G.M. Moons
supervisors

2006-11
The workings of stress

April 26, 2006

Anikó Körösi

Neurobiology of stress adaptation in the mouse. Roles of corticotropin-releasing factor and urocortin 1

B. Olivier, E.W. Roubos, T. Kozicz, J.G. Veening, J. Van der Gugten
supervisors

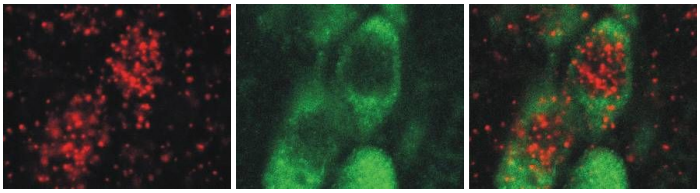
Failure of stress adaptation systems may lead to the development of stress-related physiological and mental disorders. Anikó Körösi studied the role of corticotropin-releasing factor (CRF), urocortin 1, and the Edinger-Westphal nucleus (EW) in stress adaptation. She found that the CRF over-expressing (CRF-OE) mouse is a suitable tool for investigating the role of chronically elevated CRF in the control of stress adaptation, and that urocortin 1 in the EW plays an important role in stress adaptation.

The best-known stress adaptation system is the hypothalamo-hypophyseal adrenal (HPA) axis, in which

hypothalamic corticotropin-releasing factor (CRF) plays a central role. The discovery of urocortin 1 in the EW, acting like CRF through CRF receptors, raised the question how CRF-related peptides, the HPA-axis and the EW collaborate in stress adaptation. Körösi focused on the presence and dynamics of CRF, urocortin 1, and their receptors CRF1 and CRF2, using CRF-OE mice and acute and chronic stress paradigms.

The CRF-OE mice have increased amounts of CRF peptide and mRNA, restricted to the central nervous system. This CRF over-expression is associated with an increased level of bioactive CRF in the hypothalamus, increased body temperature and heart rate, and increased food and water consumption, and therefore seems a valid model to study the role of central CRF in stress adaptation. Körösi demonstrated that CRF differentially controls the two CRF receptors, CRF1 and CRF2, with regard to mRNA expression and expression sites in the brain. The presence of both CRF1 and -2 receptor mRNAs is also demonstrated throughout the spinal cord, which leads to the assumption that CRF-related peptides act at peripheral components of the stress adaptation system, via distinct spinal cord receptors.

Körösi demonstrated that in CRF-OE mice, urocortin 1 was down-regulated in the EW. Furthermore, these neurons respond, like the HPA-axis, to an acute challenge by increased Fos and urocortin 1 mRNA expression, indicating that the HPA-axis and the EW respond in concert to acute challenges, but act in opposite ways during chronic stress. Chronic stress-induced activation of the adrenals resulted in inhibition of the urocortin 1-system in the EW, most likely through a direct action of corticosterone on EW-urocortin 1 neurons. This notion is supported by the demonstration that glucocorticoid receptors coexist with urocortin 1 in EW neurons, and that chronic corticosterone-treatment reduces the number of urocortin 1-containing EW neurons.



Glucocorticoid receptors (red) coexist with urocortin 1 (green) in EW neurons. (Courtesy of Anikó Körösi)

Anikó Körösi (March 14, 1977, Budapest, Hungary). Secondary education (Liceo Scientifico Leonardo da Vinci, Turin, Italy), 1996; Biology (University of Turin, Italy), 2001. The research leading to this thesis was started in 2002 at the Department of Psychopharmacology, Utrecht University in collaboration with the Department of Cellular Animal Physiology, Radboud University Nijmegen.

2006-12

The wolf within

April 27, 2006

Linda Van den Berg

Genetics of aggressive behaviour in Golden Retriever dogs

B.A. Van Oost, M.B.H. Schilder, P.A.J. Leegwater supervisors

There are indications that aggression in dogs is at least in part heritable. Linda Van den Berg analysed aggression in Golden Retrievers both phenotypically and genotypically. She found that the aggressive phenotype was best described through a personal interview of the owner, and that this aggression was heritable. She was however not able to identify consistent genetic aberrations in aggressive Golden Retrievers in several candidate genes for canine human-directed aggression.

The recent upsurge in reports of aggressive Golden Retrievers, in this otherwise fairly docile dog race, seems to point towards a strong genetic involvement in dog aggression. Van den Berg set out to find the proper phenotypic characterisation of human- and dog-directed aggression of Golden Retrievers. She compared the results of a behavioural test of the dogs, a dog owner questionnaire, and a personal interview with the owner, and found that the owner's interview provided consistent phenotypes regarding both dog- and human-directed aggression. Using the phenotypes obtained by the owner's interview, she obtained evidence that both types of aggression were largely genetically determined (325 dogs, heritability estimate for dog- and human-directed aggression was 0.81 and 0.77, respectively).

Alterations in brain serotonin metabolism were described in aggressive dogs. Therefore Van den Berg performed genetic analysis of four serotonergic genes, serotonin receptor 1A, - 1B and - 2A, and serotonin transporter, as candidate genes for Golden Retriever (human-directed) aggression. She performed mutation screens and linkage analysis, and was able to identify several polymorphisms. However, none of the genetic polymorphisms could be linked to aggression in groups of aggressive and non-aggressive dogs. Therefore she deems it unlikely that these genes are involved in aggression. Van den Berg about this in itself disappointing result: "Although we carefully selected the candidate genes, the candidate gene approach was similar to looking for a needle in a haystack. Further progress in this project is expected with genome-wide analysis, which is now under way for Golden Retrievers. It will be very interesting to study the genetic variation with respect to the phenotypic measures that we obtained in our studies."

Playful aggression in a Golden Retriever (left). (Courtesy of Linda Van den Berg)

Linda van den Berg (August 1, 1977, Oosterhout). Secondary education (Mgr. Frencken College, Oosterhout), 1995; Biology (Utrecht University), 2000. From 2001-2005 she worked on the project of thesis at the Dept of Clinical Sciences of Companion Animals, Faculty of Veterinary Medicine, Utrecht University.

news

Rudolf Magnus Graduate School Certificates

The Director and the Research Training Committee of the Graduate School took pleasure in presenting the Rudolf Magnus Graduate School Certificate to the following Doctor:

Boudewijn J. Kollen (March 14, 2006)

Ponsen & Looijen: Personal advice on thesis production and discounts on thesis printing

Ponsen & Looijen Printers in Wageningen is a firm with a long tradition in the high quality reproduction of PhD theses. Moreover, Ponsen & Looijen is recommended by the Netherlands Organisation for Scientific Research (NWO). The Rudolf Magnus Institute has drawn up a contract with Ponsen & Looijen, to be able to help PhD students in the complex process of book production. This can save time and money during this, often hectic, last phase of the graduation. This offer is only valid for PhD students of our Institute, but notice that there is no obligation to make use of the services of Ponsen & Looijen.

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- offer a 12.5% discount on the printing of the thesis to each PhD students of our Institute.
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- provide PhD students with a book '*How to make a PhD thesis!*', with very clear instructions and information of all aspects of the book production.

All written information as well as the personal advice is available in Dutch and English. For any questions contact Joke Ploos van Amstel, Ponsen & Looijen, utrecht@p-l.nl, tel. 06-5310 5267.

agenda

April 7, Helmholtz Lecture

Kalanit Grill Spector (Stanford University, USA)
'Fine scale organisation of the human vertral system'
'Rode zaal', Ruppert Building, Leuvenlaan 19, Utrecht, 16:00-17:00
Contact, v.maassen@fss.uu.nl

April 19-27, Course on Neuropsychopharmacology

Venue, partly in Amsterdam, Weesp, and Utrecht. Programme, http://www.rudolfmagnus.nl

April 20, Psychopharmacology Colloquium

Filip van den Bergh (Psychopharmacology, Pharmaceutical Sciences, Utrecht) 'Breaking down impulsivity: Response inhibition deficits and delay aversion'
12:00-13:00, Room N016, Went Building, Sorbonnelaan 16, Utrecht
Contact, k.b.e.bocker@pharm.uu.nl

April 25-26, Symposium 'Mind the Brain'

Keynotes by **Fernando Lopes da Silva** and **Bernhard Hommel**. A symposium organised by the Master Students Neuroscience and Cognition.
Venue, UMC Utrecht,
Programme, http://mindthebrain.neuroscience-cognition.org

May 4-5, Meeting of the Association of European Psychiatrists - Neuroimaging Section

'Neuroimaging Change over Time in Psychiatry'
Academy Building, Domplein 29, Utrecht
Programme and registration, http://www.rudolfmagnus.nl

August 28-29, Rudolf Magnus-Helmholtz Summerschool

Conference Centre Ottone, Kromme Nieuwegracht 62, Utrecht.
Programme to be announced
Check our website for updates, http://www.rudolfmagnus.nl

September 8-9, Brain Days

A two-days meeting with international experts on the theme, **'Brain plasticity in children'**.
UMC Utrecht. Programme to be announced
Check our website for updates, http://www.rudolfmagnus.nl

November 8, Rudolf Magnus Symposium

Including the Rudolf Magnus Lecture 2006 by **Frans De Waal** (Emory Univ. Atlanta, USA) and the announcement of the winner of the Rudolf Magnus Research Award 2006.
UMC Utrecht, 13:30-17:15. Programme to be announced
Check our website for updates, http://www.rudolfmagnus.nl
Contact, m.vandenadort@med.uu.nl

November 8, Rudolf Magnus Evening

A unique mixture of social and scientific events is organised following the Rudolf Magnus Symposium.
UMC Utrecht, 18:00-21:30, programme will include diner, details to be announced. The evening programme is only (and freely) accessible for all members of the Rudolf Magnus Institute. Registration is required. Contact, m.vandenadort@med.uu.nl

November 16-22, Introductory Course for PhD students in Neuroscience

Information and registration, http://www.rudolfmagnus.nl

November 23-24, Annual Meeting PhD students

Venue, Conference Centre Woudschoten, Zeist.
Information and registration, http://www.rudolfmagnus.nl

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