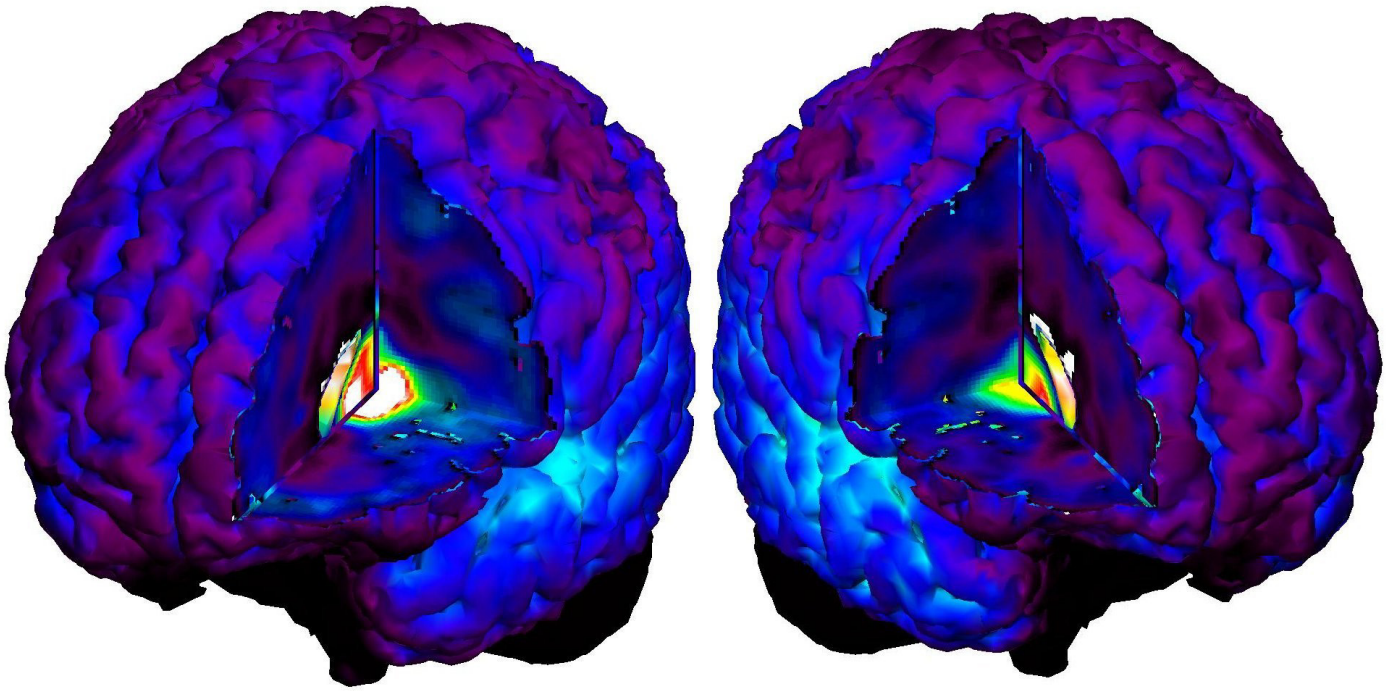
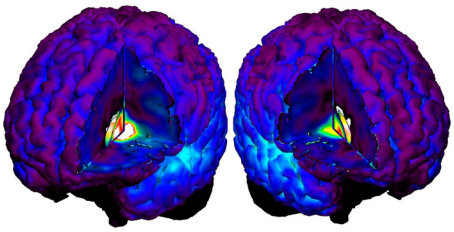
Neurobiology Research Unit

Dept. Neurology, Neuroscience Centre
Copenhagen University Hospital, Rigshospitalet

Annual Report 2014





Cover image showing central serotonin 4 (5-HT4) receptor binding of [^{11}C]SB207145 in the brain of a healthy volunteer at baseline and after three weeks of intervention with the selective serotonin reuptake inhibitor fluoxetine. The decreased binding of [^{11}C]SB207145 after flouxetine, which increases central serotonin levels, indicates that [^{11}C]SB207145 PET can be used as an in vivo molecular imaging marker of serotonergic tone in humans. The image appeared as the cover image of Molecular Psychiatry in April 2014. Courtesy of Mette Ewers Haahr.

Preface

Dear reader,

I am pleased to present you with the 2014 annual report describing the activities in 2014 within the Neurobiology Research Unit (NRU). I would like to take this opportunity to acknowledge and thank all of the NRU staff for their dedicated work as well as to thank all our highly valued national and international collaborators and the funding agencies that support our work. These have all been essential factors to ensure that 2014 was another very successful year for NRU.

In June 2014, Jens D. Mikkelsen, senior researcher at NRU since 2008, was inaugurated as professor in translational neuropharmacology at the University of Copenhagen. He held a well-attended inaugural lecture entitled "*New pharmaceuticals against brain disorders: From animal models to patients*". Professor Mikkelsen is successfully leading the Danish Strategic Research Council project COGNITO, described separately on page 16.

The past year has also been a year with substantial research output from the group. NRU-affiliated researchers have presented their work at >40 international congresses, conferences, and meetings, and in total the group has published 48 peer-reviewed scientific publications and 2 book chapters. The full 2014 publication list can be found on page 23. Currently, we have >40 papers in the pipeline so we expect an equal high amount of publications in 2015.

With respect to research training, we have in 2014 organized several pre- and post-graduate programmes with international speakers and a well-attended program. In terms of pregraduate training, two of our medical students obtained the highest grade when defending their research year reports and two of our non-medical students obtained their degree after a successful defence of their master theses in human biology. Further, two psychology students and two medical technologist students have been trained in internships at NRU. In terms of postgraduate training in 2014, NRU senior staff members have supervised more than 20 national and international PhD students and post docs, and three courses have been given within the auspices of NRU. In March, we hosted our yearly one-week PhD course "*Basic Kinetic Modelling in Molecular Imaging*", in August we organized together with the MGH/HST Athinoula A. Martinos Center for Biomedical Imaging, Boston, USA a three-day course in the use of FreeSurfer which is software for analysis of brain imaging data. Furthermore, in September, we hosted a three-day course related to our activities in Cimbi: "*The Emotional Brain: Functional and structural dimensions of emotion processing and emotional disorders*" with 9 international speakers and 20 participants. Finally, professors Olaf B. Paulson, Jens D. Mikkelsen, and Steen G. Hasselbalch and lecturer Morten Skøtt Thomsen for the third time contributed to the Sino-Danish Center for Education and Research by lecturing at the Master degree program 'Neuroscience and Neuroimaging' at the University of Chinese Academy of Sciences in Beijing.

The Center for Experimental Medicine Neuropharmacology (NeuroPharm) is a new research center under the auspices of Rigshospitalet and University of Copenhagen. It is funded by the Innovation Fund Denmark and starts on Jan 1st, 2015 with NRU as coordinator. Other partners in NeuroPharm include four additional Danish academic partners, one from the University of Copenhagen and three from university hospitals in the Capital Region of Denmark. International partners include Massachusetts General Hospital, Imperial College London and the British-based small-medium sized enterprise Imanova Limited. Furthermore, NeuroPharm have several affiliated partners from the drug industry. You can read more about the new center on page 17.

I hope that you will enjoy reading this 2014 annual report and encourage interested readers to stay tuned at www.nru.dk.

On behalf of the NRU management group

Gitte Moos Knudsen
Professor, Head of Department

Contents

About Neurobiology Research Unit

Mission

The mission of NRU is to conduct translational neuroscience research on brain neurotransmission at an internationally competitive level with the aim to promote preventive, diagnostic and therapeutic advances. We make use of in vivo molecular, structural, and functional brain imaging to uncover disease mechanisms and risk correlates as well as to determine drug effects. Also, we make use of animal and cell models as well as human brain tissue to investigate drug effects and diagnostic value in the clinic. We bring discoveries from cells and animals into healthy volunteers and patients as early as possible.

Activities

The activities within NRU fall in nine different categories:

1) Basic neurobiological and translational neuroscience research
2) Development and validation of new in vivo imaging probes
3) Neuropharmacological imaging research
4) Development and optimization of data analysis methods
5) Neuroimaging research studies of patients with neurological or psychiatric disorders
6) Diagnostic brain imaging of neurological patients
7) Neuropsychology research and neuropsychological testing
8) Education and training
9) Dissemination of results

We see our role at Rigshospitalet and in the Capital Region of Copenhagen as a key unit to conduct innovative diagnostic, therapeutic and preventive neuropharmacological research. This takes place in close interaction with the hospital clinics, academia and industry enabling immediate subsequent implementation of prevention strategies, diagnostics and innovative drugs as well as non-pharmacological treatments of patients with brain disorders. NRU collaborates with many other national and international research institutes.

NRU is a major training site for pre- and postgraduate students. We aim to attract physicians and people with other relevant educations to the neuroscience field and to educate and train research staff, in particular medical students, graduate students, PhD students and post docs. We organize PhD courses and regular meetings and seminars where the pre- and postgraduate students are expected to present their work.

Relative to the number of staff members NRU has an outstanding scientific output. Our most important form of dissemination is through publications in high impact peer-reviewed journals. We also communicate our results at national and international meetings and thereby establish and maintain national and international recognition. We edit and contribute chapters to Danish medical textbooks within brain-related topics. Broader public dissemination is also prioritized. Together with the "Science Theatre", we have for a decade arranged a 2-hour session related to brain disorders. We also contribute with articles in popular journals, give public lectures, and participate in interviews for newspapers and TV.

Facilities

NRU has four separate locations on Rigshospitalet, Blegdamsvej, and has access to scanning facilities at additional two sites at the hospital.

At Juliane Maries Vej 28, in the Rockefeller building (see photo below), NRU covers 590 m^2, that includes 15 offices, a conference room with kitchen, a laboratory for handling human specimens, and two sound-insulated rooms with facilities for neuropsychological and -physiological testing. We have also access to shared changing facilities and meeting rooms in the building.

The Rockefeller has since August 2013 been housing NRU on the 3rd floor.

The NRU experimental laboratory resides in Building 93, Juliane Maries Vej 20, where we have 270 m^2 of well-equipped facilities for basic neuroscience work (in vitro and in vivo studies). Of these facilities, an office and five laboratory rooms are allocated for NRU while another eight rooms are shared with the other research groups in the building. Equipment in the laboratory includes lab benches with hoods and standard equipment, a cell culture room, microscopes, small animal storage facilities, gamma- or beta-counters, facilities for testing animal behaviour, cell harvester, autoradiography, and much more.

The SPECT laboratory of NRU is located at the Department of Neurology on the 8th floor in the main complex of Rigshospitalet. The laboratory consists of a room for the 3-headed Philips IRIX SPECT scanner, a type B approved isotope laboratory, and two offices, a total of 124 m^2.

Storage and additional freezers for biobank material are located in Building 61.

NRU has a close collaboration with the PET and Cyclotron Unit at Rigshospitalet, which provides NRU with access to radiochemistry production and to PET- and MR-PET scanner facilities. NRU also uses MR-scanner facilities at the Department of Diagnostic Radiology, in close collaboration with the staff there.

Finances

The vast majority of NRU research is funded from external sources; most notably through the establishment in 2006 of the 10-year Lundbeck Foundation *Center for Integrated Molecular Brain Imaging* (Cimbi, page 8), supported by 80 mio DKK, and *Novel treatments of cognitive dysfunction* (COGNITO, page 17), funded 2012-2017 by the Danish Council for Strategic Research with 18 mio DKK. On page 26, all current external funding sources are acknowledged.

About Neurobiology Research Unit

Organization and Staff

NRU is chaired by Professor Gitte Moos Knudsen since 2004. Professor Jens D. Mikkelsen is laboratory leader and responsible for the basic neuroscience section, and Chief Engineer Claus Svarer is responsible for the data analysis section. The Chief Technologist in the SPECT laboratory is Gerda Thomsen. Professor Olaf B. Paulson, Ass. Professor Lars H. Pinborg, PhD Vibe G. Frøkjær, and PhD Morten Skøtt Thomsen have in 2014 been members of the NRU leader group. The staff employed by or otherwise involved in the work at NRU in 2014 consisted of:

Head
Gitte Moos Knudsen, professor, MD, DMSc

Senior Researchers
Claus Svarer, chief engineer, PhD
Jens D. Mikkelsen, professor, MD, DMSc
Klaus K. Holst, biostatistician, PhD (half time)
Matthias Herth, chemist, PhD
Morten S. Thomsen, human biologist, PhD
Lars Pinborg, associate professor, MD, DMSc (half time)
Olaf B. Paulson, professor, MD, DMSc
Patrick Fisher, neuroscientist, PhD
Vibe G. Frøkjær, MD, PhD

Administration
Birgit Tang
Dorthe Givard
Peter S. Jensen

Post Docs
Agnete Overgaard, human biologist, PhD
Anders Ettrup, human biologist, PhD
Cornelius Donat, biologist, PhD
Hanne D. Hansen, molecular biologist, PhD
Linda Blomster, biomedical chemist, PhD
Ling Feng, engineer, PhD
Melanie Ganz-Benjaminsen, computer scientist, PhD

PhD Students
Brenda Mc Mahon, MD
Christian G. Jensen, psychologist
Dea S. Stenbæk, psychologist
Jo Henningsen, biochemist
Liv V. H. Brüel, psychologist
Louise M. Jørgensen, MD
Majbrit M. Jensen, human biologist
Marie Deen Christensen, MD
Mette T. Foged, MD
Mona El-Sayed, human biologist
Per Jensen, MD
Sophie da Cunha-Bang, MD
Valdemar L. Andersen, pharmacist
Vincent Beliveau, neuroscientist

Research Assistants
Agnete Dyssegaard, pharmacist
Christinna V. Jørgensen, molecular biomedicine
Jon Lansner, psychologist
Maria Arvaniti, human biologist
Mille D. Andersen, biomedicine
Nina A. Frimer, human biologist
Sara R. Jørgensen, biomedicine

Technical Research Personnel
Anders D. Olsen
Gerda Thomsen, chief technologist
Glenna Skouboe
Hans Jørgen Jensen
Lone I. Freyr
Louise Nielsen
Maria R. Nørnberg
Svitlana Olsen
Søren Iversen

Visiting Scientists
Dan Peters, PhD, CEO at DanPET, Sweden
Maciej Salaga, PhD student, Lodz, Poland
Kishore Vakamudi, New Mexico, US
Stefan Posse, professor, New Mexico, US

Pregraduate Researchers and Students
Anine T. W. Skibsted, medicine
Camilla B. Larsen, medicine
Charlotte B. Mikkelsen, engineer
Emil Andersen, psychology
Erik Perfalk, medicine
Franziska Wichern, human biology
Gunild Vulpius, medicine
Gustav R. Jakobsen, medicine
Janus H. Magnussen, human biology
Jonas Villadsen, molecular biomedicine
Lea Bäcker, liberal arts and sciences
Lola Torz, biology
Louise V. A. Holde, psychology
Mathias Kølvraa, medicine
Martin K. Madsen, medicine
Rasmus Rydbirk, biology
Rebecca Margolinsky, medicine
Simon Sanggaard, neuroscience and psychology
Tatiana G. Fjaellingsdal, neurocognitive psychology
Vibeke N. H. Dam, psychology
Zuhal Filikci, medicine

Positions of Trust

Gitte Moos Knudsen: Chairman for the steering group for research laboratories at Rigshospitalet from 1999; Scientific Advisory Board Member of the Strategic Research Council from 2010; Vicepresident of the European College of Neuropsychopharmacology (ECNP) since 2013; Field Editor at the International Journal of Neuropsychopharmacology since 2013; Board of Directors of the Brain Prize.

Olaf B. Paulson: Auditor for Danish Society for Neuroscience; Member of the editorial board of the Scientific World Journal; Referee for several international journals; Member of review committee for Italian Ministry for Education, University and Research (MIUR).

Jens D. Mikkelsen: Member of the Danish Medical Research Council since 2013; Member of the chairman committee for external evaluations of medical educations in Denmark; Member of the Academy for Technical Sciences; Regularly expert panel scientist for the EU commission, Brussels.

Vibe G. Frøkjær: Appointed member of The Young Academy of Denmark since 2011.

Awards, Grants and Honours

Recipient of the Carlsberg Foundation Research Prize 2014

Gitte Moos Knudsen
Professor, MD, DMSc

Prof. Gitte Moos Knudsen received the Carlsberg Foundation Research Prize 2014 because of "*her crucial contribution to the basic research in the human brain's communication systems*". The ceremony was held Sep 9 at the Ny Carlsberg Glyptotek and among others attended by HRH Princess Mary, the education and research minister Sofie Carsten Nielsen and the Carlsberg Foundation Chairman Flemming Besenbacher.

Post doc grant recipient

Hanne Demant Hansen
Molecular biologist, PhD

During my PhD at NRU, I have worked mainly with evaluating novel PET radioligands for the serotonin 2A (5-HT_{2A}) and 7 (5-HT_7) receptors but I also had the opportunity to learn about and perform experiments on the combined PET-MR scanner at the A.A. Martinos Center for Biomedical Imaging in Boston, USA. In these experiments we investigated whether the serotonin 1B (5-HT_{1B}) receptor radioligand [^{11}C]AZ10419369 was sensitive to changes in endogenous serotonin and what effects small doses of competing 5-HT_{1B} receptor ligands had on the binding of the radioligand and the blood volumen in the brain of the animals.

The results of these experiments gave the idea to my postdoc project where the concept of investigating the competition between [^{11}C]AZ10419369 and other drugs will be the same, but the effects will be studied in healthy volunteers at the PET-MR scanner at Department of Clinical Physiology, Nuclear Medicine and PET at Rigshospitalet. The competing drugs in these clinical studies will be the 5-HT_{1B} receptor agonists that are used in the treatment of migraine attacks. With the simultaneous PET and MR measurements we can obtain a specific finger print and predict the efficacy and mode of action of drugs, which will be a major step forward for the development of novel drugs for treatment of brain disorders. My two-year postdoc project was funded by the Lundbeck Foundation until 2016. It will be an integrated part of NeuroPharm (described on page 17).

Post doc grant recipient

Agnete Overgaard
Human biologist, PhD

The year of 2014 began with the defence of my PhD thesis entitled "*Characterisation of the kisspeptin system: the role of sex, obesity, and endocrine disruptors*". During my PhD at NRU, I studied the kisspeptin system in various rodent models to investigate whether kisspeptin was sensitive to various factors suspected to compromise fertility, such as obesity and environmental pollutants. We found that both pubertal timing and kisspeptin mRNA levels were sensitive to both early-life under- and overfeeding, which suggest that changes in kisspeptin expression may be involved in the altered pubertal timing. In adult rats fed either normal diet or high-fat diet, we found a strong negative correlation between kisspeptin expression and plasma triglyceride levels, suggesting that it is the high plasma triglycerides and not the diet or bodyweight *per se*, which affect kisspeptin expression.

After my PhD, I defined together with MD PhD Vibe Frøkjær and Professor Jens Mikkelsen from NRU and Professor Liisa Galea from University of British Columbia a postdoc project on postpartum depression, which received funding from both the Lundbeck Foundation and The Danish Council for Independent Research. The project deals with the impact of hormone fluctuations during and after pregnancy on depressive behaviour and on the balance of the serotonin system in two different rat models. The aim of the project is to gain knowledge of the mechanisms behind depressive symptoms during the postpartum period. Professor Liisa Galea is an expert in monitoring behavior in the rat models of postpartum depression, and I will therefore spend the first year in her laboratory, monitoring maternal and depressive behaviour in the rats. The last 1.5 years of the project will be carried out at NRU, where I will investigate pre- and postsynaptic markers of serotonin signaling in the same animals.

Center for Integrated Molecular Brain Imaging

www.cimbi.org

Director: Gitte Moos Knudsen

The Center for Integrated Molecular Brain Imaging (Cimbi) is founded on collaborations between various research institutions in Copenhagen and was established in 2006 and extended in 2011 through two generous 5-year grants totalling 80 mio DKK from the Lundbeck Foundation. The institutions involved in Cimbi span many scientific fields and provide the strong foundation needed to carry out the diverse activities undertaken in the Center.

Cimbi Center Director is Professor Gitte Moos Knudsen.

Besides NRU, the Cimbi core institutions are:

- Danish Research Centre for Magnetic Resonance (DRCMR), Copenhagen University Hospital, Hvidovre. DRCMR has expertise with conduction of MRI research and provides MRI resources and expertise in fMRI, brain morphology and diffusion tensor imaging.
- Department of Drug Design and Pharmacology (FARMA), Faculty of Health and Medical Sciences, University of Copenhagen. FARMA provides the Cimbi basis for cold chemistry facilities and expertise in organic syntheses.
- DTU Compute, Department of Applied Mathematics and Computer Science, Technical University of Denmark (DTU) conducts research in mathematical modelling and advanced signal processing, especially within the field of medical imaging.
- PET and Cyclotron Unit, Department of Clinical Physiology, Nuclear Medicine & PET, Copenhagen University Hospital, Rigshospitalet provides both expertise and infrastructure for radiochemistry, PET- and MR-PET scanner facilities.

A number of additional Danish and international collaborating institutions have been associated with Cimbi throughout the years.

The research in Cimbi focuses on the neural bases of personality dimensions that predispose individuals to affective and substance use disorders, with special emphasis on the serotonergic neurotransmitter system. The serotonin system is involved in a large variety of psychophysiological functions, including feeding, mood, aggression, and pain. Serotonin is also a critical neurotransmitter in brain development and in the generation and regulation of emotional behaviour. Individual differences in trait effect and personality are for a large part genetically determined, and they are critical in shaping complex human behaviour, social interplay and also in overcoming challenges from the ever-changing environments. Such individual differences may also serve as important predictors of vulnerability to neuropsychiatric disorders, including depression, anxiety and memory disorders.

An important component is to identify the underlying mechanisms driving variability in brain circuit function. During the initial period of Cimbi, (Cimbi-I, 2006-2010), a number of relevant correlations were demonstrated by means of molecular (PET), and structural and functional (MRI and fMRI) brain imaging studies of human subjects together with complementary studies using animal models. These correlations include predictive correlations between on the one hand genetic or behavioural characteristics and on the other hand regional conditions in the brain's structure, activation or serotonergic markers. Advanced informatics techniques, new tracer compounds, and novel serotonergic challenge paradigms have also been developed in Cimbi-I.

Here in the second operative period of the Center (Cimbi-II, 2011-2015) we are focusing more on longitudinal and interventional studies in order to better address causal relationships and to substantiate the predictive value of brain imaging as biomarkers. Specifically, Cimbi-II operates with five interacting themes of relevance for the serotonergic transmitter system: Mood and Emotions, Biorhythms, Affective Cognition, Brain Development, and Decision-making. In addition, two platforms are included, one for radioligand development and validation, and another for data analysis. The established Cimbi database and biobank are internationally recognised as unique and continues to constitute a valuable resource for researchers within and outside of Cimbi, e.g. for new hypothesis driven studies.

The past year has again been a year with substantial research output from the Center. In 2014, a total of 34 peer-reviewed articles were published. Also, one Cimbi-associated PhD thesis was successfully defended and 2 were accepted for defense in early 2015. Currently 16 Cimbi-associated PhD students are enrolled. Economically, Cimbi managed impressively to raise additional funding for Cimbi projects of more than 12.8 mio DKK.

Cimbi is now entering the final year of its Lundbeck Foundation support and we are planning a large seminar to be held in the Royal Society for Sciences and Letters on September 2-4, 2015. The branding of Cimbi is well-established and there will at least be three lines of research that will carry on, namely 1) the nomenclature of radioligands developed within the framework 2) the Cimbi database and 3) the Cimbi biobank.

Photo from the 2014 Cimbi Annual Meeting which was held in September as a 2-day retreat meeting at Comwell Borupgaard in Snekkersten.

Mood and Emotions

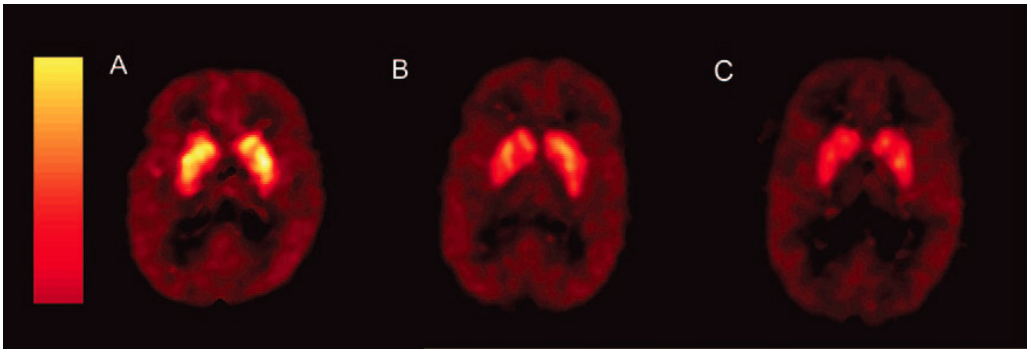
by Vibe G. Frøkjær, NRU

An important endeavor in Cimbi is to advance the understanding of brain functions that drives processing of emotions and regulation of mood and stress responses in the context of risk and resilience for neuropsychiatric disorders. The serotonergic system is organized in a fashion that enables it to modulate such brain functions and also allows integration of hormonal signals, e.g., stress and sex-hormone information [9, 16]. Serotonergic signaling is considered important for an appropriate adaption to environmental challenges and thus may be critical for the maintenance of mental health and development of neuropsychiatric diseases, e.g., major depression in the context of environmental stress and changes in sex-hormone milieu.

Through a combination of cross-sectional work and longitudinal experimental modeling of risk mechanisms we investigate within Cimbi the relations between familial risk for mood disorders and markers of serotonergic tone, neuroendocrine control of stress hormone release, and of the serotonergic effects of sex-hormone fluctuations.

In 2014, we have by means of [11C]SB207145 PET, which is a molecular imaging marker of serotonergic tone [23], shown that familial risk for major depression (MDD) is associated with serotonin type 4 receptor (5-HT4R) binding in striatum in a "risk-dose" dependent manner [33], as illustrated in Figure 1. This suggests that the higher the risk load the higher the serotonergic tone, which might represent a successful neurobiological compensatory mechanism. However, this hypothesis would have to be tested in future longitudinal work, e.g., in high-risk individuals and patients with manifest depression.

Figure 1: The distribution of [11C]SB207145 binding potentials in 3 young (24–30 yrs) males scanned at the HRRT scanner. Binding levels are high in the striatum, intermediate in the temporal and limbic areas, and low in the neocortex. (A) The distribution in a subject without a family history of depression. (B) Lower binding potentials in a subject who reported to have one first-degree relative with MDD, and (C) even lower binding potentials in a subject who reported to have 2 first-degree relatives with MDD. Figure from [33], Copyright © The Author 2014.



Further, we have demonstrated that in MDMA (Ecstasy) users, who we argue represent a model of serotonin deficiency, stress hormone release to awakening is higher than in healthy non-users and linked to prefrontal serotonin transporter binding [16], see Figure 2. These findings establish a coupling between prefrontal markers of serotonergic projections and stress hormone responses. We speculate that this coupling predominantly emerges from early brain development since it appears present independent of serotonergic manipulation as in MDMA-use. This highlights potential critical mechanisms of risk for neuropsychiatric disorders, which are linked to serotonin signaling and stress coping capacities arising form early brain development. Such mechanisms might be sensitive to potential supportive environments, e.g., parental bonding quality though infant life [42].

In conclusion, our 2014 results highlight that alterations in serotonin brain signaling may critically influence the capacity to adapt to stress or risk load of other types and thus reflect a mechanism by which risk or resilience for mood disorders works. Mapping the interplay between serotonin signaling, hormone biology and familial risk factors and the mental health consequences is paramount to support future stratification of populations of at-risk or depressed patients and thus support targeted treatment or preventive strategies in high-risk populations.

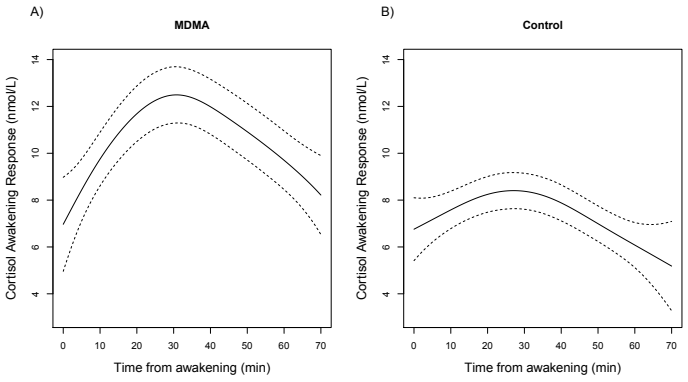


Figure 2: Cortisol awakening response (CAR) in 18 MDMA users (A) vs. 32 non-using controls (B). Average curve estimated from an additive mixed model of salivary cortisol concentration over time. Dashed lines represent point-wise 95% confidence bands. Figure from [16], Copyright © CINP 2014.

Biorhythms

by Patrick M. Fisher, NRU

At Cimbi we have multiple studies aimed at revealing how neurobiological mechanisms change over time. Whether in response to a drug, exposure to a bright-light lamp or simply changes in season, these projects focus on biorhythms of the human brain, a core theme of Cimbi. Data collection for many of these studies is now complete and we are actively working through these rich datasets to determine the different ways in which they help us to better understand biorhythms of the human brain.

One such study aims to elucidate how seasonal changes affect the brain serotonin system in healthy individuals and may contribute to the emergence of depressive symptoms during winter time, which is known as seasonal affective disorder (SAD). We recruited both healthy individuals and individuals with SAD to complete two PET neuroimaging scans looking at two aspects of the serotonin system: the 5-HT_4R, measured with [^{11}C]SB207145 PET and the serotonin transporter (5-HTT), measured with [^{11}C]DASB. Participants completed scans during the summer and winter months allowing us to ask whether the brain serotonin system responds to seasonal changes differently in people with SAD compared to healthy controls. Although analyses related to this study are being finalized, we can share that we see preliminary evidence for differences in how the serotonin system is regulated in SAD individuals compared to healthy controls. This type of longitudinal study is unique and particularly useful for elucidating how environmental factors such as season shape critical brain neurotransmitter systems.

Previously, we reported that 5-HT_4R imaging with [^{11}C]SB207145 PET is a useful measure for changes in brain serotonin levels following three-week exposure to fluoxetine, an antidepressant acting on the serotonin system [23]. In follow-up, we recently demonstrated that an individual's change in brain serotonin levels is associated with the change in how a key brain area responds to emotional stimuli [Fisher, Neuropsychopharmacology, 2015]. Using functional magnetic resonance imaging (fMRI) we measured the response of the amygdala, a critical brain structure for regulating emotional state, to emotionally salient faces, expressing fear or anger. We found that individuals showing a greater increase in serotonin levels following fluoxetine administration also showed a significant decrease in the amygdala response to emotional stimuli (Figure 3). This finding provides novel evidence that serotonin signaling is centrally involved in the brain's response to emotionally salient information. Furthermore, this represents the first direct demonstration that fluctuations in brain serotonin levels and the neural response to emotional stimuli are intertwined.

Another central aim of Cimbi is the identification of biomarkers that predict either treatment response or individual variability in neuroimaging markers. We recently applied a technique known as imaging genetics to link common genetic polymorphisms with individual differences in 5-HT_4R levels, which we are particularly interested in given our previous finding that this measure reflects differences in brain serotonin levels. We demonstrated that an individual's status for two commonly studied genetic variants (the 5-HTTLPR and BDNF val66met polymorphisms) was significantly predictive of brain 5-HT_4R binding [15], see Figure 4. This finding suggests that both of these behaviorally relevant genetic polymorphisms play an important role in shaping individual differences in brain serotonin levels, which may be critically important for understanding how these polymorphisms shape emotional behavior and risk for neuropsychiatric disorders.

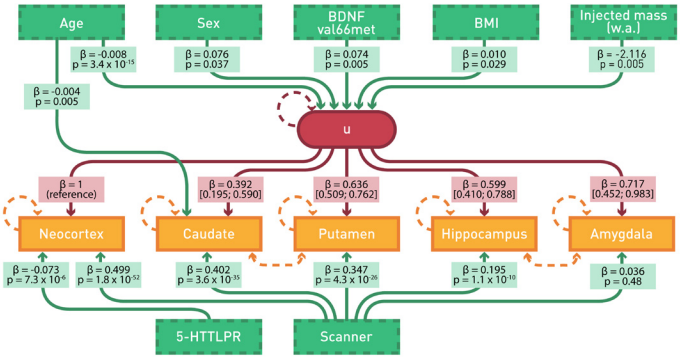


Figure 4: Latent variable model of genetic effects on [11C]SB207145 binding. Our final LVM is depicted with model paths. Green boxes indicate observed predictors. The red circle reflects the latent variable (u). Orange boxes represent measured log-transformed regional [11C]SB207145 binding potential values. Age, sex, BMI, weight-adjusted (w.a.) injected mass, and BDNF val66met genotype all map onto the latent variable whereas 5-HTTLPR, scanner type, and an age effect on caudate directly affect regional binding potential values. BDNF val66met val/val and 5-HTTLPR LL groups are reference for respective genotype parameter estimates. Orange hatched lines between (1) caudate and putamen and (2) amygdala and hippocampus indicate additional shared correlation; circular red and orange hatched lines reflect error estimates included in the model. The parameter estimate, ß, for each model path is noted in respective boxes (95% confidence intervals indicated for estimates between latent variable and regional binding estimates). Significance of parameters estimates, p, is also noted. All regions loaded significantly onto the latent variable (all factor loadings: $P<9.8 \times 10^{-5}$). Figure from [15], Copyright © 2014 Wiley Periodicals, Inc.

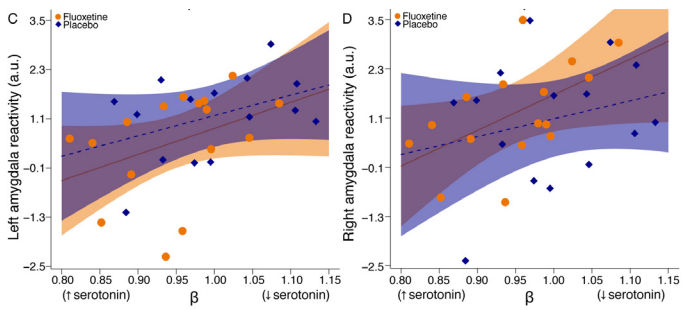


Figure 3: Change in [11C]SB207145 binding (ß) associated with (left panel) left and (right panel) right threat-related amygdala reactivity. A ß<1 reflects decreased specific binding from baseline to rescan (i.e., putatively increased serotonin levels), whereas a ß>1 reflects increased specific binding (i.e., putatively decreased serotonin levels). The mean left and right threat-related (i.e., fear and angry—neutral contrast) amygdala reactivity values at rescan, adjusted for baseline, are plotted for all 31 participants (orange points: fluoxetine group, blue points: placebo group). Independent best fit lines and 95% confidence intervals are shown for fluoxetine (solid line) and placebo (hatched line) groups. Figure from [Fisher, Neuropsychopharmacology, 2015], Copyright © 2015 American College of Neuropsychopharmacology.

Affective Cognition

by Dea S. Stenbæk, NRU

An important endeavour in Cimbi is to advance an interdisciplinary understanding of risk for increased levels of mental distress and psychopathology. A major aim of the cognitive section at NRU is therefore to characterize psychological risk factors; i.e. personality traits, levels of perceived stress, life experiences and cognitive and emotional processing associated with serotonergic neurotransmission. Besides being a core function in Cimbi, the cognitive section at NRU contributes to other NRU-related activities.

In the past year we have continued our work of validating and improving the Cimbi test battery, which comprises an extensive compilation of affective and non-affective cognitive tests administered to research participants during a standardized three-hour test procedure. This included a comprehensive psychometric validation of our in-house developed 24-word Verbal Affective Memory Test (VAMT-24) and an investigation of the association between cerebral 5-HT_4R binding and VAMT-24, thereby advancing our understanding of the serotonin system's influence on affective memory processing which is of relevance for e.g. affective disorders. As part of the validation process with the Cimbi test battery, and for use in other Cimbi projects, we also evaluated a novel method of acute tryptophan depletion (ATD), using a gelatin-based protein mixture.

In addition to studying affective cognitive processes, we also investigated personality factors associated with increased risk of mental distress and psychopathology, i.e. Neuroticism and Harm Avoidance. Obese individuals seeking surgical treatment for their obesity were found to have higher Neuroticism scores than obese individuals who did not seek treatment and normal weight controls [43]. In sub-fertile women undergoing assisted reproductive technology, higher Neuroticism scores at baseline predicted increased levels of mental distress during treatment, and were associated with the probability of positive pregnancy outcome (Figure 5) [44]. Furthermore, in a large survey sample of healthy adults (n=518), recollected parental bonding was significantly associated with increased levels of trait Harm Avoidance; a personality trait highly correlated to Neuroticism, and Harm Avoidance significantly mediated indirect effects of recollected parental bonding on levels of mental distress [42]. These findings point to the significance of personality traits and their developmental underpinning in understanding risk mechanisms for mental distress, treatment seeking behaviours and treatment outcomes, and potentially psychopathology.

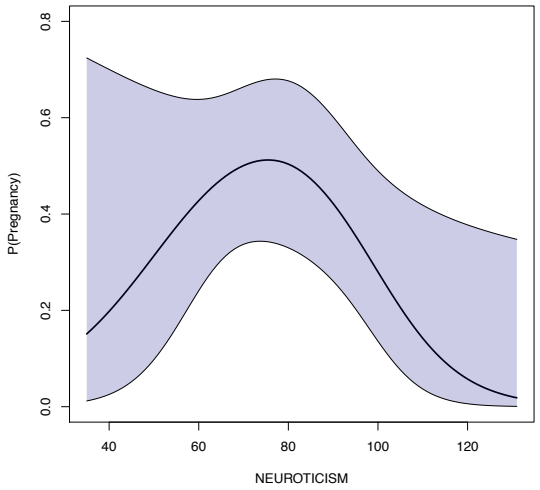


Figure 5: A non-linear association was observed, so that women with high or low Neuroticism scores at baseline showed a significant trend (p=0.028) towards lower chances of pregnancy (defined as HCG>50). This was examined using a logistic regression model with the effects of Neuroticism modeled using a natural cubic spline basis with a single knot located at the median. Although similar trends were observed using different statistical approaches, significance levels varied in both directions with the number and placement of knots, polynomial degree, and type of test (Wald/Likelihood). Figure from [44], Copyright © The Author 2014.

The Multimodal, meditation-based stress course: "Open and Calm"

Prolonged psychological stress is a risk factor for illness and constitutes an increasing public health challenge creating a need to develop public interventions specifically targeting stress. The Open and Calm project lead by PhD student Christian Gaden Jensen was a randomized controlled trial evaluating health effects of a novel program. The multimodal, meditation-based course was publicly entitled "Open and Calm" (OC) since it consistently trained relaxed and receptive ("Open") attention, and consciously non-intervening ("Calm") witnessing, in two standardized formats (individual or group) over nine weeks.

A total of 72 participants who complained to their general practitioner about reduced daily functioning due to prolonged stress were randomly assigned to OC or treatment as usual, i.e. involving for example unstandardized consultations with their general practitioner. Intent-to-treat analyses showed significantly larger improvements in OC than in controls on all outcomes. Also, treatment effects on self-reported outcomes (perceived stress, depressive symptoms, quality of life, sleep disturbances, mental health) were sustained after three months and were not moderated by age, gender, education, or course format. The dropout rate was only 6%. The standardized OC program reduced stress and improved mental health for a period of three months. Further testing of the OC program for public mental health promotion and reduction of stress-related illnesses is therefore warranted. A larger implementation in the City of Copenhagen is now in progress.

Brain maturation in children and adolescents

by Katrine Skak Madsen, DRCMR

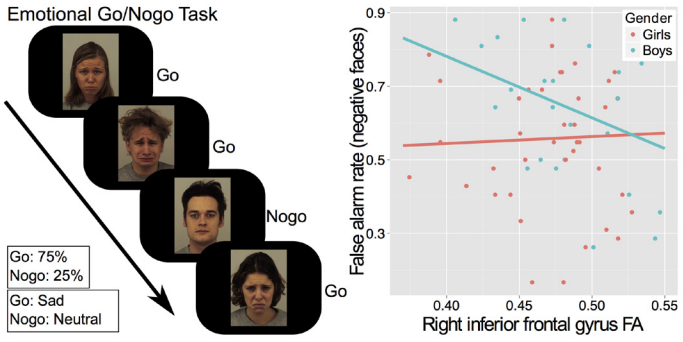
In this project we capitalize on the wealth of cross-sectional and longitudinal data collected in a large group of typically-developing children and adolescents within the HUBU (*Hjernens Udvikling hos Børn og Unge*) project. Within Cimbi, our major interest is to define the degree of variability in the maturational trajectories of specific brain networks, and to link these to developing cognitive, emotional and neuroendocrine functions. Additionally, we investigate the impact of genetic polymorphisms, personality trait characteristics, hormones, and environmental (e.g. physical activity, stress, alcohol use) factors. Between 2007 and 2012, we successfully completed with a six-month interval a total of ten assessments of more than 65 participants, who at baseline were in the ages of 7-13 years. In 2013 we completed the 11th assessment including 43 adolescents.

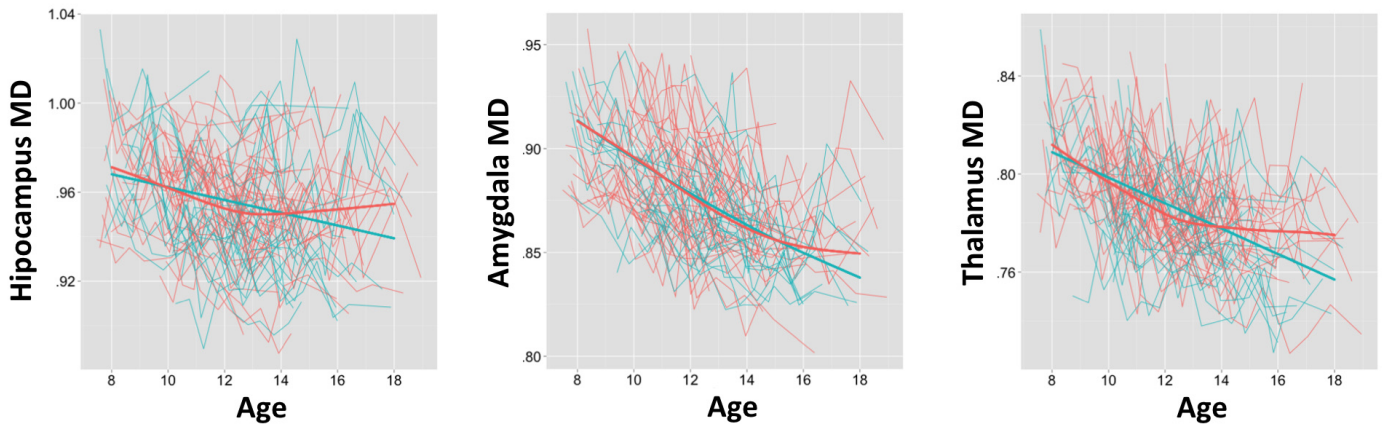
In 2014 much of our time was spend on analysing the longitudinal data. In our first analysis, we investigated the maturational trajectories of subcortical grey matter structures using diffusion tensor imaging. In 88 children and adolescents aged 7-19 years and examined two to eleven times (713 scans) we observed significant sex-specific age-related decreases in mean diffusivity (MD, thought to reflect cell density) for all subcortical regions (Figure 7). Females showed a non-linear decrease with MD reaching a plateau around the age of 12-13 years in all regions, except amygdala. Interestingly, this is also the average age at which females reach sexual maturity. In contrast, males showed a linear or almost linear maturational decrease in MD throughout the studied age range. These sex differences may be mediated by differences in sex hormones or when the sexes enter puberty. Future studies will examine how changes in sex hormones during puberty are related to sex differences in the maturational trajectories of different brain regions.

In another study, we used cross-sectional data to investigate how emotional content impacts our ability to stop pre-potent or reflective behaviour. An example could be not to retract or freeze when confronted with an angry person/face. The ability to overcome this natural instinct is a crucial part of engaging in social interaction. Experimentally, we used the emotional Go/NoGo task to study how emotional face stimuli affect our ability to regulate and inhibit responses (Figure 8). In 63 adolescents aged 10-15 years, we examined the association between their ability to inhibit their responses to negative (angry, fearful, sad) faces and regional white matter microstructure. Better inhibition performance (fewer false alarms) to negative faces was correlated with higher fractional anisotropy (FA, thought to reflect axonal density, diameter, myelination and organisation) in the white matter underlying the right inferior frontal gyrus, a key structure in the inhibition network, in boys but not in girls. Future studies will examine how individual differences in the maturational trajectories of regional FA are correlated with response inhibition performance.

Figure 7: Spaghetti plots showing the maturational trajectories of mean diffusivity (MD) in the subcortical ROIs across the age range 7 to 19 years. The thick lines represent the fitted maturational trajectories for males (blue) and females (red). MD significantly decreased with age in all ROIs, but appeared to reach a developmental plateau around the age of 12-13 years in females, except for the amygdala, whereas in males MD continued to develop throughout the studied age range. Courtesy of Kathrine Skak Madsen.

Figure 8: The emotional Go/Nogo task contains eight conditions pairing emotional (happy, angry, fearful, sad) with neutral face stimuli. (Left) Example of condition in which sad faces were go stimuli and neutral faces were nogo stimuli. Participants were required to respond as fast as possible to emotional faces, while trying to withhold their responses to neutral faces and visa versa. A total of 56 faces are shown for each condition, containing 75% go and 25% nogo. (Right) Scatter plot of false alarm rate to negative (angry, fearful, sad) faces against right inferior frontal gyrus fractional anisotropy (FA). In boys (blue), higher FA in the white matter underlying the right inferior frontal gyrus was associated with lower false alarm rate (i.e. fewer incorrect presses on nogo stimuli), while no significant associations were observed in girls (red). Courtesy of Kathrine Skak Madsen.





Neural correlates of risky decisions and reward

by Julian Macoveanu, DRCMR

Changes in neural activity during various brain processes can be captured with functional magnetic resonance imaging. Under fMRI participants perform tasks that involved specific brain regions. In order to identify the role serotonin plays when humans take risks or experience a monetary reward or loss, we have conducted a combined fMRI and serotonin manipulation study in healthy volunteers [32]. In this study, the volunteers were investigated twice with fMRI while performing a gambling task, before and following a three-week period when they were randomly assigned to receive either a selective serotonin reuptake inhibition drug (SSRI) or placebo. Compared to the placebo group, the SSRI group showed reduced activations to risky decisions in a region critical for the integration of cognitive and emotional information, namely orbitofrontal cortex. The SSRI treated group also showed reduced response in raphe, the main serotonin producing brain region (Figure 9). Our results suggest that SSRI treatment might reduce emotional engagement by reducing reward related activity. Our findings may explain the side effects observed in clinical treatment with SSRIs which report reduced affective arousal to pleasant and rewarding events.

Using fMRI we further evaluated differences in the brain response to risky decisions between twins at low and high familiar risk for depression. The studied twins had either a co-twin with diagnosed depression (high-risk group) or no first-degree family members with depression (low-risk group). When taking risky gambles, the high-risk twins showed reduced activity in the middle insula extending into temporal cortex, compared to the low-risk twins (Figure 10). This abnormal risk-taking activity in insula may reflect an increased vulnerability to affective disorders.

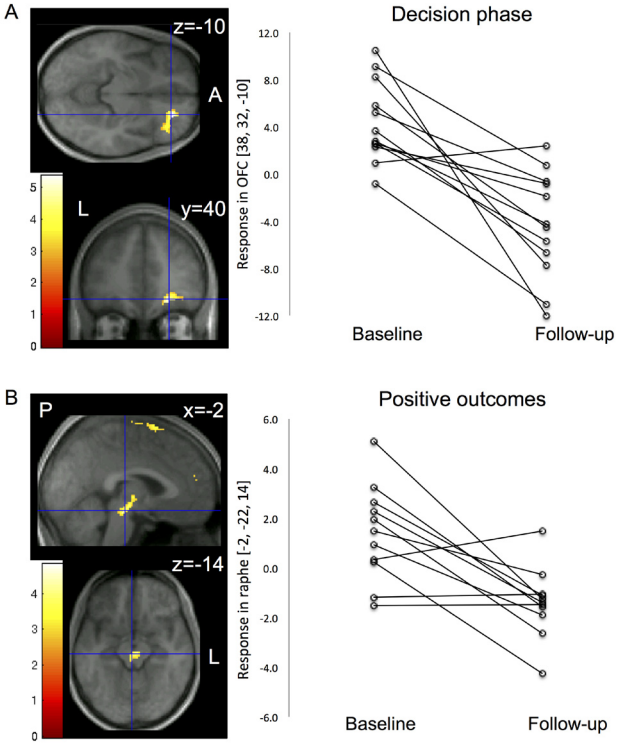


Figure 9: Brain regions showing a change in task-related activity after the SSRI intervention compared to placebo. A) Choice phase: In the right orbitofrontal cortex, the three-week SSRI intervention reduced the risk-related activity. B) Outcome phase: The three-week SSRI intervention attenuated the responsiveness of midbrain raphe nuclei to positive outcomes (monetary rewards). Left panels: Color-coded statistical parametric maps of brain regions showing an interaction between type of intervention (SSRI vs. Placebo) and time (baseline vs. follow-up). The color bar indicates t-scores. Right panels: Individual parameter estimates of task related activity at baseline and after the three-week SSRI intervention. Figure from [32], Copyright © 2014 Elsevier Inc.

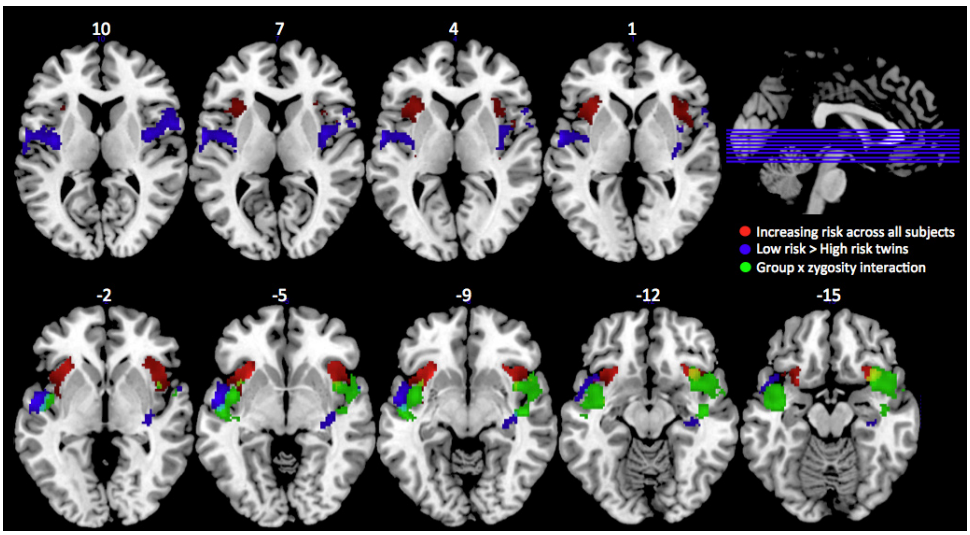


Figure 10: Group differences in brain response during the choice phase. Blue - The blue clusters regions where high-risk twins display an attenuated increase in choice related activity with higher gambling risk relative to low-risk twins. Reduced risk-related activity was located in the middle part of the insula extending into superior temporal cortex. Green – Regions in the insula showing an interaction effect between familial risk and zygosity. There was a reduced influence of risk on choice related activity in dizygotic high-risk twins as opposed to dizygotic low-risk twins, but no such difference was present in the monozygotic group. Red – risk-related increase in insula's response during the choice phase across all participants. Courtesy of Julian Macoveanu.

Platform 1: Design, Radiosynthesis, and In Vivo Evaluation of PET radioligands for Detection of 5-HT Release

by Hanne D. Hansen, NRU

The collaboration in Cimbi Platform 1 between NRU and the Department of Drug Design at University of Copenhagen and the PET and Cyclotron Unit at Rigshospitalet has in 2014 lead to the successful synthesis, radiolabelling and in vivo evaluation of 13 different PET radioligands. These radioligands were targeted towards a wide range of receptors and enzymes but the main targets of interest continues to be the serotonin 2A (5-HT_{2A}) and the serotonin 7 (5-HT_7) receptor.

The 5-HT_{2A} receptor agonist PET radioligand [^{11}C]Cimbi-36 was developed within the collaboration of Platform 1 and has previously been evaluated in pigs and published in 2011, and recently now also in non-human primates [13] and in humans [11]. The first-in-human clinical trial with [^{11}C]Cimbi-36 describes how [^{11}C]Cimbi-36 selectively images the 5-HT_{2A} receptors in humans (Figure 11) and that quantification of binding can be accomplished with reference tissue modelling.

Although we have now identified a successful 5-HT_{2A} receptor agonist PET radioligand, the work with compounds targeting the 5-HT_{2A} receptor continues. It is our goal to also develop an ^{18}F-labeled analogue of the successful [^{11}C]Cimbi-36. Having an ^{18}F-labeled version would allow for longer scan time because of the longer half-life of the isotope and also allow for the distribution of this new radioligand to other PET imaging centres.

In the search of a PET radioligand for the 5-HT_7 receptor, we published the evaluation of three compounds developed by our collaboration partner Prof. Marcello Leopoldo at the University of Bari Aldo Moro in Italy [20, 30]. Unfortunately these radioligands proved unsuccessful. However, successful data in pigs was obtained with [^{11}C]Cimbi-712 and [^{11}C]Cimbi-717, as illustrated in Figure 12 [19]. In pigs, we observed a dose-dependent decrease in binding when the animals were pretreated with a 5-HT_7 receptor antagonist demonstrating that [^{11}C]Cimbi-717 selectively images the 5-HT_7 receptors in the pig brain.

In 2014 we also published the evaluation of two clinical drugs as PET-radioligands: The multimodal antidepressant compound called vortioxetine [3] and the 5-HT_{2A} receptor inverse agonist pimavanserin which is used to treat psychoses in Parkinson's disease patients [4]. Both drugs are well-tolerated and clinical efficacious, but as PET radioligands the compounds have high non-specific binding and the tracer kinetics of both ^{11}C-labelled drugs is unfavourable for quantification of binding.

Figure 11: Representative [11C]Cimbi-36 PET brain images from a healthy volunteer at baseline and after ketanserin pretreatment. PET images show radioligand uptake from 40 to 120 minutes and scaled to standardized uptake values (SUVs; unit: g/mL), and images are superimposed on an MRI template of the brain. Figure from [11], Copyright © 2014 ISCBFM.

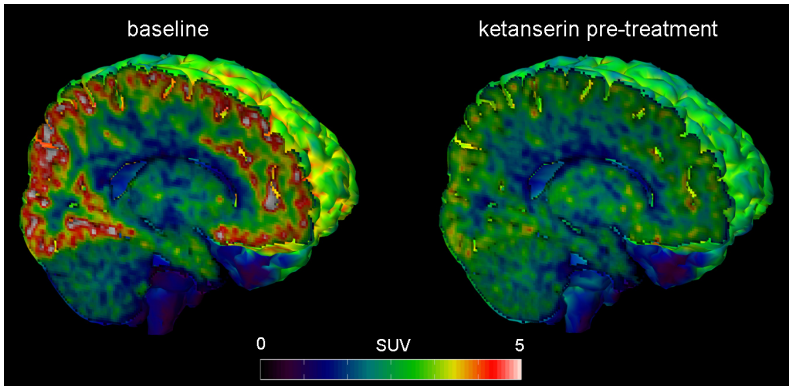
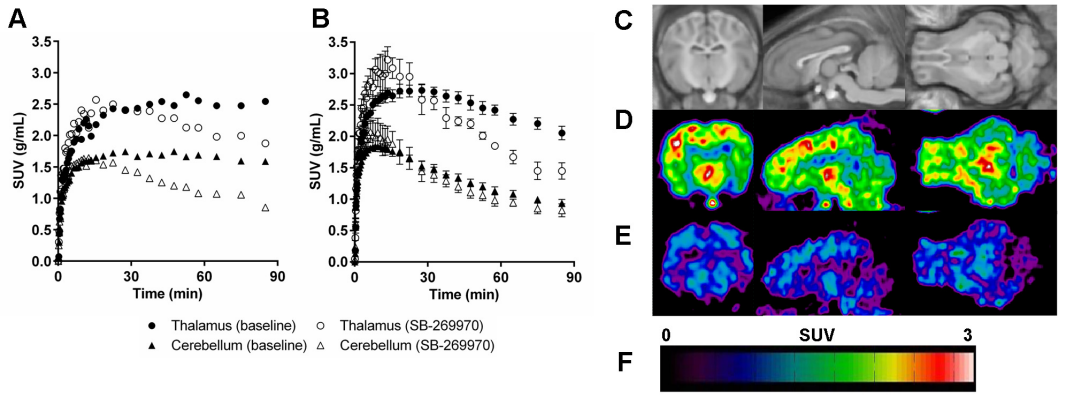


Figure 12: (A) Time-activity curves for [11C]Cimbi-712 at baseline (n=2) and after SB-269970 pretreatment (1.0 mg/kg/h, n=2). (B) Time-activity curves for [11C]Cimbi-717 at baseline (n=6) and after SB-269970 pretreatment (1.0 mg/kg/h, n=3). (C) MR-based atlas of pig brain. (D and E) Summed [11C]Cimbi-717 PET images from 0-90 min at baseline and after SB-269970 pretreatment, respectively. (F) Color bar of standardized uptake value (SUV) (g/mL). Error bars = SEM. Figure from [19], Copyright © 2014 SNMMI.



Platform 2: Data Analysis

by Claus Svarer, NRU,
Klaus K. Holst, NRU,
Lars Kai Hansen, DTU

The NRU group lead by Claus Svarer has worked on a project aimed at building a high-resolution multi-receptor serotonin atlas which can be queried by pattern recognition and machine learning tools and hence can accelerate the deciphering of the serotonin system and its involvement in human disease. Together with the FreeSurfer lab at the Martinos Center for Biomedical Imaging, MGH, Boston, USA, new PET surface-based analysis tools implemented in the neuroimaging software package FreeSurfer (surfer.nmr.mgh.harvard.edu) have been developed and published [17]. The accessibility of these tools in combination with the one-of-a-kind database available through Cimbi has put us in the unique position to create a high-resolution atlas of the binding potential of the serotonin transporter and four different serotonin receptors (5-HT_{1A}, 5-HT_{1B}, 5-HT_{2A} and 5-HT_4). We expect to be able to publish the atlas during 2015.

The NRU group has also been involved in a biostatistics project run by Klaus Holst. This project dealt with development of a new data model for representing multi-dimensional scientific data such as fMRI and PET data. Neuroimaging studies are characterized by collection of big data, and special attention on how to represent and store such data much be given. Relational database systems are generally not suitable for multi-dimensional array type data, and to facilitate the use of common datasets for different statistical programs the neuroCDF data model has been developed at NRU. It is based on the platform independent network Common Data Form (netCDF), which is a file based model tailored for representing multi-dimensional array-orientated (space-time) scientific data, e.g. fMRI and PET data. With native support in MATLAB which is often used for preprocessing of neuroimaging data, and with interfaces for all major programming languages such as R, Python, C++, Java, and Fortran, the neuroCDF format allows data to be transported or shared by dissimilar computer systems and different statistical programs. The neuroCDF package in R (http://github.com/kkholst/neurocdf) provides the building blocks for importing data from SPM into the netCDF format, and allows data to be read, visualized and analyzed in R (using parallel computing). Hereby the vast amount of statistical packages in R can be used in both voxel-wise and ROI analyses. Results can be written to the neuroCDF file again, and may subsequently easily be imported in another program (e.g. MATLAB/Octave) for further processing or visualization.

The DTU group lead by Lars Kai Hansen continued the work on real-time brain imaging based on portable and mobile smartphone solutions for emotional, social, and mental state mapping. The work combines basic research, technical developments and applications. An important research component is improved precision of the methods for converting the EEG scalp measurements to a comprehensive "four dimensional" information stream illustrating brain activity. Sofie Therese Hansen, Michael Riis Andersen and Lars Kai Hansen worked on new imaging methods aimed at sharper images and faster computation, and Finn Aarup Nielsen and Lars Kai Hansen worked on real-time annotation of mental states to help the user understand what the four dimensional stream means.

Applications of real-time EEG are starting to emerge. In 2014, a project (http://www.grandchallenges.ca/grantee-stars/0338-04/) was initiated applying the smartphone brain scanner in underdeveloped countries with Farrah Mateen, MGH, Boston, USA. Andreas Trier Poulsen, Simon Kamronn, Ivana Konvalinka and Lars Kai Hansen worked on an application of the smartphone brain scanner for measurement of joint attention to digital media. The joint attention signal earlier reported for single, sequential viewing sessions was demonstrated under quasi-natural conditions in a classroom setting, see photo below. This study further demonstrated the smart phone brain scanner's versatility and robustness.

Photo showing the joint attention to digital media experiment. Courtesy of Simon Kamronn and Andreas Trier Poulsen.

Director: Jens D. Mikkelsen

The COGNITO project lead by Professor Jens D. Mikkelsen is supported by the Danish Strategic Research Council and aimed to evaluate the efficacy of new pharmacological treatments for cognitive dysfunctions. Apart from NRU, 4 other Danish academic partners, 2 associated partners and 3 companies are involved in this project. The project was initiated in 2012 and will continue until the end of 2017.

The overall aim of COGNITO is to identify and validate novel treatments for cognitive disorders, in particular those diseases characterized by dysfunction in working memory and decision making. Novel pharmacological treatments for CNS disorders such as those characterized by cognitive dysfunction are based on animal models where the novel treatments are first tested. However, it has turned out that such animal models might be important in understanding some fundamental mechanisms in drug action and memory processes, but rather poor to predict clinical efficacies in the patients. An important objective of the COGNITO project is to evaluate novel models that better predict effects in man. To that end we are interested in studying the effect of cognitive enhancers and the targets which they interact in human tissues rather than in animals.

With regard to treatment of cognitive disorders, one of the most promising novel treatment opportunities are alpha7 nicotinic acetylcholine receptor (α7nAChR) modulators for treatment of schizophrenia and Alzheimer's disease. In 2013, randomized clinical studies have revealed that treatment with the α7nAChR partial agonist EVP6124 (encenicline) has significant effect on certain cognitive domains, in particular those where prefrontal function is involved. The compound proceeded into final stages of clinical development and is expected to represent a novel treatment for cognitive disorders in the years to come. The COGNITO project has focused interest to understand the mechanisms underlying the documented effect of the α7nAChR partial agonist, with the aim to assess whether other types of α7nAChR modulation including allosteric modulation could have better effects on these patients.

Research highlights from 2014

The novel drugs are discovered from screening on the homomeric α7nAChR consisting of 5 identical α7nAChR subunits. However, the target in a human cell may possess different properties either because it interacts with other proteins not expressed in artificial cell systems or because it is distributed differentially within the cell. We have shown that the α7nAChR subunit binds to the beta2 nAChR subunit in the human brain. From anatomical data the two subunits are co-expressed in the cholinergic neurons of the basal forebrain, and this is interesting because they are considered to be central in control of the cortex during memory consolidation. Interestingly, our studies in cellular systems demonstrate that co-transfection of cDNA encoding both the beta2 and the α7 subunit has profound consequences for the pharmacological effects. Also proteins of the Ly6 family interact with the α7nAChR, but the pharmacological consequences are unknown.

Our data strongly suggest that the human α7nAChR is different from both the receptor on which novel drugs have been screened in cells as well as the α7nAChR in animals. It is therefore necessary to study the effect of novel medications on human cells and tissues before these are tested in man, and we may also learn from effects and mechanisms these drugs may have in our novel models. We have also shown that the gene, *chrfam7a,* that encodes a truncated version of the α7nAChR subunit is expressed in the human brain. This gene is only present in humans, and thus represents an important regulator of α7nAChR in the human brain that is not possible to study in animal models. When integrated into α7nAChR it is believed that this gene product functions as a dominant negative subunit. However, because this gene is only present in humans, it may also represent an evolutionary important gene involved in cognition.

Another important aim of the COGNITO project is to analyze populations in order to identify patients with cognitive disorders that would mostly benefit from treatment with cognitive enhancers. Along the same lines, we are interested in correlating cognitive abilities in a normal population with variations in the *chrna7 and chrfam7a* genes. We have found correlation between variations in the gene encoding the α7nAChR subunit, *chrna7*, and patients with schizophrenia.

One method to test effect of novel treatments is the use of inducible pluripotent stem cells (iPSC). These originate from human cells and can be differentiated into neuronal phenotypes. We have used this technology to study the effect of α7nAChR agonists and demonstrated that the cells are responding. The goal will be to generate iPSC from patients with cognitive dysfunction, and then test if various medications are effective, but this will take longer than the COGNITO project.

We continued to evaluate and optimize methods in order to develop the current PET tracer 11C-NS14492 with the intention to use it clinically. We have evaluated *Kd* and *Bmax* on the pig and humans using tritiated NS14492, and the results have shown strong species differences and relatively low binding capacity in human brain. We have therefore paused the development of this tracer for human use.

The COGNITO project has commercial perspectives in that we together with the industry are working to identify the products that modulate α7nAChR in an appropriate manner. Studies on the α7-beta2 heteromeric receptor were done in collaboration with Eli Lilly & Co.

The interdisciplinary approach has been a key factor in 2014, and COGNITO is delighted to have associated partners from the Department of Neuroscience, Ohio State University (Professor John Bruno) and Caltech, Pasadena (Professor Henry A Lester), USA. Several new collaborations have been established with international groups including the Pasteur Institute, Paris (Professor Uwe Maskos) and Barrow Neurological Institute, Phoenix, USA (Professor Jie Wu).

Photo of the participants at the 2014 COGNITO annual meeting. The meeting took place in January 2015 at Schæffergården in Gentofte.

Center for Experimental Medicine Neuropharmacology

www.neuropharm.eu

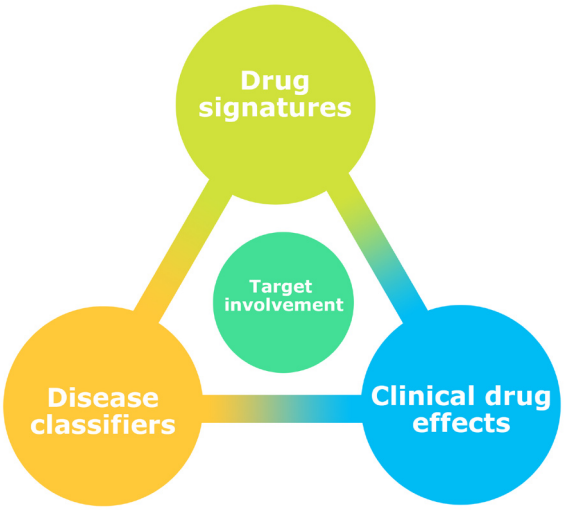
Director: Gitte Moos Knudsen

The Center for Experimental Medicine Neuropharmacology (NeuroPharm) is a new research center under Rigshospitalet and University of Copenhagen. It is funded by the Innovation Fund Denmark and inaugurated on Jan 1st, 2015. NRU is the coordinating partner in the Center and other partners in NeuroPharm include four additional Danish academic partners, one from the University of Copenhagen and three from university hospitals in the Capital Region of Denmark. International partners include Massachusetts General Hospital/Harvard, Imperial College London and a British-based small-medium sized enterprise, Imanova. NeuroPharm also have several affiliated partners from the drug industry.

The aim of NeuroPharm is to develop and validate new human experimental medicine models in brain disorders in order to identify ways to safely assess novel treatments and intervention outcomes in humans. This will be done by studying brain neurobiology and the brain's response to neuropharmacological interventions.

The research in NeuroPharm is divided into four work packages. The specific scientific objectives of these are to

- predict the outcome of a pharmacological intervention (WP1)
- assess novel treatment interventions for brain disorders (WP2)
- develop and validate neuroimaging methods for experimental medicine approach (WP3)
- build statistical and predictive models of outcomes (WP4)



By means of PET and MR brain scanning we will image brain receptors, receptor occupancy, and the brains regional interactions, i.e., functional connectivity. The ability to *simultaneously* measure drug occupancy and brain reactivity directly in humans provides a completely novel approach to assess interventional effects. We will employ these brain imaging tools in patients with, e.g., depression and migraine. We will make use of existing data and biological samples to be analysed in the context of a multivariate data analysis framework. Generation of predictive statistical models will allow for a more informed use of data acquired within the Center and will provide a foundation for better study designs. Through this research, we expect to answer pertinent and basic questions regarding human brain disease mechanisms and predict brain responses to categories of neuromodulatory interventions as well as treatment efficacy.

The diagram below summarizes the overall framework of NeuroPharm, in the context of drug assessments where the main goal is to identify *target involvement*. Based on data outcome from the various methods available within the Center, e.g., brain imaging techniques we will gain novel insight into the specific patterns characteristic for individual brain-targeting drugs (*Drug signatures*). The same endophenotype data can also critically aid to stratify patients and categorize disease subtypes (*Disease classifiers*) which will enhance prediction of treatment efficacy (*Clinical drug effects*). Conversely, individuals' clinical outcome after drug intervention can itself be part of the drug signature and can help inform the disease classification.

In order for Denmark to remain an active global partner within clinical research it is mandatory to improve conditions for early clinical trials and experimental treatment. This will ensure industry-financed proof of concept studies. In this sense NeuroPharm is timely and superbly aligned with recent initiatives from the Danish government in support of attracting more early clinical trials to Denmark.

The vision of NeuroPharm is to establish itself as an internationally leading hub for experimental medicine in brain disorders.

Clinical Neurology

Lars H. Pinborg

Olaf B. Paulson

Epilepsy surgery registry data

Patients with medically intractable epilepsy with a focal start in the brain are possible candidates for epilepsy surgery. In Denmark epilepsy surgery is centralized at Rigshospitalet, and annually approximately 100 patients are evaluated here and at the Epilepsy Hospital Filadelfia in Dianalund.

In 2013 we started a retrospective study on the outcome of the Danish epilepsy program from 1992-2009. Funding from the Lundbeck Foundation allowed PhD student Mette Thrane Foged to collect clinical and paraclinical data on 113 patients with pathologically verified mesial temporal lobe sclerosis. We expect to complete data acquisition and analysis in spring 2015. In 2014 epilepsy research nurse Minna Litman was important in collecting data including questionnaires addressing quality of life. Preliminary results imply that 67% of the operated patients were seizure free (Engel class 1a) one year after surgery, corresponding to the best international numbers.

In 2013 we also started the construction of a prospective database for patients enrolled in the Danish Epilepsy Surgery program. The Danish Council for Independent Research supports the initiative. The database covers many aspects of importance for patients with epilepsy from sociodemographic data, seizure semiology, intra- and extra-cranial EEG, MEG, MRI including fMRI, molecular imaging, and psychological and psychiatric data. More than 25 members of the Danish Epilepsy Surgery team, including close collaborators at Hvidovre Hospital, Glostrup Hospital and the Epilepsy Hospital Filadelfia in Dianalund, have contributed to the content of the database. The database will be hosted at Rigshospitalet and programming will begin in 2015. To support future research and exchange of data between countries the design of the database is coordinated with close collaborators in Nordic and European countries.

EEG-fMRI in epilepsy

The implementation and integration of multimodal functional imaging techniques in the diagnostic workup of epilepsy surgery candidates is key for stepping forward both with respect to the number of patients we can offer surgery and the number of patients that surgery render free of disabling seizures.

In 2014 we have continued our collaboration with professor Henrik Larsson from the Functional Imaging Unit at Glostrup Hospital. EEG-fMRI combines the unique temporal resolution of EEG with the spatial resolution of MRI. Supported by the Lundbeck Foundation Professor Stefan Posse from the University of New Mexico in Albuquerque revisited NRU during the summer 2014. Supported by the Juchum Foundation and the Simon Fougner Hartmanns Familiefond a new 256 channels MRI compatible EEG system was purchased. In collaboration with professor Carsten Thomsen from the Department of Radology at Rigshospitalet the new system is being implemented for EEG-fMRI research.

We expect to benefit from the new high density EEG system epilepsy source location in the epilepsy surgery evaluation process (Figure 14). Associate Professor Sandor Beniczky is an important collaborator for electrical source imaging and we expect to strengthen our collaboration with both the Epilepsy Hospital Filadelfia in Dianalund and Aarhus University where MEG is performed.

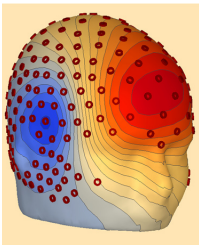


Figure 14: Voltage map (256 EEG electrodes) in an epilepsy patient with frequent seizures despite optimal treatment with antiepileptic drugs. The classical, visual assessment of the standard (32 channels) EEG would lead to misinterpretation of the spike localisation by tracing only the negative peak (blue) which in this case is in the right temporal region. The new voltage map clearly shows also a concommittant positive pole (red), suggesting a tangential dipole with the source located between the negative and the positive peaks. The electrical source was found to co-localize with a vascular malformation in the right frontal lobe demonstrated on MRI. Courtesy of Sandor Beniczky.

Imaging of neuroinflammation

As part of our involvement in the INMiND consortium (described on page 20) we have started several projects testing different aspects of neuroinflammation in different neurological diseases using the SPECT tracer [^{123}I]CLINDE. This particular tracer binds to the translocator protein (TSPO) site formerly known as the peripheral benzodiazepine receptor. The upregulation of TSPO on activated microglia provides the background for imaging of neuroinflammation in neurological disease. An example is anti-NMDA receptor encephalitis (Figure 15) which is a disease first described in 2007 but now known to have an incidence rate larger than herpes encephalitis and a comparable morbidity and mortality [26].

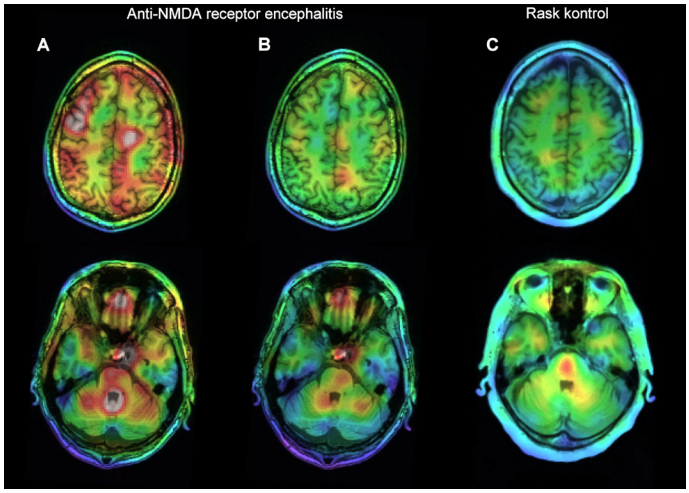


Figure 15: [^{123}I]CLINDE SPECT and coregistered MRI in a patient with anti-NMDAR encephalitis. (A) At the start of immunotherapy [^{123}I]CLINDE-binding to TSPO was strongly increased in cortical and subcortical brain regions but almost normalized (B) after seven weeks of immunotherapy compared to (C) a healthy volunteer. The patient is a 35-year-old man who presented with psychiatric symptoms dominated by perceptual difficulties and delusions that resemble symptoms following intoxication with NMDA antagonists. Anti-NMDA-receptor antibodies were detected in CSF. Cerebral MRI was unremarkable. After seven weeks of immunotherapy the patient was back at work part-time as a computer scientist despite mild cognitive problems. The image appeared as the front cover of Neurology in February 2015. Figure from [25], Copyright © 2015 American Academy of Neurology.

The SPECT Laboratory

Gerda Thomsen

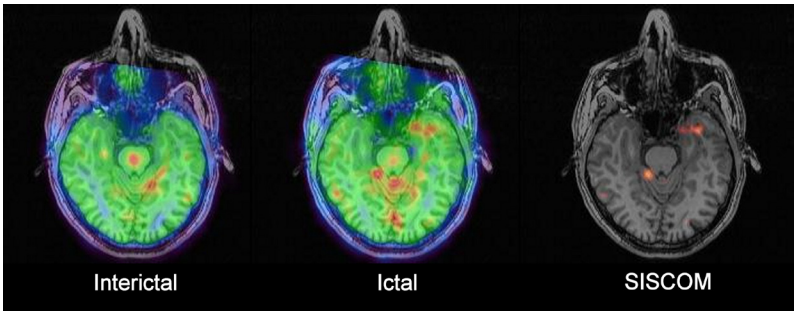
Clinical work in the SPECT-lab

Patients with neurological disorders are referred to the SPECT-lab for diagnostic SPECT-scanning, mostly from the Department of Neurology, Rigshospitalet, but also from Dianalund, Hillerød and other hospitals in Denmark. In 2014, the SPECT-lab conducted more than 200 diagnostic scans.

One type of the clinical SPECT scans conducted at the SPECT-lab is DaTSCAN which is striatal dopamine transporter (DAT) imaging with the ligand [^{123}I]FP-CIT. This technique is used in evaluation of adult patients with suspected parkinsonian syndromes since it may help differentiate essential tremor from idiopathic Parkinson's disease, multiple system atrophy and progressive supranuclear palsy. Furthermore, the technique can help differentiate Lewy body dementia from Alzheimer's disease. For each of these investigated patients, the SPECT-lab conducts the DAT SPECT scan and performs a semi-quantitative analysis of the resulting scan based on an in-house developed method which correlates the actual scan to a database of age-matched healthy subjects. Finally a trained clinician evaluates the scan visually, guided by the outcome of the semi-quantitative analysis. In 2014, the SPECT-lab performed a total of 145 DaTSCAN investigations.

Another type of clinical SPECT scans performed by the SPECT-lab is blood flow imaging with the ligand [^{99m}Tc]HMPAO. This technique is unique since after injection of [^{99m}Tc]HMPAO the lipophilic compound crosses the intact blood-brain barrier, distribute in proportion to cerebral blood flow with a peak brain activity within 2 min after injection. At present, we mostly use the technique for brain perfusion by SPECT in the presurgical detection of the epileptic focus in patients with complex partial seizures refractory to medical treatment. The SPECT-lab is highly specialized in presurgical epilepsy surgery work-up, since it is the only laboratory in Denmark to conduct ictal-interictal SPECT imaging with co-registration to MRI. The technique is called SISCOM (Subtraction Ictal SPECT Coregistred to MRI) and it has proven to be a highly valuable diagnostic tool to non-invasively localize the seizure-onset zone (see Figure 16). The SISCOM technique also applies to investigations in children. In 2014, the SPECT-lab performed a total of 25 SISCOM-analyses.

Figure 16: (Left) Interictal and (middle) ictal [^{99m}Tc]HMPAO SPECT imaging with co-registration to MRI, and (right) resulting SISCOM image. Courtesy of SPECT-lab.



Research in the SPECT-lab

In 2014, the SPECT-lab has been engaged in different research projects and directly involved in several new peer-reviewed publications. In the period 2008-10 we collected data from healthy individuals and together with EU researchers built reference databases of healthy controls, e.g., the ENC-DAT database of [^{123}I]FP-CIT SPECT scans (European Normal Control Database of DaTSCAN), for comparison of the outcome in patients to assist the clinicians' evaluation quantitatively. In 2014, the ENCDAT project resulted in two new NRU-related publications, the first one studying the association between central serotonin transporter availability and body mass index [22], and the second one age and gender dependencies of the binding of [^{123}I]FP-CIT in the thalamus and pons [28]. The first study confirmed previous PET findings of an altered central serotonergic tone depending on BMI, as a probable pathophysiological mechanism in obesity, while the other study demonstrated gender effects for binding in the thalamus and an age-related decline in radiotracer binding for both thalamus and pons.

SPECT-lab is also actively engaged in the EU-funded INMiND project (described on page 20), and last year we contributed by performing several [^{123}I]CLINDE SPECT investigations in both healthy subjects and in patients expected to show microglial activation (Figure 17), e.g., stroke patients during recovery, multiple sclerosis patients, and patients with anti-NMDA receptor encephalitis.

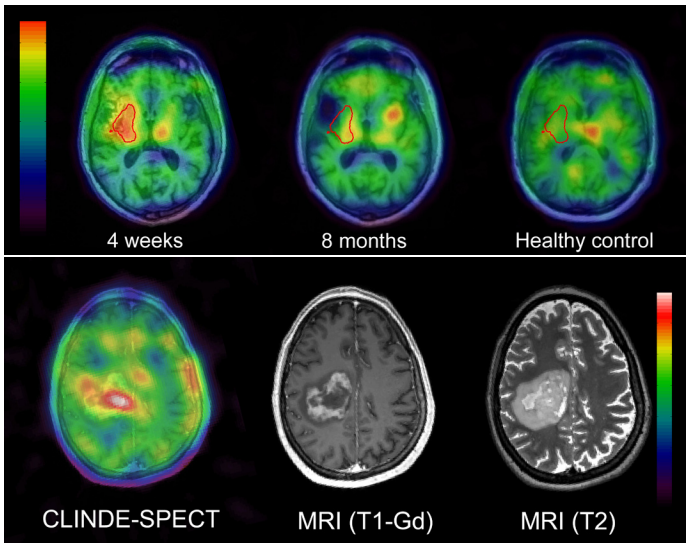


Figure 17: (Upper panel) [^{123}I]CLINDE SPECT images showing the spatial and temporal development of inflammatory responses (i.e. TSPO expression) in an MCA stroke patient 4 weeks (left) and 8 months (middle) after a stroke. Clearly, 8 months after the stroke TSPO expression in the region adjacent to the infarct (red circle) was reduced to near normal level as compared to (right) an age, gender and TSPO-genotype matched healthy volunteer. (Lower panel) [^{123}I]CLINDE SPECT and two MRI scans from a 31 year old female patient with multiple sclerosis. Clearly, an increased binding of [^{123}I]CLINDE to TSPO is seen in the severe plaque that is present. Courtesy of Ling Feng and Per Jensen.

Strategic Collaborations

PET and Cyclotron Unit, RH

For several years, NRU has had an outstanding collaboration with Professor Liselotte Højgaard and her dedicated staff (see photo below) at the PET and Cyclotron Unit at Department of Clinical Physiology, Nuclear Medicine & PET.
The collaboration covers both research and developmental activities and provides NRU with both excellent expertise and infrastructure for radiochemistry, as well as PET- and MR-PET scanner facilities. We highly appreciate this crucial collaboration and look forward to continuing the joined research activities in 2015 and beyond.

The staff at the Department of Clinical Physiology, Nuclear Medicine & PET with whom NRU has an excellent collaboration.

Martinos Center, MGH, US

The Athinoula A. Martinos Center for Biomedical Imaging was launched in 2000 under the Directorship of Professor Bruce R. Rosen, MD, PhD, and the Center has been pioneering brain imaging with MRI. The Center is located on the MGH research campus in the Charlestown Navy Yard with a satellite facility on the MIT campus in Boston, US. In 2011, director Bruce Rosen was awarded the Kirsten and Freddy Jørgensen Prize at Rigshospitalet, and a fruitful collaboration was established between the two sites and further strengthened at later retreat meetings, the first one in Boston in 2012 and the second one in Copenhagen in 2014 (see group photo form that meeting below). Bilateral exchange of scientists has also taken place since 2011, to conduct scientific work within:

- PET-MR brain imaging under pharmacological challenges
- Improvement of technical performance of PET-MR, including attenuation correction and motion correction
- Testing novel PET radioligands
- Quantification of PET imaging data using FreeSurfer

Group photo from the MR-PET retreat meeting held June 2014 at Hotel Bella Sky in Copenhagen with participants from Rigshospitalet and the Martinos Center, US. The workshop included presentations from both sites and fruitful discussions about future collaboration projects.

DanPET AB

DanPET AB (www.danpet.eu) is a Swedish company with focus on biomarkers and preclinical programs within the areas of nicotine and monoamine reuptake. It is founded and directed by Dan Peters who has been working at NRU during May-October 2014 as an associated researcher in an EU-program called CCJobs (Creating Competitive Jobs, http://ccjobs.se/en). CCJobs is an Interreg project between Swedish and Danish universities and business organizations who want to test a new method to utilize research and increase companies' ability to absorb it.

The main purpose of Dan Peters' project was to mature the alpha7 PET-ligand 11C-NS14492 so it can be used in the clinic in the future. Dan's project at NRU also generated the biomarker 3H-NS14492 as a poster and publication that also became a product, with customers all over the world, commercialized together with Novandi Chemistry AB in Stockholm. Professor Jens D. Mikkelsen and CCJobs are very much acknowledged for this achievement.

Future collaboration between NRU and DanPET AB will include investigation of two new potential [^{18}F] alpha7 PET-ligands (NS14552 and NS14562) as well as the supply and research-implementation of new ligands of relevance within the nicotinic field, e.g. the alpha6 nicotine area.

INMiND Consortium

NRU is part of the INMiND (**I**maging of **N**euroinfla**m**mation **I**n **N**eurodegenerative **D**isease) consortium consisting of 22 mostly European academic partners and 6 SMEs, i.e. small and medium enterprises. Since 2012 the INMiND consortium has been supported by the EU 7th Framework. The purpose of INMiND is to identify mechanisms linking neuroinflammation and neurodegeneration and to make this knowledge useful in a clinical context for the benefit of neurological and psychiatric patients. NRU is involved in several work packages from the cellular level to TSPO imaging using [^{123}I]CLINDE SPECT in neurological patients, and from training activities to dissemination of knowledge.

The precise quantification of [^{123}I]CLINDE cerebral binding in humans is essential for exploring its clinical implications to assist diagnostic and therapeutic purposes, and the possibility of further translating the knowledge into clinical application and patient benefit. In 2014 we demonstrated that a 2-tissue compartment modelling of data from a 90-min dynamic SPECT scan with arterial blood sampling was the optimal approach to quantify [^{123}I]CLINDE binding [12]. Further, we demonstrated that [^{123}I]CLINDE binding was influenced by TSPO polymorphism and the volume of distribution in high-affinity binders was 7-times higher than in the low-affinity binders. No effect of blood-brain-barrier disruption was found on the binding of [^{123}I]CLINDE.

Teaching and Training

NRU is a major teaching and training site for pre- and postgraduate students. In terms of pregraduate training, the following list of NRU-affiliated students successfully defended their theses or research year reports in 2014:

- Anine Terese Westh Skibsted, "*Neural correlates of human reactive aggression - An fMRI study using the Point Subtraction Aggression Paradigm*", Master's thesis and research year report, Faculty of Health and Medical Sciences, University of Copenhagen
- Erik Perfalk, "*Cerebral serotonin 4 receptor and sex hormones in healthy men - a [(11)C]SB207145 PET-study*", Master's thesis and research year report, Faculty of Health and Medical Sciences, University of Copenhagen
- Janus Houe Magnussen, "*In vivo and in vitro imaging of the alpha7 neuronal nicotinic receptor*", Master's thesis, Faculty of Health and Medical Sciences, University of Copenhagen
- Nina Almegaard Frimer, "*Estradiol fluctuations and implications for serotonergic neurotransmission*", Master's thesis, Faculty of Health and Medical Sciences, University of Copenhagen

In terms of postgraduate training, NRU senior staff members have supervised more than 20 national and international PhD students and post docs. For the third time we have contributed to the Sino-Danish Center for Education and Research by lecturing at the Master degree program 'Neuroscience and Neuroimaging' at the University of Chinese Academy of Sciences in Beijing. Also, three different PhD courses have been given within the auspices of NRU. In March, we hosted our yearly one-week course "*Basic Kinetic Modelling in Molecular Imaging*", in August we organized together with our collaborators at the Martinos Center for Biomedical Imaging in Boston, USA a three-day course in the use of the FreeSurfer software tool and finally, in September, we hosted a three-day course "*The Emotional Brain: Functional and structural dimensions of emotion processing and emotional disorders*" related to our activities in Cimbi. More information about a few of these events are given below.

The Master degree programme "Neuroscience and Neuroimaging" in Beijing

The Sino-Danish Center for Education and Research (SDC) is a joint project on education and research between the eight Danish universities, the Danish Ministry of Science, Innovation and Higher Education, the University of the Chinese Academy of Sciences and the Chinese Academy of Sciences. The overall aim of SDC is to promote and strengthen collaboration between Danish and Chinese learning environments and increase mobility of students and researchers between Denmark and China.

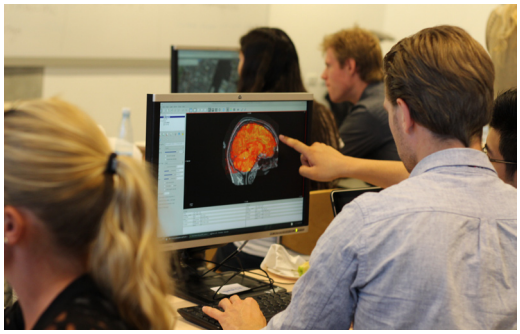
In 2012 SDC established in Beijing an international Master of Science (two-year) programme in Neuroscience and Neuroimaging. This is offered to up to 30 students every year, and ideally half should be admitted via the University of the Chinese Academy of Sciences and half via Danish (Aarhus) universities. A semester runs over 13 full weeks, and NRU staff have since the start been responsible for two and a half weeks of teaching with 12 one hour sessions per week. Since 2012 the teaching has predominantly been lectures, however, in 2014 the first semester was reorganized and the teaching made more interactive with compressed lectures and time for the student to present important aspects of the curriculum.

Since the start, Professor Olaf B. Paulson has had the main responsibility for planning of the education and the written exam. The topics of the NRU teaching have primarily covered molecular neurobiology of neuroimaging, cerebral blood flow, glucose metabolism, and translational neuropharmacology. In 2014, Professors Jens D. Mikkelsen and Olaf B. Paulson were in China to teach.

FreeSurfer Course Copenhagen

In August 2014 NRU organized together with the Department of Computer Science, University of Copenhagen, a PhD course on the popular neuroimaging analysis package, FreeSurfer. FreeSurfer is a freely available and open source suite of tools for the analysis of neuroimaging data that provides an array of algorithms to quantify the functional, connectional and structural properties of the human brain. The course was very popular with a total of 61 participants; 20 PhD students from all around Denmark and 41 Danish professionals and PhD students from other European countries, see group photo below.

The FreeSurfer course Copenhagen was given as an intensively interactive course that covered both the theoretical and practical aspects of functional and structural brain image analysis using FreeSurfer. Lectures were interleaved with hands-on practical sessions that gave the attendees an experience of carrying out the analysis on real data. The lectures were held by the developers of the FreeSurfer software package from the Laboratory for Computational Neuroimaging of the Athinoula A. Martinos Center for Biomedical Imaging, Massachusetts General Hospital and Harvard Medical School, Boston, USA. The course description and lectures are still online and can be found at the course home page: https://fscph2014.nru.dk/index.html.



Other highlights in 2014

DHL relay in Fælledparken

The yearly DHL relay in Fælledparken was held August 25, and this time NRU participated with four incredibly fast running teams. The DHL relay is a good athletic activity and a fantastic social event.

NRU retreat meeting in Sweden

October 3-4, 2014, NRU held a two-day retreat meeting in Sweden with participation from more than 30 of the staff members. First, we visited the Lund University BioImaging Center (LBIC) where we got an introduction to and a tour around their new 7T MR facilities. Afterwards we went to Örestrand Hostel in Höganäs where the rest of the program was devoted to team building activities and social gathering.

Publications 2014

As is evident from the lists in this section, NRU has in 2014 published a total of 2 PhD dissertations, 2 book chapters, and 48 scientific peer-reviewed papers (including 6 papers that were first formally published in early 2015).

NRU collaborators in Cimbi published 14 papers without NRU affiliation, and these external Cimbi publications are listed separately below but with a continued number from the NRU list in order to ease the general referencing in this report.

NRU PhD dissertations

- Agnete Overgaard. Characterisation of the kisspeptin system: The role of sex, obesity, and endocrine disruptors. University of Copenhagen, Faculty of Health and Medical Sciences. Defended Jan 17, 2014
- Mette H. Lauritzen. Imaging Cardiac Metabolism using Hyperpolarised 1-13C-pyruvate MRS. University of Copenhagen, Faculty of Health and Medical Sciences. Defended Mar 26, 2014

NRU Book chapters

- Frøkjær VG. Molecular Imaging of Depressive Disorders. In: Dierckx RAJO, Otte A, Vries EFJ, Waarde A, Boer JA (Eds.) PET and SPECT in Psychiatry (2014). Springer, New York (USA), ISBN: 9783642403835
- Knudsen GM, Hasselbalch SG. Imaging of the Serotonin System: Radiotracers and Applications in Memory Disorders. In: Dierckx, R.A.J.O., Otte, A., de Vries, E.F.J., van Waarde, A., Luiten, P.G.M. (Eds.) PET and SPECT of Neurobiological Systems (2014). Springer, New York (USA), ISBN: 9783642420139

NRU Papers in peer-reviewed journals

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2 Andersen SB, Holst KK, McMahon B, Madsen MK, Møller P, Hageman I, Knudsen GM. Sweet taste sensitivity is influenced by 5-HTTLPR genotype and affected in seasonal affective disorder. Psychiatry Research. 2014 Dec 15;220(1-2):727-9

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Acknowledgements

NRU kindly thanks the following public and private foundations, organizations and companies for generous support for our current research activities:

- A.P. Møller Foundation for the Advancement of Medical Science
- Alfred Benzon Foundation
- Arvid Nilsson's Foundation
- Augustinus Foundation
- Aase and Ejnar Danielsen's Foundation
- Biomedical Laboratory Scientist Education and Research Fund
- Capital Region of Denmark, Foundation for Health Research
- Carlsberg Foundation
- Danish Agency for Science, Technology and Innovation:
    - The Danish Council for Independent Research – Medical Sciences
    - The Danish Council for Strategic Research
- Danish Society for Neuroscience
- Desirée og Niels Ydes Fond
- Eli Lilly
- Fondation Juchum, Det Bøhmske Legat
- Health Foundation
- Kirsten og Freddy Johansens Fond (KFJ prize)
- Lennart Gram's Mindefond
- Lundbeck Foundation
- Mathematisches Forschungsinstitut Oberwolfach
- Nordea-fonden
- Novo Nordisk Foundation
- PharmaDanmark
- Research Council of Rigshospitalet
- Rigshospitalets Jubilæumsfond
- Royal Danish Academy of Sciences and Letters
- Savværksejer Jeppe Juhl og Hustru Ovita Juhls Mindelegat
- Simon Fougner Hartmanns Familiefond
- University of Copenhagen – Faculty of Health Sciences

International research funding:
- EU 7th Framework Program: INMiND (HEALTH-F2-2011-278850)

Cimbi is very grateful to the **Lundbeck Foundation** for their generous support of 80 mio DKK for the establishment and development of the Center in the period 2006-2015. Also, Cimbi is grateful to all of the other public and private foundations, organizations and companies who since 2006 have generously provided additional funding for the activities in the Center.

Support achieved by NRU for the Cimbi research activities carried out in 2014 is included in the list above, while additional funding achieved by the other Cimbi core institutions in 2014 is listed below.

- Danish Council for Independent Research - Technology and Production Sciences
- H. Lundbeck A/S
- Hvidovre Hospital Research Fund
- John and Birthe Meyer Foundation
- Tehnical University of Denmark - Department of Informatics and Mathematical Modeling
- University of Copenhagen – Faculty of Pharmaceutical Sciences
- University of Copenhagen – Mobility Stipends

This report is published by

Neurobiology Research Unit
Copenhagen University Hospital Rigshospitalet
Section 6931
Blegdamsvej 9
2100 Copenhagen Ø
Denmark

tel: (+45) 3545 6711
fax: (+45) 3545 6713
web: www.nru.dk

Editing
Gitte Moos Knudsen
Peter Steen Jensen

Layout
Peter Steen Jensen

Annual Report 2014