Neurobiology Research Unit

Dept. Neurology, Neuroscience Centre
Copenhagen University Hospital, Rigshospitalet

Annual Report 2013

THE LUNDBECK FOUNDATION

Cimbi
Center for integrated molecular brain imaging

COGNITO
Det Strategiske Forskningsråd

Preface

You have in front of you the new format of our annual report where we have gathered all the activities in 2013 from the Neurobiology Research Unit (NRU) and its main projects, Cimbi and Cognito, in one report. 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 2013 was a very successful year for NRU.

In August 2013, NRU moved its offices from the old "villa" that had housed us since the mid 1990'ies, to newly renovated facilities on third floor in the Rockefeller building next door, at Juliane Maries Vej 28. The move was quite complex and required a great deal of patience from all parts. In the new premises, NRU has now two fully equipped sound-proof neuropsychology test rooms installed and a laboratory for handling human specimens is currently under establishment.

I am very proud that two of the NRU senior staff members last year obtained new appointments as professors at the University of Copenhagen. Jens D. Mikkelsen was appointed as professor in translational neuropharmacology and Steen G. Hasselbalch as professor in cognitive neurology and dementia. A number of honorary awards were given to NRU staff members in 2013: the 2013 Mayo Clinic Distinguished Alumni Award was assigned to senior staff member Prof. Olaf B. Paulson (see photo below), the ECNP Fellowship Award 2013 to post doc Patrick Fisher, and the first prize in "Prisopgaven i psykiatri 2013" to medical student Camilla B. Larsen. These assignments and awards illustrate that NRU is staffed with both talented senior researchers and promising younger researchers who are all able to compete at a high level, both nationally and internationally.

Photo of Andrew Engel (left) and Olaf Paulson (right) taken at a private black-tie dinner at the Mayo Foundation House in Rochester in October. Andrew Engel and Olaf Paulson worked together in 1972/73, and Olaf Paulson was proposed to the 2013 Mayo Clinic Distingushed Award by Andrew Engel and colleagues.

With respect to research training, 2013 has been yet another productive year for NRU with several pre- and post-graduate programmes being successfully completed. In terms of pregraduate training, 2 of our medical students obtained the highest grade when defending their research year reports and 4 of our non-medical students obtained their degree after a successful defence of their master theses in biomedicine (2), engineering (1), and psychology (1). Further, 4 psychology students and 2 medical technologist students have been trained in internships at NRU, and finally we have had shorter trainee visits by students from DTU and high school. Gladly, we have been able to keep most of our talented graduated students employed in positions as research assistants or PhD students. In terms of postgraduate training in 2013, NRU senior staff members have supervised more than 20 national and international PhD students and post docs, and two PhD 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*" and in September, we hosted a 3-day course related to our activities in Cimbi: "*The Emotional Brain: Functional and structural dimensions of emotion processing and emotional disorders*" with >10 international speakers and more than 30 participants. As a new initiative, we will in 2014 together with the Martinos Center, MGH, Boston, USA be arranging a 3-day course in the use of FreeSurfer which is software for analysis of brain imaging data.

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 58 peer-reviewed scientific publications and several books and book chapters. Also, three of our PhD students have successfully defended their PhD theses. The full 2013 publication list can be found on page 26. Currently, we have >40 papers in the pipeline so we expect an equal high amount of publications in 2014.

I hope that you will enjoy reading this 2013 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

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About Neurobiology Research Unit

Organization

The research group is chaired by MD, DMSc, Professor Gitte Moos Knudsen since 2004. MD, DMSc, Professor Jens D. Mikkelsen is laboratory leader and responsible for the basic neuroscience section, and Chief Engineer, PhD Claus Svarer is responsible for the data analysis section.
MD, DMSc, Professor Olaf B. Paulson, MD, DMSc, Professor Steen Hasselbach, MD, DMSc, Ass. Professor Lars H. Pinborg, MD, PhD Vibe G. Frøkjær, and MSc, PhD Morten Skøtt Thomsen have in 2013 been members of the NRU leader group. The Chief Technologist in the SPECT laboratory is Gerda Thomsen.

Mission & Activities

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.

The activities within NRU fall in seven different categories:

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

We see our role at Rigshospitalet (RH) 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 postdocs. 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

Since June 1996 NRU has been located at Juliane Maries Vej 24 in an old villa named Building 92 at the Rigshospitalet campus (see photo below), where we had approx. 500 m^2 of space. In August 2013 in conjunction with the general building plans for Rigshospitalet the research unit had to leave the old villa and move to Juliane Maries Vej 28 at 3rd floor in the Rockefeller building (see photo below). In the new premises we have 350 m2 which include offices and facilities for data analysis, a conference room with kitchen, a laboratory for handling human samples, and two sound-insulated rooms with facilities for neuropsychological testing.

The old villa which housed NRU in the period 1996-2013.

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

The NRU experimental laboratory resides at the ground floor in Building 93, Juliane Maries Vej 20, where we have approx. 200 m^2 of well-equipped facilities for basic neuroscience work (in vitro and in vivo studies). Specifically, four laboratory rooms are allocated for NRU while another three rooms and two offices are shared with the other research groups in the building. Equipment in the laboratory includes lab benches, small animal storage facilities, gamma- or beta-counters, behavioural animal labs, 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 an office. Further office and laboratory facilities are shared with other employees at the department.

NRU has a close collaboration with the PET and Cyclotron Unit at Department of Clinical Physiology, Nuclear Medicine & PET both in research and developmental activities, which provides NRU both expertise and infrastructure for radiochemistry, PET- and MR-PET scanner facilities. In 2013, a closer collaboration with the Department of Diagnostic Radiology was initiated, giving NRU access to their MR facilities.

Finances

The vast majority of NRU research are 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 7), supported by 80 mio DKK, and COGNITO: Novel treatments of cognitive dysfunction (page 21), funded 2012-2017 by the Danish Council for Strategic Research with 18 mio DKK. On page 30, all current external funding sources are acknowledged.

Staff in 2013

Head
Gitte Moos Knudsen, professor, MD, DMSc

Senior Researchers (Management)
Claus Svarer, chief engineer, PhD
Jens D. Mikkelsen, professor, MD, PhD
Morten S. Thomsen, human biologist, PhD
Lars Pinborg, associate professor, MD, DMSc (half time)
Olaf B. Paulson, professor, MD, DMSc
Steen G. Hasselbalch, professor, MD, DMSc (half time)
Vibe G. Frøkjær, MD, PhD

Administration
Charlotte H. Münchow
Dorthe Givard
Dorthe Pedersen
Peter S. Jensen

Post Docs
Agnete Overgaard, human biologist, PhD
Anders Ettrup, human biologist, PhD
Dea Adamsen, biochemist, PhD
Karine Madsen, MD, PhD
Klaus K. Holst, biostatistician, PhD (half time)
Linda Blomster, biomedical chemist, PhD
Ling Feng, engineer, PhD
Matthias Herth, chemist, PhD
Mette E. Haahr, MD, PhD
Patrick Fisher, Neuroscientist, PhD

PhD Students
Brenda Mc Mahon, MD
Christian G. Jensen, psychologist
Dea S. Stenbæk, psychologist
Hanne D. Hansen, molecular biologist
Jo Henningsen, biochemist
Majbrit M. Jensen, human biologist
Martin A. Santini, human biologist
Mette T. Foged, MD
Mona El-Sayed, human biologist
Per Jensen, MD
Sophie da Cunha-Bang, MD
Valdemar L. Andersen, pharmacist

Research Assistants
Anna P. Nielsen, MD
Birgitte Bertelsen, human biology
Christinna V. Jørgensen, molecular biomedicine
Liv V. H. Brüel, psychology
Louise M. Jørgensen, MD
Maria E. K. Lie, biomedicine
Mille D. Andersen, biomedicine
Sara R. Jørgensen, biomedicine
Vincent Beliveau, neuroscience

Technical Research Personnel
Anders D. Olsen
Agnete Dyssegaard
Gerda Thomsen, chief technologist
Glenna Skouboe
Hans Jørgen Jensen
Kenneth Nielsen
Lone I. Freyr
Louise Nielsen
Maria R. Nørnberg
Mikkel L. Schiøth
Svitlana Olsen
Søren Iversen
Vibe Hansen

Visiting Scientists
Caroline Ancel, PhD, Strasbourg, France
Cornelius Donat, PhD, Leipzig, Germany
Douglas Greve, associate professor, Boston, US
Pantaleo Di Pilato, PhD-student, Bari, Italy
Stefan Posse, professor, New Mexico, US
Todd Ogden, associate professor, New York, US

Pregraduate Researchers and Students
Amalie H. Ditlevsen, psychology
Anine T. W. Skibsted, medicine
Camilla B. Larsen, medicine
Cecilie Hedegaard, psychology
Charlotte B. Mikkelsen, engineer
Christopher Steiness, high school
Elena J. J. Hoebeke, psychology
Emil Andersen, psychology
Feline Tokman, high school
Franziska Wichern, human biology
Gunild Vulpius, medicine
Hrefna Einarsdottir, medicine
Janus H. Magnussen, human biology
Jesper B. Madsen, psychology
Jon Lansner, psychology
Jonas Villadsen, molecular biomedicine
Maria Arvaniti, human biology
Maria G. Heede, medicine
Martin K. Madsen, medicine
Michelle D. Olsen, psychology
Mikael Agn, engineer
Nina A. Frimer, human biology
Per T. Ørskov, psychology
Rashid A. S. Ahmad, engineer
Rasmus Rydbirk, biology
Rebecca Margolinsky, medicine
Signe P. Ringkøbing, psychology
Silja K. Back, psychology
Taha N. F. Ammar, engineer

Cimbi - Center for Integrated Molecular Brain Imaging

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 million 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.

NRU constitutes the backbone of Cimbi with Professor Gitte Moos Knudsen being the Center Director. Besides housing the management of Cimbi, NRU's role in Cimbi is to conduct research within molecular brain imaging of neurotransmission, receptor binding, cellular characterization and quantification, tracer validation, kinetic modelling, and advanced analysis of brain imaging data.

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, Univ. CPH. FARMA provides the Cimbi basis for cold chemistry facilities and expertise in organic syntheses.
- Informatics and Mathematical Modelling (IMM), the Technical University of Denmark. IMM 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.

The Danish and international collaborating institutions associated with Cimbi include:

- Dept. Biostatistics, Faculty of Health Sciences, Univ. CPH
- Medical Psychology, Department of Public Health, Univ. CPH
- Dept. Medical Genetics, Faculty of Health Sciences, Univ. CPH
- Dept. Psychology, Univ. CPH
- Research Unit for Affective Disorders, Department of Psychiatry, Copenhagen University Hospital, Rigshospitalet
- The Neuroscience, Cognition & Learning Consortium, Learning Lab Denmark
- Rotman Institute-Baycrest Centre and University of Toronto, Canada
- Dept. Clinical Neuroscience, Karolinska Institute, Stockholm, Sweden
- Institute of Medicine, Research Center Jülich, Germany
- Dept. Pharmacology, Oxford University, UK
- MRC Cognition and brain sciences unit, University of Cambridge, UK
- Neuroscience and Psychiatry Department, University of Manchester, UK
- Center of Excellence for Stress and Mental Health, San Diego Veteran's Affairs Healthcare System, US
- Martinos Center for Biomedical Imaging, Massachusetts General Hospital, Harvard University, Boston, US

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. Finally, it plays a prominent role in the inhibition of impulses. 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 2013, Cimbi published a total of 37 peer-reviewed publications and currently has an equal number of papers in the pipeline. Also, three Cimbi-associated PhD theses were successfully defended and currently 15 Cimbi-associated PhD-students are enrolled. Economically, Cimbi managed to raise additional funding of more than 8.7 million DKK, including funding for 2 Post docs, 3 PhD students and 2 scholar stipends.

The prospects for the overall project are excellent with a completed data collection in most of the Cimbi-II studies and a structured plan for the remaining. This leaves the overall time-schedule on track to complete data analyses and manuscript writing over the next two years. An important task in 2014-15 and beyond is to ensure that Cimbi research will be well-implemented and embedded within the involved institutions. We are confident that we will be successful in that if we manage to keep the number of talented researchers that have been trained in Cimbi employed here for the next years.

[11C]SB207145: Development and application of a tool for measuring endogenous serotonin levels in humans

By Patrick M. Fisher, Ph.D, Post-doc and CoI representative for the Cimbi-Biorhythms section.

This section presents this year's main Cimbi story which illustrates when Cimbi's individual research projects through a collaborative and cross-disciplinary effort across several research groups provide a successful "big picture story".

A core theme of Cimbi is to develop our understanding of the neurobiological mechanisms that contribute to the emergence of inter-individual differences in features of behavior and personality. The development, validation and implementation of novel tools for measuring relevant neurobiological mechanisms provides unique opportunities to better observe these processes and understand their role in the neural architecture that shapes behavior and contributes to risk for neuropsychiatric illnesses. Positron emission tomography (PET) provides unmatched resolution of the living human brain as a molecular neuroimaging tool and offers a window into the distribution of receptors, transporters and enzymes. This is an extremely valuable tool because it is uniquely able to quantitatively measure, with outstanding precision, features of the serotonin system including its various receptors and the serotonin transporter. Additionally, molecular neuroimaging with PET makes it possible to measure release of endogenous neurotransmitter in brain. However, this is easier said than done and in Cimbi we have focused on promising PET radioligands for measuring endogenous serotonin release in the living human brain. This is exemplified by the detailed description in the Cimbi annual report 2012 (also available on www.cimbi.org) of the development of [11C]Cimbi-36 as a potential tool for measuring endogenous serotonin release. Here we will share with you how Cimbi has completed a set of studies aimed at systematically characterizing a PET radioligand known as [11C]SB207145, which binds to the serotonin type 4 receptor (5-HT4R). From the determination of how to quantify the signal from [11C]SB207145 PET scans to initial evidence that it may also represent a proxy for inter-individual differences in brain serotonin levels, the collaborative effort across research groups within Cimbi underscore its abilities to move biomedical research forward.

Radioligand development and validation

The compound SB207145 was first developed at GlaxoSmithKline. The radio-labeling of the compound with a carbon-11 positron emitter, making [11C]SB207145, was described in 2008 along with the determination that it was a suitable radioligand for measuring 5-HT4R with PET (Gee et al., 2008). The prospect of a novel radioligand capable of measuring a previously unavailable feature of the serotonin system, which had also been associated with antidepressant actions, sparked a collaboration between Cimbi and GlaxoSmithKline.

Following initial studies in pig here in Cimbi, supporting the promise of [11C]SB207145 as a tool for measuring 5-HT4R binding (Kornum et al., 2009), this radioligand was taken into humans. The human studies were led by the former Cimbi researcher Dr. Lisbeth Marner. Building on earlier work describing [11C]SB207145 in both animal models and human, Dr. Marner and colleagues completed a set of studies at the PET and Cyclotron Unit at Rigshospitalet, a core Cimbi institution, further characterizing the [11C]SB207145 PET signal (Marner et al., 2009, 2010). The specificity of [11C]SB207145 to 5-HT4R in the human brain was affirmed with this set of studies along with a suitable method for quantification of the PET signal, providing compelling evidence for its continue study in humans.

Genetic and pharmacological studies in mice

Despite its intense study for decades, the ability to measure endogenous serotonin release in humans has remained elusive. Dr. Marner and colleagues reported that 5-HT4R in humans was not sensitive to putative increases in serotonin levels following an acute pharmacological challenge with a selective serotonin reuptake inhibitor (SSRI) and serotonin 1A receptor antagonist. However, alternative evidence suggesting that 5-HT4R binding measured with SB207145 may be a proxy for differences in serotonin levels was suggested by a set of studies within the NRU animal laboratory and international collaborators.

While working with a rat model for depression, the former Cimbi researcher Dr. Cecilie Löe Licht showed that an acute challenge of the serotonin system did not affect 5-HT4R levels, but a statistically significant 16-47% down-regulation of 5-HT4R levels was observed following 14-21 day treatment with the SSRI, paroxetine (Licht et al., 2009). Dr. Licht and colleagues also presented evidence that depletion of serotonin levels in this rat model significantly increased 5-HT4R levels in some brain regions. Following this study and in collaboration with Dr. Trevor Sharp's laboratory at Oxford University, we reported that genetic manipulation of the gene coding for the serotonin transporter (5-HTT), a key regulator of serotonin levels, affected 5-HT4R levels (Jennings et al., 2011). Together, these data converge in support of a model wherein 5-HT4R levels are inversely linked to alterations in brain serotonin levels. That is, increases in brain serotonin levels result in decreased 5-HT4R binding while decreases in brain serotonin levels result in increased 5-HT4R binding. These findings added to our interest in pursuing the applications of this novel PET molecular neuroimaging tool.

Imaging genetics and behavioral relevance

In parallel with the on-going characterization of SB207145 and its potential application for measuring long-term changes or differences in brain serotonin levels, we also pursued studies supporting its behavioral relevance. In 2012, former Cimbi researcher Dr. Mette Haahr completed a study evaluating the association between 5-HT4R binding in humans and memory performance. This study was motivated by evidence that 5-HT4R signaling critically affected learning and memory and our newly developed capacity to quantitatively measure 5-HT4R binding in humans, *in vivo*. Consistent with this association, Dr. Haahr and colleagues reported a significant negative association between 5-HT4R binding in the hippocampus and short term memory performance (Haahr et al., 2012). The hippocampus is an essential brain structure for learning and memory and these results reinforced a link between serotonin signaling and memory, alluding to 5-HT4R as an important mediator of this process.

Building on work suggesting a role for 5-HT4R signaling in feeding and food-related reward and addiction, Dr. Haahr and Cimbi colleagues evaluated its association with body weight. Consistent with a relation between 5-HT4R and feeding related behaviors, we reported that body-mass index, a measure of size relative to height, was significantly positively associated with 5-HT4R binding in humans (Haahr et al., 2012). Notably, this positive association was observed within key reward-related brain regions including the ventral striatum and prefrontal cortex. These findings further link serotonin signaling and

risk for obesity, pointing toward a possible effect on reward-processing related to feeding behavior.

In light of our observation in mice that genetic manipulation of serotonin levels affected 5-HT4R levels, which is consistent with SB207145 as a potential proxy for measuring endogenous serotonin levels, we evaluated a similar effect using imaging genetics from data contained in the Cimbi database. Current Cimbi post doc Dr. Patrick Fisher and Cimbi colleagues applied an imaging genetics framework, which is an approach for identifying genetic factors that significantly contribute to inter-individual differences in neurobiological mechanisms. In the case of 5-HT4R, we evaluated a commonly studied genetic polymorphism known as 5-HTTLPR, which affects the gene coding for the serotonin transporter. The shorter S-allele of this polymorphism shows relatively decreased serotonin transporter expression and this we used as a model for elevated brain serotonin levels. Consistent with this model, we found that individuals with at least one copy of the S-allele showed 9% lower 5-HT4R binding in the neocortex compared to individuals with two copies of the longer L-allele (Fisher et al., 2012). This finding is particularly intriguing because the 5-HTTLPR polymorphism has been widely studied and related to aspects of brain function, behavior and risk for neuropsychiatric illnesses including depression and responsiveness to common antidepressant treatments. Thus, our findings provide evidence for a molecular mechanism through which this polymorphism may contribute to these behavioral and clinical phenotypes.

Evidence for sensitivity to endogenous serotonin levels

2013 at Cimbi witnessed a culmination of extensive work aimed at understanding and applying our understanding of 5-HT4R binding with [11C]SB207145 PET. Drs. Patrick Fisher and Mette Haahr within NRU led a double-blind, placebo-controlled study evaluating the effects of pharmacologically increased serotonin levels on 5-HT4R binding (Haahr [30]). This study randomly assigned 32 healthy males to receive daily doses of either placebo or the SSRI, fluoxetine, which increases brain serotonin levels. Building on the animal work that was just described, we hypothesized that fluoxetine, by increasing brain serotonin levels, would significantly decrease 5-HT4R binding, supporting this PET measure as a proxy for brain serotonin levels in humans.

To determine this effect we included 5-HT4R binding data estimated in multiple regions throughout the brain, including the hippocampus, amygdala, neocortical brain regions and the striatum. Comparing the receptor binding levels before and after intervention, using what is known as an "occupancy plot", we estimated the change in 5-HT4R binding following SSRI intervention. Consistent with SSRIs inducing an increase in brain serotonin levels, we observed (**Figure 1**) that 5-HT4R binding was decreased 5.2% in individuals who received fluoxetine. Importantly, the group that received placebo did not show a significant change in 5-HT4R binding, consistent with a specific effect resulting from fluoxetine modulating brain serotonin levels. In addition to these results being published in the prestigious journal, *Molecular Psychiatry*, these findings garnered public attention and were highlighted in different media.

Future directions

The progression in characterizing [11C]SB207145 as a PET radioligand for measuring 5-HT4R in humans and its application as a proxy for endogenous serotonin levels highlights many core themes of Cimbi. The systematic characterization and validation of [11C]SB207145 contributes the arsenal of tools available to scientific research for probing the serotonin system in humans, *in vivo*. The serotonergic manipulation studies in animal models underscore the translational capabilities of Cimbi and the fostering of fruitful collaborations extending outside Cimbi's borders. Critical to the application of novel molecular neuroimaging tools is identifying behavioral relevance, which has been reinforced for 5-HT4R with studies related to memory function and body-weight, bringing together data collected through the Cimbi neuropsychology test battery and molecular neuroimaging. The imaging genetics study in humans was facilitated by the well-organized and rich Cimbi database, providing access to potentially relevant measures across Cimbi disciplines. Also, related findings provide critical insight into how genetic variability shapes serotonin signaling and implicates a molecular mechanism mediating established links between genetic variation, brain function and behavior. Finally, the pharmacological challenge study provides novel evidence that this molecular neuroimaging tool may be a useful proxy for endogenous serotonin levels, a measure which has remained elusive, despite great effort. Within the following pages we will highlight other exciting findings that during 2013 have emerged from the various Cimbi-related research projects. Building on these findings and collaborations, 2014 holds great promise for Cimbi and we look forward to sharing those findings within next year's report.

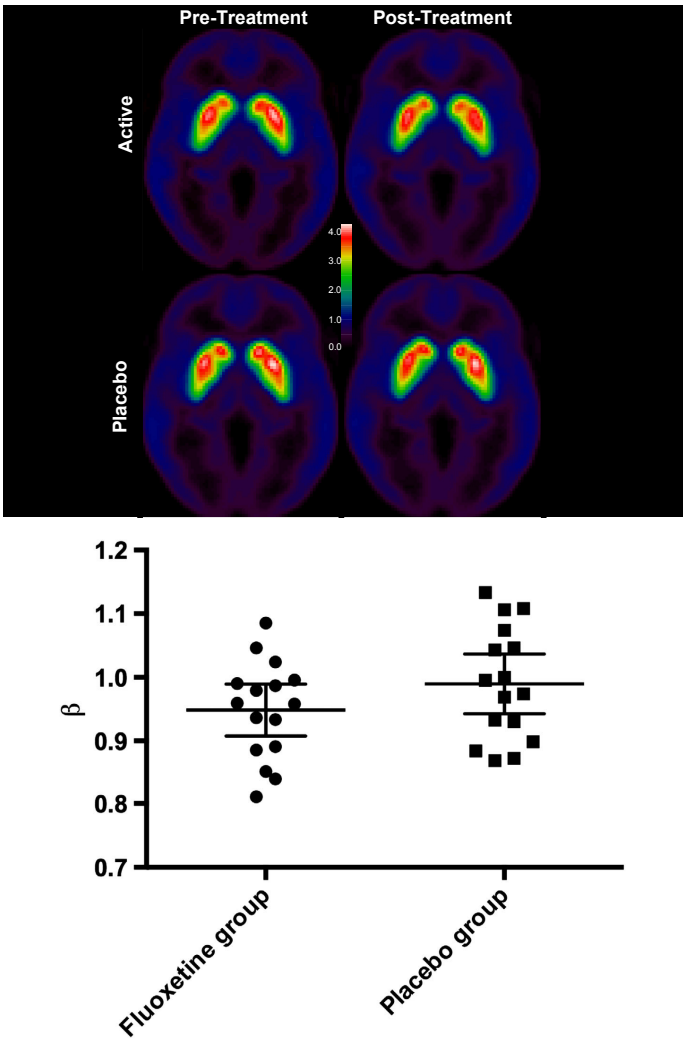


Figure 1: (Upper) The fluoxetine (active) and placebo group effects illustrated by voxel-based analysis using averaged parametric images representing specific 5-HT4 receptor binding all normalized to Montreal Neurological Institute (MNI) space. The left panel shows the pretreated state, whereas the right panel shows the post-treated state. The color bar represents BPND (binding potential (non-displaceable)) units from 0 to 4.25. (Lower) The estimated β values in the 32 included individuals divided into the fluoxetine and the placebo groups. The bars indicate the estimate of the group mean β with the 95% confidence intervals (fluoxetine group: P=0.017, placebo group: P=0.65, one sample t-test, β≠1; difference between groups: P=0.17, two sample t-test). Figures from Haahr [30], Copyright © 2014 Macmillan Publishers Limited.

Mood, Emotions and Biorhythms

An important endeavor in Cimbi is to advance the understanding of functional brain circuits that are involved in the processing of emotions and regulation of mood, stress responses, aggression, and biorhythms such as light dependent diurnal rhythms of e.g. hormone secretion. The serotonergic system is organized in a fashion that enables it to modulate key neural circuits, including a corticolimbic circuit comprising parts of the prefrontal cortex, anterior cingulate cortex and amygdala that orchestrate stress and fear responses (Fisher [16]). These functional circuits are 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.

To advance the mechanistic understanding of how serotonin modulates these important brain functions through research in Cimbi-II, we pursue and elaborate on hypotheses generated from cross-sectional observations in Cimbi-I with an emphasis on longitudinal work where we apply challenges and interventions. Intervention studies where serotonergic signaling is manipulated e.g. pharmacologically or by bright light exposure are important in studying how changes in serotonin levels affect mood, processing of emotions and biorhythms.

By integrating state of the art structural and functional neuroimaging including frontier molecular imaging of key serotonin signaling markers, some of which are developed within Cimbi (Haahr [30], Paterson [52]), we have obtained unique datasets enabling us to ask questions such as: 1) Does bright-light exposure affect the neural circuitry of fear processing? 2) Are dynamic stress hormone responses to stimuli likely to be under prefrontal serotonergic control? 3) Are prefrontal serotonin signaling markers related to trait aggression? The results and prospects of these evaluations are presented below.

Bright-light intervention significantly affects threat-related brain function

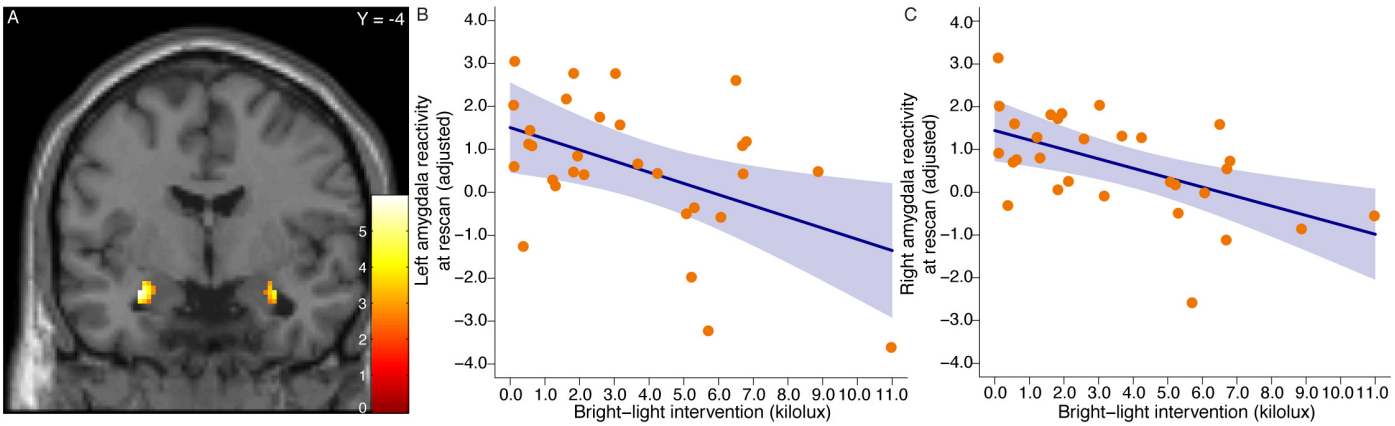
Despite the successful application of bright-light therapy for treating depressive disorders such as seasonal affective disorder for over 30 years, the neurobiological pathways through which it has its anti-depressive effects have not been evaluated. The effect of seasonal variation as a stressor and/or risk factor for depression is particularly relevant in Scandinavia because of the extreme changes in daylight minutes throughout the calendar year. Prompted by these observations, Cimbi has been conducting studies aimed at illuminating the effects of daylight and light exposure on relevant neural pathways and serotonin signaling.

One of these studies probed the effects of a three-week bright-light intervention protocol on aspects of brain function. A group of 32 healthy male participants were asked to self-administer light therapy every day for three weeks. To determine dose-dependent effects, we modeled the light dose that each participant received based on the lamp they used and other relevant parameters. Additionally, participants were genotyped for the 5-HTTLPR (L_A/L_A vs. L_G- or S-carriers) to probe whether serotonin moderated light effects. Participants completed the gender matching emotional faces fMRI paradigm collected across many datasets from Cimbi where participants view faces with angry, fearful or neutral expressions. This paradigm is used as a probe for measuring the response of the brain to aversive or threatening stimuli. Within this study we focused on the amygdala and medial prefrontal cortex because of their critical role in processing aversive stimuli. Participants completed the fMRI paradigm both before and after the light intervention, allowing us to determine the effect of light intervention on the brain response to this task.

In a dose-dependent manner, bright-light intervention negatively affected the response of the amygdala and medial prefrontal cortex to threatening compared to neutral faces (**Figure 2**). Additionally, functional coupling, a metric from our fMRI task that we use as a measure of coordinated communication between brain regions, showed that amygdala-prefrontal and prefrontal-prefrontal functional coupling were significantly positively affected by bright light in a dose-dependent manner. Finally, we found (**Figure 3**) that the effect of light dose on prefrontal-prefrontal functional coupling was significantly moderated by 5-HTTLPR genotype status (L_AL_A individuals vs. carriers of at least one L_G or S allele) (Fisher [17]).

These novel findings provide the first evidence for neurobiological mechanisms affected by a clinically relevant dose of bright-light therapy. Identifying neurobiological mechanisms that may affect risk for seasonal disorders depends critically on understanding the neural pathways sensitive to light. Our data suggest that the interplay between the amygdala and prefrontal cortex in response to threat is one such pathway.

Figure 2: Effects of bright-light intervention on amygdala reactivity. (A) Statistical parametric map highlighting amygdala response to task at baseline across all participants (blood oxygen level-dependent contrast: angry and fear - neutral faces). Color bar represents t scores. Bright-light dose received was negatively associated with (B) left and (C) right amygdala reactivity at rescan, accounting for baseline reactivity values. Thirty individual data points shown in orange and blue shading represent 95% confidence limit of regression line.Figure from Fisher [17], Copyright © 2014 Society of Biological Psychiatry.



Prefrontal serotonergic signaling is linked to stimulated stress-hormone release

In two cross-sectional Cimbi database studies we investigated the potential relation between prefrontal serotonergic signaling and hypothalamus-pituitary-adrenal (HPA) axis stress hormone output. This relationship is of particular interest in understanding the risk architecture behind the development of e.g. mood disorders. We hypothesized that prefrontal serotonergic tonus would act as a modulator of the HPA-axis stress hormone output in daily life conditions, and not only in the context of more extreme stress exposures. Further, we hypothesized that such a relationship would be changed in an off-balance condition of serotonin deficiency. Therefore, we compared two Cimbi populations in the evaluation, namely 32 healthy volunteers with no psychostimulant use and 18 mentally healthy MDMA (Ecstasy) users that we claim offer a human model of chronic serotonin depletion as previously published by Cimbi. Thus we wanted to test if prefrontal serotonin transporter (SERT) binding would be associated with HPA-axis output differently in healthy volunteers compared to an off-balance state of chronic serotonin depletion reflected in MDMA-users.

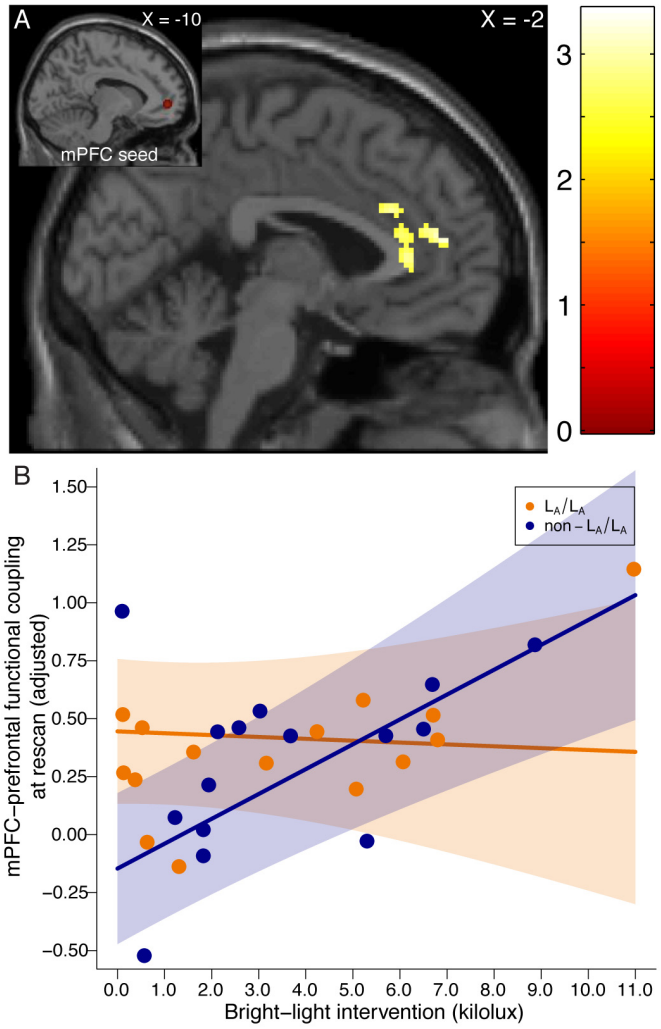


Figure 3: Serotonin transporter-linked polymorphic region (5-HTTLPR) moderates effect of bright-light intervention on medial prefrontal cortex (mPFC)-prefrontal functional coupling. (A) Statistical parametric map highlighting mPFC cluster, wherein the positive effect of bright-light intervention on functional coupling with our mPFC seed was significantly moderated by 5-HTTLPR genotype status (mPFC seed outlined within inset). Color bar represents t scores. (B) Plot of bright-light intervention by 5-HTTLPR interaction effect showing mean functional coupling estimate across 394 voxels and is intended only for visualization of interaction effect. Thirty individual data points are shown in blue or orange. Blue and orange shading represents 95% confidence limit of regression lines for LG or S carriers (non-LA/LA) and LA/LA individuals, respectively. Figure from Fisher [17], Copyright © 2014 Society of Biological Psychiatry.

The serotonin transporter used as PET-marker here is key in regulating synaptic levels of serotonin and thus influences serotonergic tonus. The functionality of our brain circuits of interest is influenced by serotonin transporter genotypes, e.g. the high and low-expressing serotonin transporter promoter variants (5-HTTLPR), however not necessarily exclusively through direct translation of those genes to serotonin transporter protein levels in the adult human brain. It may as well be through more complex neurodevelopmental effects in early life brain maturation of serotonin, e.g. on brain structure and architecture involved in regulation of stress and fear responses, or more likely, a combination.

With inspiration in earlier observations of an association between frontal receptor (2A) markers of serotonergic function and the personality trait Neuroticism, which indexes the ability to cope with emotional stressors and is a risk factor for developing major depression, we wanted a measure of the endocrine stress hormone release. Therefore, we initiated the collection of the cortisol awakening response (CAR) as a standard procedure in the healthy volunteers program in Cimbi-I. The CAR is a distinct feature of HPA-axis activity that is distinguishable from the basal diurnal rhythm of cortisol secretion. Awakening in the morning provokes a profound 50-75% rise in plasma cortisol that peaks at around 30 minutes after awakening and returns to baseline levels within about 60 minutes. CAR appears associated with HPA-axis response to psychosocial stressors.

When taking advantage of the SERT-binding and CAR dataset that have been build-up so far in Cimbi populations, we observed the following. First, in 32 healthy volunteers we demonstrated that prefrontal SERT binding is positively associated with cortisol responses to awakening (Frokjaer [18]). This coupling was not dependent on 5-HTTLPR genotype status. Notably, a voxel-based analysis further supported the regional brain pattern of the association and emphasized a particularly strong association between CAR and SERT binding in the subgenual anterior cingulate cortex. In this particular brain region structural and functional deficits have consistently been linked to mood disorders, and, remarkably, deep brain stimulation targeting this exact region improved depressive symptoms in 60% of patients with treatment resistant depression one year after surgery. Our data raise the question whether deep brain stimulation targeting subgenual anterior cingulate cortex acts over serotonergic pathways by dampening dysfunctions in HPA-axis responsiveness.

Second, in the group of MDMA users who we argue represent a model of serotonin deficiency, we have (as illustrated in **Figure 4**) demonstrated (1) a higher CAR relative to non-users and (2) a positive coupling between prefrontal SERT and CAR, similar to the coupling previously seen in healthy non-users (Frokjaer et al., accepted early 2014).

In conclusion these findings independently replicate and thus converge to establish a coupling between prefrontal serotonin transporter availability and HPA-axis responses. We speculate that this coupling predominantly emerges from early brain development since it appears independent of serotonergic manipulation as in MDMA-use. Further, since CAR was clearly elevated in MDMA-users, we speculate that the inhibitory control on HPA-axis output is less efficient in the off-balance state established by recent MDMA use, most likely through mechanisms other than those that can be compensated by lowering SERT levels.

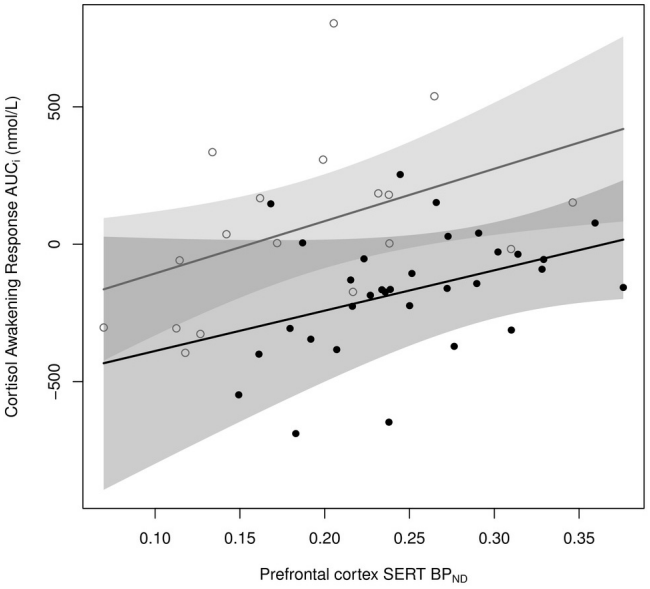


Figure 4: Scatter plot of the association between cortisol awakening response (CAR) and prefrontal serotonin transporter (SERT)-binding in healthy volunteers (black circles) and 3,4-Methylenedioxymethamphetamine (MDMA) users (open circles) modeled as a 'prefrontal SERT by group' interaction (adjusted for age). No significant interaction is present. Shaded areas represent point-wise 95% confidence bands. Figure from (Frokjaer et al., 2014), Copyright © CINP 2014.

Trait aggression and trait impulsivity is not associated with prefrontal serotonin 2A receptor binding in healthy volunteers

Aggression plays a critical role in the manifestation of violence and criminality, and given the extensive morbidity of this behavior there is a need for better understanding the underlying neurobiological mechanisms. Experimental studies in rodents strongly imply that the serotonin transmitter system plays a role in modulating aggressive behavior with most studies pointing to an inverse relationship between serotonin and aggression, in particular impulsive aggression (i.e., unplanned or reactive acts of aggression). A few human studies have indicated that higher frontal serotonin 2A receptor binding characterizes patients with personality disorders that ostensibly display compromised impulse control and higher scores on impulsive aggression than healthy individuals. However, in such clinical populations with manifest psychopathology and maybe even prior medication we cannot separate trait from state or scar characteristics of the disease and hence a healthy PET-population as provided by Cimbi can offer important insight.

Therefore we evaluated, in the first sample of healthy individuals so far, the potential coupling between frontal serotonin 2A receptor binding and trait aggression and trait impulsivity. We demonstrated that in healthy individuals, with trait personality, aggression and impulsivity scores in the normal spectrum, frontal serotonin 2A receptor binding does not correlate with trait aggression and impulsivity in a manner detectable in a large sample of 94 individuals (da Cunha-Bang [8]). Consequently, our findings support that serotonin 2A receptor availability does not play a selective role for aggression and impulsivity traits in mentally healthy individuals. This raises the question whether aggression could be a function of frontal serotonin 2A receptor availability only in vulnerable populations. To further address the associations between impulsive aggression and the serotonin system, a study using more direct behavioral measures of impulsive aggression in patients with heightened levels of aggression compared to healthy individuals will be needed.

Affective Cognition

The cognitive section at NRU was established as a core facility for Cimbi in 2012. It specializes in research on relationships between the brain's serotonergic system, stress-regulation and affective cognition, focusing on interdisciplinary studies of neurobiological and psychological factors in human health. During 2013, equipment and test-facilities were improved with two new sound-insulated test rooms and an external video-linked test monitor station. This process was part of optimising the flow of psychophysiological and neuropsychological test sessions, as the need for testing has steadily increased over the past years. The cognitive section conducts a standardized, three-hour neuropsychological test battery with participants in Cimbi projects and 2013 was characterized by a strong and successful effort to finish data collection in a series of research projects.

In 2013, the cognitive group comprised two senior researchers, two PhD-students, and four full-time pregraduate psychology students. One student graduated in 2013 with her thesis on cognitive-affective changes in seasonal affective disorder and received an excellent review of her Cimbi-based project. These data are currently in preparation for submission. Due to an increased number of intervention studies, seven additional psychology students worked as research assistants in the group. Furthermore, the group was also able to house and supervise two master-internships from the Institute of Psychology at University of Copenhagen and to supervise two high-school students as part of the "Forskerspirer" project at University of Copenhagen.

Validation of a method for Acute Tryptophan Depletion

As part of Dea Siggaard Stenbæk's Ph.D. project, the Cimbi test battery was applied in an acute tryptophan depletion (ATD) study with two primary aims: 1) To validate an in-house protocol for ATD using a dietary collagen peptide (CP) drink and a low amino acid diet and; 2) To study changes in affective cognition in response to reduced tryptophan in peripheral blood (Trp). The results from this study which confirm ATD as a valid, reversible, and non-invasive way to manipulate the serotonin system are to be published soon.

Validation of the Verbal Affective Memory Test

As part of Christian Gaden Jensen's Ph.D. project, the group has worked on validating the first Danish test of affective memory (The 24-word Verbal Affective Memory Test, VAMT-24). In 2013, data collection for three studies was finished. Study 1 validated the perceived valence of the 48 words included in VAMT-24 in 87 healthy individuals, and two 24-word verbal lists were constructed. Study 2 was a psychometric study of VAMT-24 in 137 healthy subjects in collaboration with the Department of Psychology, University of Copenhagen. Study 3 was a study on seasonal changes in affective recall (VAMT-24) in 28 individuals diagnosed with Seasonal Affective Disorder (SAD) and 30 healthy controls. Since VAMT-24 was not related to affective symptoms in healthy individuals, a new 26-word version of VAMT was developed, involving a larger proportion of affective words. This test was applied in several projects in 2013, e.g. the tryptophan depletion project, the meditation-based health promotion project (see below), and a longitudinal brain imaging study of adolescents at Hvidovre Hospital (the 'HUBU-project', described later).

Neuropsychopharmacology

The effects of assisted reproductive technologies (ART) on

levels of mental distress in two commonly used protocols (long GnRH agonist and short GnRH antagonist) were examined in 83 women undergoing their first ART-cycle. The role of personality indices of negative emotionality, such as Neuroticism, for levels of mental distress was also investigated and whether levels of mental distress and Neuroticism predicted probability of pregnancy.

Meditation-based health promotion for stress

In 2013, we completed also a randomized, controlled intervention study of 72 participants with moderate degrees of self-reported stress recruited from general practitioners in Copenhagen and randomized to individual meditation treatment, group-based treatment, or waitlist controls. The course book 'Open and Calm' was produced for the purpose of this study (Jensen CG, 2013). The primary outcome was the cortisol awakening response, and secondary outcomes included computer-tests of attention, affective verbal recall (VAMT-26) and affective priming. The project also collected individual data on 5-HTTLPR-polymorphisms, which is related to risk of depression after stress. The project included five data waves: two at baseline, two post-treatment, and one at 3-month follow-up. In addition, information on participants' use of medication is documented via a national register from 1 year before randomization until 1 year after randomization. The project was conducted in collaboration with the Institute of Psychology, Copenhagen University and followed by a national TV-documentary ('Længere ind i sindet', DR-dokumentar).

Brain maturation in children and adolescents

The major aims of this longitudinal Cimbi project are to define the degree of variability among typically-developing children and adolescents in the maturational trajectories of specific brain networks, and to link these to developing cognitive, emotional and neuroendocrine functions. Further, the impact of genetic polymorphisms, personality trait characteristics, and environmental (e.g. physical activity, stress, alcohol and drug use) factors are investigated. The project capitalizes on the wealth of cross-sectional and longitudinal data collected within the HUBU (*Hjernens Udvikling hos Børn og Unge*) project, which started in 2007. Between 2007 and 2012, we have successfully completed 10 assessments with 6-month intervals of more than 65 children and adolescents, who at baseline were between the ages of 7 and 13 years. In 2013 we conducted an 11th assessment including 43 adolescents. We implemented an emotional face Go/Nogo fMRI paradigm, in which participants are required to respond as fast as possible to emotional faces (happy and sad), while they try to withhold their response to neutral faces and vice versa. The emotional Go/Nogo task may be used to study how emotional stimuli affect our ability to regulate and inhibit responses and to discriminate between stimuli. Extended versions of this task (also including angry and fearful faces) are used as behavioural paradigms outside the scanner in both the HUBU cohort and in the adult Cimbi cohorts.

In 2013, research year student Jonathan Holm-Skjold investigated the link between white matter microstructure and measures of emotional regulation and discrimination from the behavioural emotional face Go/Nogo task in 63 children aged 10-15 years. We previously observed that higher trait neuroticism scores were associated with left-right asymmetry in cingulum fractional anisotropy (FA – an estimate of axonal myelination, diameter, density and organisation) in the HUBU cohort, and that this association was opposite in boys and girls. Thus, we focused specifically on left-right FA asymmetry within an emotional network consisting of regions-of-interest (ROIs) in the cingulum bundle, uncinate fasciculus and white matter underlying the ventromedial prefrontal cortex. We observed that better emotional discrimination of negative emotional faces (angry, fearful, and sad) relative to neutral faces was linked to higher left relative to right FA in the white matter underlying the ventromedial prefrontal cortex. Moreover, faster reaction time to negative emotional faces was linked to higher left relative to right ROI FA in boys, but not in girls. Effects persisted when controlling for neutral conditions, suggesting that the observed effects were not due to general differences in emotional discrimination or reaction time per se, but may be linked to the emotional valence of the stimuli.

In 2013, research year student Troels Lukassen studied the moderating effect of a common polymorphism in the brain-derived neurotrophic factor gene, the BDNF *val66met* polymorphism, on the association between physical activity and white matter microstructure. Sixty-four children aged 10-16 years from the 7th HUBU assessment were included. A parent-reported questionnaire assessed the child's organized sports activities within the last year. The minutes spent on sports activities were multiplied with the intensity-adjusted metabolic equivalent task values of the reported activities, estimating physical activity as the energy expended on sports within the last year. Higher physical activity was associated with lower mean diffusivity (MD – an estimate of the density of cellular membranes) in an estimate of global white matter in *met*-carriers, while no significant effects were observed in *val/val* homozygous children. This was corroborated by voxel-wise analysis, which revealed that higher physical activity was significantly associated with lower MD in ~50% of the skeleton voxels in the *met*-carriers.

Also last year, we investigated the impact of the BDNF *val66met* polymorphism on white matter microstructure in 173 healthy subjects aged 7-50 years. This study utilized and combined existing data from the Cimbi and HUBU cohorts. Mean FA values were extracted from ROIs in the cingulum bundle and uncinate fasciculus. No significant effects were observed for the uncinate fasciculus. In the cingulum, *met*-carriers had significantly higher FA than *val/val* homozygotes. Interestingly, there was also a significant age by genotype interaction effect on cingulum FA with *val/val* homozygotes exhibiting a steeper developmental trajectory relative to the *met*-carriers. Additional analyses of male and female subjects separately revealed that the observed effects were driven by the male subjects (**Figure 5**), as no significant effects were found in females. Future studies will investigate if this apparent effect of BDNF genotype on the maturational trajectory of the cingulum can be replicated in the longitudinal HUBU data.

In 2013, we also continued our work on the analyses of the longitudinal data in HUBU. With our collaborators in Utrecht (Alexander Leemans, University Medical Center Utrecht, the Netherlands), we validated an automated longitudinal intra-subject analysis (dubbed ALISA) approach to robustly extract white matter fibre tracts in subjects assessed at multiple time points using whole-brain deterministic fibre tractography (Aarnink [68]). The ALISA method was compared to manual fibre tract segmentations as well as to an automated inter-subject analysis. In the analyses we included diffusion tensor imaging data sets from 10 healthy children scanned five times with 6-month intervals and from one control subject scanned ten times with weekly intervals. Extracted fibre tracts included the cortical-spinal tract, forceps major, uncinate fasciculus, and the superior segment of the cingulum. We demonstrated that

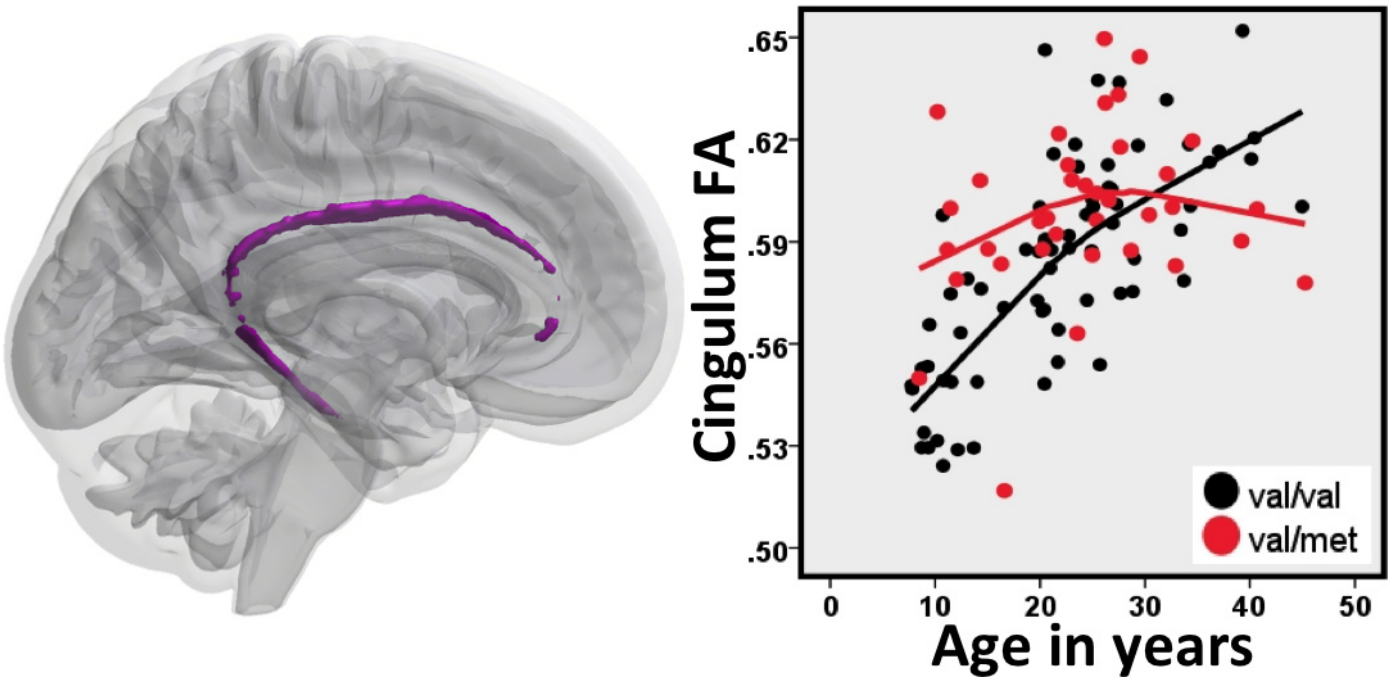


Figure 5: (Left) Smoothed version of the cingulum region-of-interest depicted together with a representation of left hemisphere. (Right) Scatterplot showing cingulum fractional anisotropy (FA) against age for BDNF val/val homozygous (black) and met-carrying males. The val/val homozygous males have a significantly steeper cross-sectional maturational trajectory than the met-carrying males. Courtesy of Kathrine Skak Madsen, DRCMR.

the increased efficiency provided by ALISA did not compromise the high degrees of precision and accuracy that can be achieved with manual fibre tract segmentations. Further, automated inter-subject analyses did not provide similarly accurate segmentations as the ALISA approach.

Neural correlates of social and value based decision

The neural response elicited during various brain processes can be captured with functional magnetic resonance imaging (fMRI). Using specific activation paradigms we are able to investigate neural processes by mapping related regional brain activation. By employing pharmacological manipulations of the serotonergic system while performing paradigms under fMRI, we were in CIMBI-I able to identify brain regions dependent on serotonergic modulation, which were involved in risk taking, reward, and emotional processing.

In Cimbi-I, healthy individuals were investigated using fMRI while performing three different tasks under different manipulations of their serotonergic system. Brain responses were hence measured at a “low”, “normal” or “high” serotonin level, as well as under blockade of a specific serotonin receptor (5-HT2A). This work resulted in a series of publications during 2013. Emotional face processing was found to be altered by serotonin manipulation. A network of regions, including the amygdala, responded more to fearful faces in the control session but lost its specificity for fear after reduction of serotonin levels (Grady [19]). Administration of the 5-HT2A receptor antagonist Ketanserin reduced the response of medial orbitofrontal cortex to fearful faces. This region also showed increased functional coupling with the left amygdala during processing of fearful faces depending on the amount of blocked 5-HT2A receptors (Hornbøll [27]). Gambling behaviour was also affected by serotonin manipulation; we found altered neural response in amygdala and dorsomedial prefrontal cortex to both monetary gains and losses with acute tryptophan depletion and acute SSRI intervention (Macoveanu [43]). We further found that blockade of the 5-HT2A receptors in healthy individuals increased risk aversion and altered underlying neural correlates (Macoveanu [44]).

Building on this knowledge, in Cimbi-II we have developed and investigated several novel activation paradigms specifically designed to increase our understanding of serotonin’s involvement in emotional processing and social behavior. The analyses are planned to integrate other markers of serotonergic function, such as receptor densities as measured with PET, and genotype status on relevant loci. Three novel fMRI paradigms have been developed and investigated. One task studies the integration of unconscious emotional information in decision-making (Emotional Choice). Another task examines reversal learning on the basis of social and non-social feedback (Social Reversal Learning), while the third task was designed to tap into social decision-making (Punishment or Impunity).

In the Emotional Choice task, by viewing pictures of different people, subjects are asked to choose one person they would like to engage in social contact with. The options are primed with a fearful, happy or a neutral face presented for a very short period that is below the threshold for awareness. Our results show that even though there was no change in the frequency at which the different options were selected, we found the aversive prime to increase amygdala activity.

In the Social Reversal Learning task subjects learn the contingencies between, on the one hand, faces and outcomes (face becoming happy or angry), and on the other, symbols and abstract outcomes (pluses and minuses). The contingencies change through the experiment, enabling analysis of the neural correlates to flexible behaviour in a volatile environment. Computational models have been developed in order to compare actual behaviour with the behaviour predicted by the models. The fMRI data during the reversal learning task has been acquired in healthy subjects and is currently under

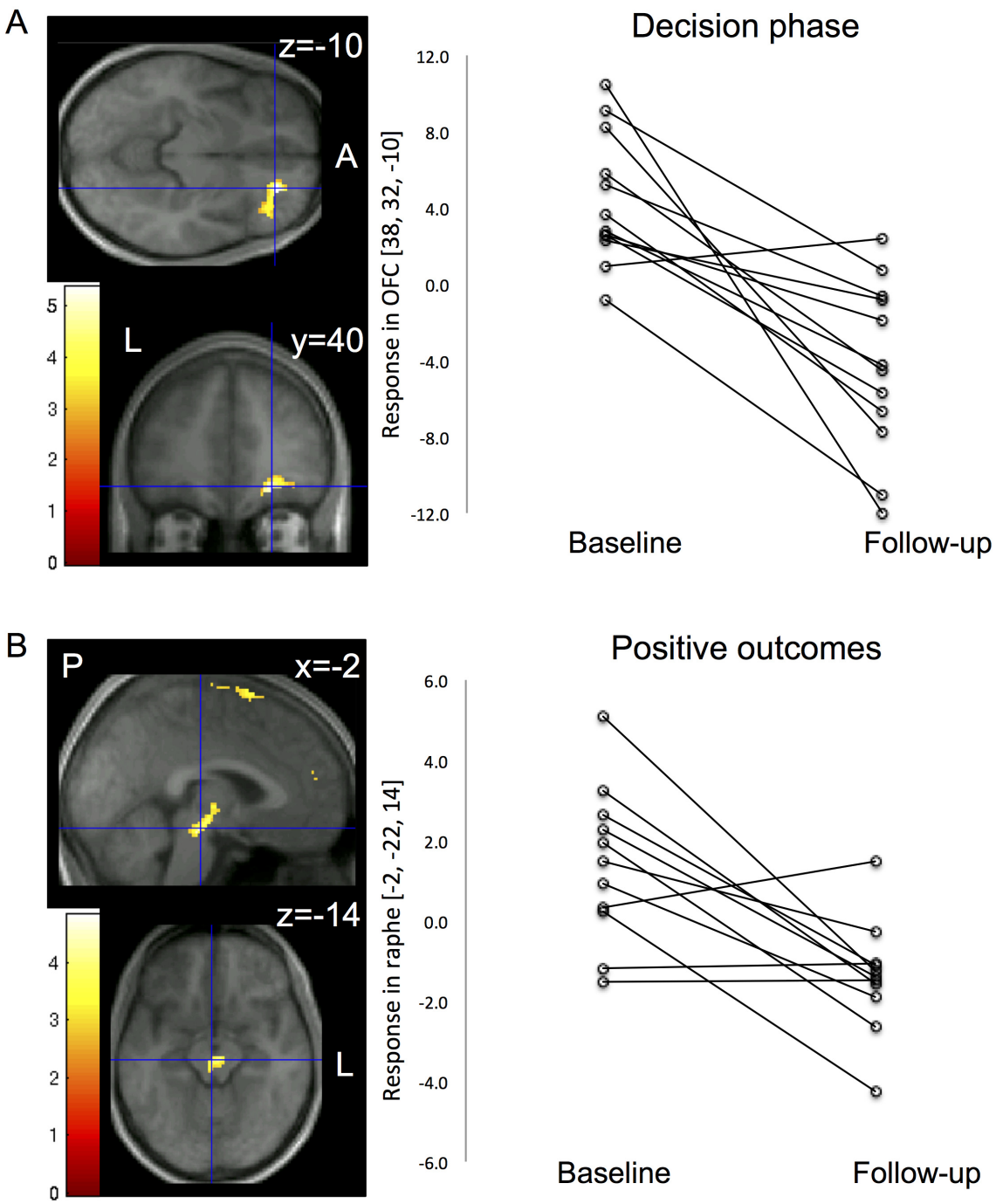


Figure 6: Brain regions showing a change in task-related activity after the SSRI intervention compared to placebo. A) Choice phase: In the right orbitofrontal cortex, the three-week SSRI intervention reduced the linear increase in activity with the risk taken during a gamble. B) Outcome phase: The three-week SSRI intervention attenuated the responsiveness of midbrain raphe nuclei to the size of positive outcomes (high reward minus low reward). 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. The parameter estimates are taken from the voxel showing a peak change at the group level. Courtesy of Julian Macoveanu, DRCMR.

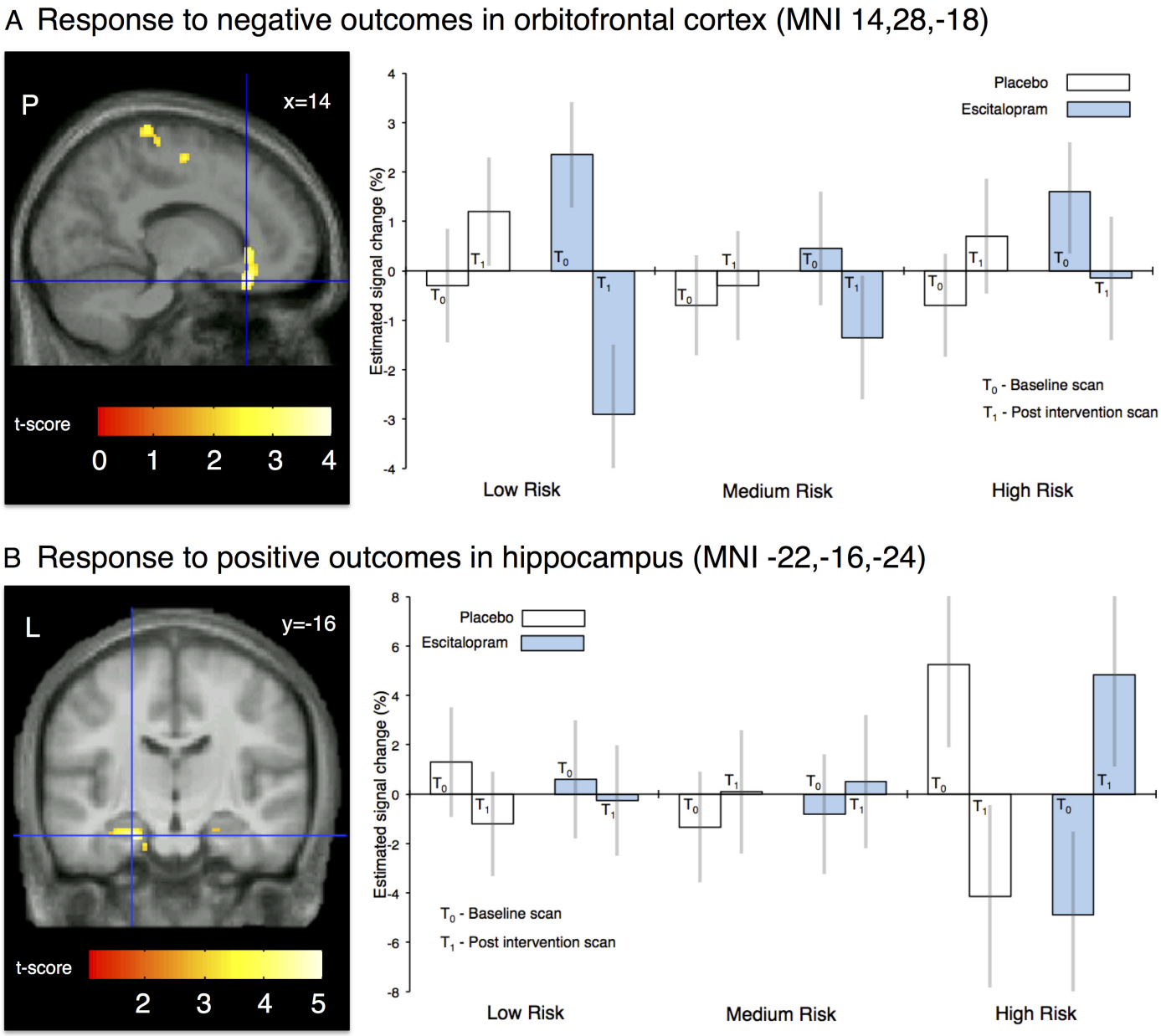
analysis. The models predicting behavioral performance are also under evaluation. In a follow up study using the same task, serotonergic manipulation is expected to affect punishment-induced learning more than learning form reward, and more so when the nature of the feedback is social.

In the Punishment or Impunity task, subjects play a game in which they decide between accepting and rejecting monetary offers of varying degrees of fairness from other persons. Rejection always leads to the subject receiving nothing rather than the proposed offer, but we manipulate whether rejection also entails punishment of the other player. By lowering central serotonergic tone with acute tryptophan depletion we will be able to understand how serotonin affects the willingness to punish, how it influences the neural response to unfairness and how it modulates the decision-making process. We hypothesize that the challenge will increase subjects' propensity to reject unfair offers relative to placebo, but only when rejection implies punishment of the proposer.

In parallel with these fMRI studies using the novel activation paradigms, the tasks validated in Cimbi-I were used in two separate studies investigating the mechanisms of repeated serotonin reuptake inhibition in healthy individuals and individuals at familial risk for depression. The participants underwent fMRI while performing a card gambling task that required them to repeatedly choose between two decks of cards associated with different risks and reward values. Both

studies employed a double-blinded parallel-group design, with participants randomly assigned to receive a daily dose of either an SSRI or a placebo. In the first study, healthy males received the SSRI fluoxetine for 3 weeks. Compared to the placebo group, these individuals 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, which is the region responsible for the bulk of serotonergic projection to cortical regions (**Figure 6**). The results suggest that SSRI treatment might reduce emotional engagement by reducing reward related activity and may therefore represent a neural correlate to the side effects observed in clinical treatment with SSRIs which report reduced affective arousal to pleasant and rewarding events. In the second study we first mapped neural changes during the same gambling task between a group of individuals with first-degree relatives diagnosed with depression and a group of healthy individuals without familial risk for affective disorders (Macoveanu [45]). The high-risk individuals demonstrated altered orbitofrontal and hippocampal response to monetary wins and losses respectively. These regions have previously been found to be affected in depression. We were further able to confirm a reversal of these aberrant activations with SSRI intervention (**Figure 7**).

Figure 7: Regions where there was a significant interaction between group (SSRI vs. placebo) and time (baseline vs. follow-up) in individuals with high risk for depression. (A) Compared to the placebo group, the escitalopram group showed decreased neuronal response to low-risk negative outcomes in orbitofrontal cortex. (B) The escitalopram group showed increased hippocampus response to high-risk positive outcomes compared to the placebo group. The estimated signal change is shown for the peak voxel and error bars represent 90% confidence interval of the mean. Figure reproduced with permission from Macoveanu [45], Copyright © Cambridge University Press 2013.



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

Positron emission tomography (PET) has unsurpassed sensitivity and specificity for measuring neurotransmitter receptors in a non-invasive way in the living human brain, and furthermore with the appropriate PET radioligand, neuroreceptor binding is inversely correlated to extracellular levels of neurotransmitters such as is validated for dopamine. This requires, however, a PET radioligand which is sensitive to changes in serotonin levels, and no such a ligand has currently been evaluated for use in humans. The process of PET radioligand development is a time and resource-demanding task that resembles that of pharmaceutical drug development in that compound may fail at any stage of the sequential development process. In Cimbi platform 1, we attempt to develop PET radioligands that enable novel and functional measures of different members of the serotonin receptor family.

The 5-HT2A receptor is the most abundant excitatory receptor in the human brain and it constitutes a prominent target for measuring serotonin levels in the human brain with PET. 5-HT2A receptor stimulation exerts the hallucinogenic effects of drugs such as LSD whilst the therapeutic effects of atypical antipsychotics are attributed to the antagonistic effects on these receptors. Compared to receptor antagonist PET tracers, receptor agonist PET tracers are hypothesized to be more sensitive to competition with endogenously released serotonin, and therefore we have through many years attempted to validate such a 5-HT2A receptor agonist radioligand for these applications. As described in last year's annual report, in 2012 we managed to take our most promising 5-HT2A receptor agonist PET radioligand, [^{11}C]Cimbi-36, all the way from medicinal chemistry through pre-clinical evaluation and to clinical studies in healthy volunteers. In 2013, we reported that radiation dosimetry in rats and pigs suggested that administration of [^{11}C]Cimbi-36 in conjunction with PET-scanning has no alarming adverse effects (Ettrup [13]). Here, we also confirmed the in vivo agonist actions of Cimbi-36 after administration in mice (**Figure 8**). Subsequent studies to test [^{11}C]Cimbi-36 in non-human primates (Finnema [15]) including studies to test sensitivity of [^{11}C]Cimbi-36 binding to increased serotonin levels were carried out in collaboration with Prof. Christer Halldin's group at Karolinska Institute in Stockholm. These studies further raised the expectations for applications in humans. The first human PET scans with [^{11}C]Cimbi-36 was conducted in October 2012, and less than 2 months later, data collection for the blocking study investigating the effect of per oral ketanserin treatment on [^{11}C]Cimbi-36 binding was completed (n=6). In 2013, a large placebo-controlled double-blinded intervention study to investigate the effect of changes in serotonin levels on cerebral [^{11}C]Cimbi-36 binding in the human brain (n=24) was initiated and also completed. Intervention to increase serotonin levels was the SSRI citalopram in combination with the 5-HT1A receptor blocker pindolol to inhibit autoreceptor-mediated attenuation of the SSRI response. Conversely, acute tryptophan depletion was applied to decrease cerebral 5-HT levels in the human brain. Data analysis and scientific communication of results from these first-in-human clinical trials are ongoing.

In parallel to our development of N-benzylated phenethylamines ([^{11}C]Cimbi-36 is an example hereof) as 5-HT2A receptor agonist PET radioligands, we have been exploring the structure-activity relationship of these compounds through a thorough medicinal chemistry approach. Syntheses of in vitro evaluation of a series of 48 compounds led to the discovery of the most selective 5-HT2A receptor agonist to date (Hansen M et al., accepted early 2014). Strategies for which compound to synthesize and evaluate were partly guided by results of computational chemistry and modelling of the ligand-receptor interaction of 5-HT2A receptor agonists (Isberg [72]).

The Cimbi platform 1 also has a strong interest for serotonin 7 (5-HT7) receptors as a target for which a PET radioligand may reflect 5-HT levels (**Figure 9**). These receptors constitute an important target since 5-HT has the highest affinity for the 5-HT7 receptors out of the 5-HT receptor family. Theoretically, this should increase sensitivity towards 5-HT for a potential radioligand, yet no 5-HT7 receptor PET radioligand is available.

Our collaboration with the medicinal chemistry group at the University of Bari Aldo Moro (Italy) led by Associate Professor Marcello Leopoldo led to the testing of several novel radioligands in the pig. Two of these radioligands unfortunately had very low brain uptake partly due to the compounds being substrates for an efflux transporter at the blood-brain barrier and therefore further work on developing a PET radioligand within this compound class was terminated (Lacivita et al., accepted early 2014). In a different compound class (biphenylpiperazine derivatives) we also tested a compound which proved to have a high brain uptake but unfortunately this compound was not selective to the 5-HT7 receptor in vivo as we could not displace the binding with the 5-HT7 receptor selective antagonist SB-269970. These results were recently accepted for publication in the European Journal of Medicinal Chemistry.

In 2013, we continued the evaluation of [^{11}C]Cimbi-717, which in pigs proved to be our top-candidate for a 5-HT7 receptor PET radioligand (Hansen [22]). Through collaboration with the A.A. Martinos Center for Biomedical Imaging (Boston, USA) this radioligand is now being evaluated in non-human primates. Although we have very good results with [^{11}C]Cimbi-717 in pigs, we continue to expand the library of potential PET radioligands by synthesizing and modifying compounds that are described in the literature to be selective for the 5-HT7 receptor and amenable for [^{11}C]- or [^{18}F]-labelling. Examples hereof are the antagonist SB-269970 and the agonist E-55888.

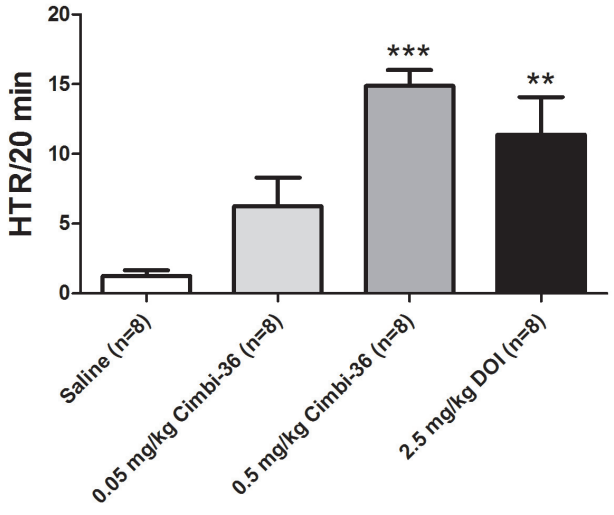


Figure 8: Cimbi-36 induces head-twitch responses (HTR) in a dose-dependent manner in mice. The HTR is a well-established and easy identifiable (shaking movement of the head, visible as rapid movement of the ears) response to hallucinogenic drugs in rodents. That a 5-HT2A receptor induces HTR strong suggests that it will be hallucinogenic in humans. Mice were injected i.p. with 0.9% saline, 2.5 mg/kg DOI (reference 5-HT2A receptor agonist), or Cimbi-36 in various concentrations, and the number of HTR was counted for 20 min. Figure from Ettrup [13], Copyright © World Molecular Imaging Society, 2013

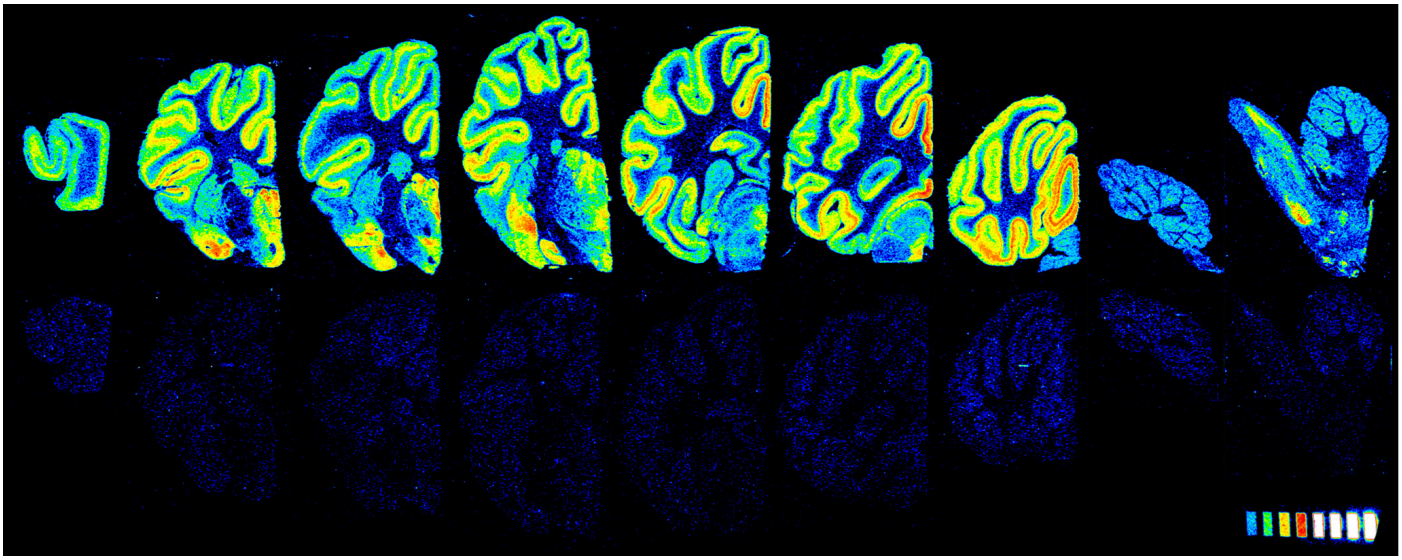


Figure 9: 5-HT7 receptor autoradiography in pig brain sections with 3H-SB269970. Coronal sections (left to right: rostral to caudal) were incubated with 5 nM 3H-SB269970 to determine total binding (upper row) and in the presence of 10 µM SB-258719 to determine non-specific binding (lower row). Figure from Hansen [22], Copyright © 2014 by the Society of Nuclear Medicine and Molecular Imaging, Inc.

Platform 2: Data Analysis

FreeSurfer surface based analysis of molecular imaging data

In most neuroimaging studies data are reported either based on a region-of-interest (ROI) analysis, which requires that a strong a priori anatomical hypothesis exists, or on a volume based SPM kind of data analysis which, because of resolution and noise issues, requires a heavy spatial filtering. As the filter for volume based analysis is applied in 3D space both GM and WM voxels are affected which further worsens the effect of the low resolution in the acquired PET images. Through collaboration with the A.A. Martinos Center for Biomedical Imaging (Boston, USA), in 2013 the NRU part of the Cimbi platform II group has developed a novel surface-based approach for analysing PET data in the software tool FreeSurfer, and this approach has been evaluated in two different studies based on Cimbi molecular imaging data. In the FreeSurfer surface-based approach, structural MR scans are first used to estimate pial and GM/WM (gray/white matter) surfaces of the brain and then PET data are co-registered to these surfaces so that finally surface-based voxelwise PET values can be extracted.

In the first study, we demonstrated how the FreeSurfer approach can be used as an exploratory surface-based analysis method for analyzing binding in cerebral cortex (Greve [20]). Sixteen dynamic 5-HT4 receptor PET scans (with the antagonist 5-HT4 receptor radioligand [^{11}C]SB207145) were included in the study and FreeSurfer results were compared to a SPM volume-based approach. It was shown how pre-processing choices (volume smoothing, cortical surface smoothing, and voxelwise partial volume correction (PVC)) affected the bias and variability of voxelwise kinetic modelling analysis of brain PET data. The multilinear reference tissue model (MRTM2) by Ichise was used to compute maps of non-displaceable binding potential (BP_{ND}) after pre-processing. The volume-based smoothing resulted in large bias and intersubject variance because it smears signal across tissue types, whereas the cortical surface-based smoothing resulted in dramatically lower bias and the least variance of the methods tested for smoothing levels 5mm and higher. When used in combination with PVC, surface-based smoothing minimized the bias without significantly increasing the variance. Surface-based smoothing resulted in 2-4 times less intersubject variance than when volume smoothing was used. Surface-based smoothing has less bias and variance because it respects cortical geometry by smoothing the PET data only along the cortical ribbon and so does not contaminate the GM signal with that of WM and cerebrospinal fluid.

As part of Mikael Agn's MSc project (DTU compute), in the second study the performance of FreeSurfer was tested at a molecular imaging dataset consisting of 122 SERT PET scans (with the radioligand [11C]DASB) from 61 healthy female subjects. Data was quantified using the MRTM2 model in a similar way as in the first study. Further, a Bayesian framework was used for building regularization into the MRTM2 model (thereby ensuring that neighbouring areas in surface space have similar outcome parameters), and this was compared to the "usual" approach where surface volumes are pre-smoothed by a Gaussian kernel before using the MRTM2 model. **Figure 10** illustrates the results for the two approaches with different levels of smoothing. It is evident that with similar filtering (one given row) the Bayesian approach seems to retain a higher resolution (visual inspection) as compared to pre-smoothing. Further studies have to be done to conclude on effects when doing e.g. GLM kind of analysis of the effect.

Latent Variable Models

In 2013, Klaus K. Holst and Esben Budtz-Jørgensen from the Biostatistics part of the Cimbi platform 2 group have continued their work on exploring the application of latent variable models on PET neuroimaging data. The principle idea is to describe regional dependence in these data by a low number of unobserved random effects, as exemplified in **Figure 11** where SERT binding potential is modelled by a single latent variable, η. In this example the latent variable may be interpreted as a common regulator of regional SERT binding potential (Holst [26]). The modelling framework provides a natural way for simultaneously describing the dependence between several different types of PET markers.

Also, work on extensions to non-linear cases has been initiated, allowing for inclusions of both binary, ordinal and time-to-event endpoints in the models. These extensions may be exploited to describe associations between neuroimaging markers and follow-up data on neuropsychiatric disease status in the CIMBI cohorts. General software implementations are published as the R-packages '*lava*' and '*mets*' (http://lava.r-forge.r-project.org/).

Multi-modal integration, networks, denoising

The DTU part of the Cimbi platform 2 group has in the past year

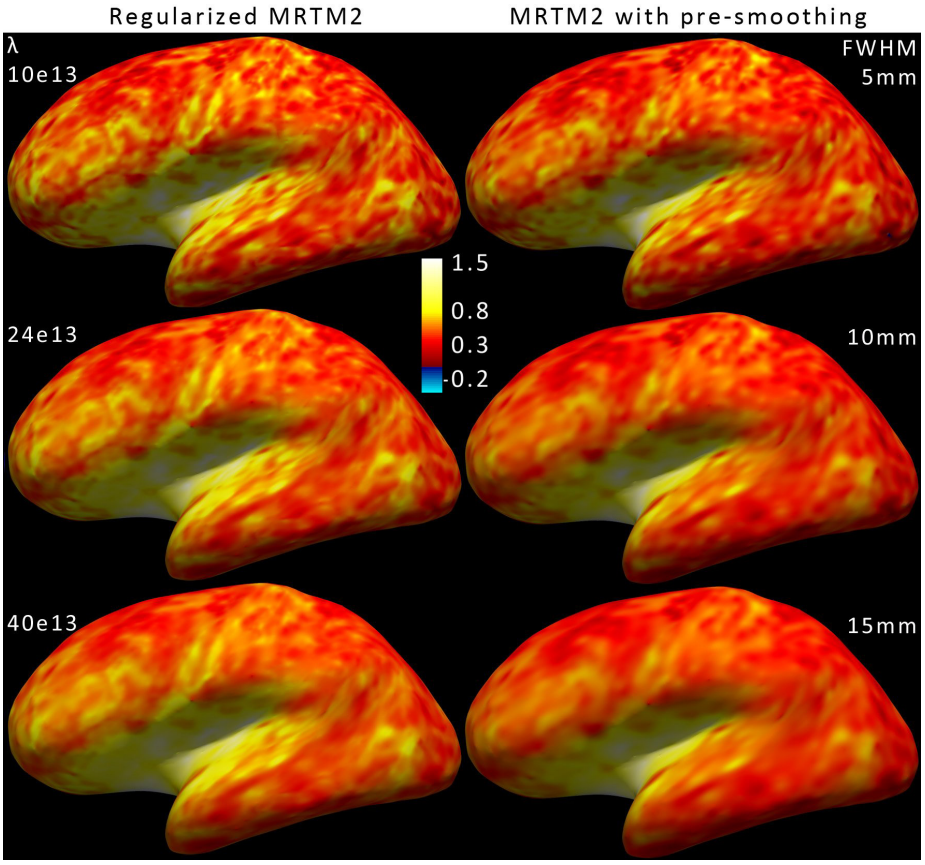


Figure 10: Comparison of FreeSurfer analysis of a SERT PET ([11C]DASB) scan from a healthy female subject based on (left panel) regularized MRTM2 and (right panel) vertex-wise MRTM2 with pre-smoothing. Each row of images corresponds to a given level of regularization (λ) and a comparable level of pre-smoothing (FWHM). Left hemisphere, lateral view. Courtesy of Mikael Agn, DTU.

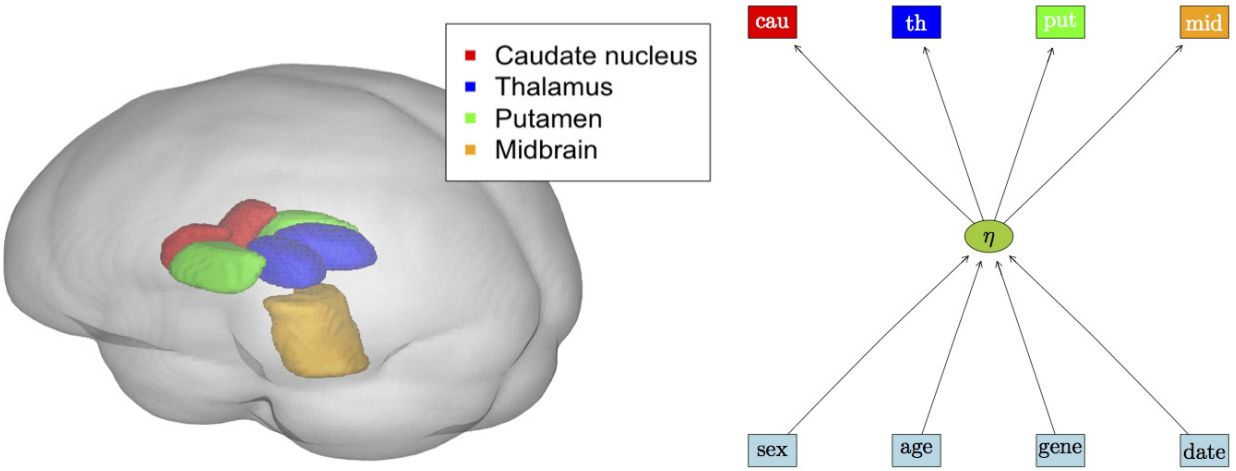


Figure 11: (Left) Brain regions of interests and (Right) latent variable model describing regional dependence in SERT binding potential. Figure from Holst [26], Copyright © Springer-Verlag 2012

also made significant scientific contribution. Trine Abrahamsen and Lars Kai Hansen have continued their work on non-linear modelling and have obtained a potentially groundbreaking result on repair of variance inflation in high-dimensional supervised learning (Abrahamsen [66]). Together with Toke Jansen Hansen, they have studied methods for non-linear de-noising in semi-supervised models, and this work has recently been submitted for publication. Also, they started work on a general manifold based model for non-linear multimodal.

Finn Årup Nielsen and Lars Kai Hansen worked on general real-time "open vocabulary" decoding of mental state (emotion, social, perceptual context) based on EEG. The hope is to provide tools for integration of brain imaging data, activation database information, and meta-data from other sources for the specific subject/context.

Real time imaging with the smartphone brain scanner

The DTU part of the Cimbi platform 2 group also continued the work on real-time brain imaging based on portable and mobile smartphone solutions for emotional, social, and mental state mapping (Stopczynski [77, 78]). An important research component is improved (precision, computational speed) methods for 3D/4D imaging for EEG (Andersen [67], Hansen [70, 71], Stahlhut [76]) and therefore the DTU group has worked on methods for real-time denoising of EEG.

Applications of real-time EEG are starting to emerge, including bio-feedback to manipulate mental state (Hansen [69], Jensen [73]). Andreas Trier Poulsen, Simon Kamronn, Ivana Konvalinka and Lars Kai Hansen undertook a large scale EEG experiment involving joint attention to video (emotion, social processing).

Last but not least, in 2013 Carsten Stahlhut from the DTU group has with Sid Kouider (Paris, DTU) published high impact work in Science on EEG imaging of conscious face processing in infants (Kouider [75]).

Cimbi Acknowledgements

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 2013 is included in the list of NRU acknowledgements on page 30, while additional funding achieved by the other Cimbi core institutions in 2013 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

Photo from the 2013 Cimbi Annual Meeting which was held in September as a 2-day retreat meeting at Comwell Borupgaard in Snekkersten.

COGNITO

COGNITO
Det Strategiske Forskningsråd

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 is supported until 2016, but it is estimated that the work will first be completed during 2017.

With regard to treatment of cognitive disorders, one of the most promising novel treatment opportunities is development of alpha7 nicotinic acetylcholine receptor (α7nAChR) modulators for treatment of schizophrenia and Alzheimer's disease. Recently, randomized clinical studies have reported that an α7nAChR partial agonist produced significant effect for certain cognitive domains, in particular some in which sufficient prefrontal function is required. The COGNITO project is currently aimed to understand the mechanisms underlying the documented effect of the α7nAChR partial agonist, and to assess whether other types of α7nAChR modulation including allosteric modulation could have better effects on these patients.

Research highlights from 2013

The novel drugs are discovered from screening on the homomeric α7nAChR consisting of 5 identical α7nAChR subunits. We have shown that the α7nAChR subunit binds to the beta2 nAChR subunit in the human brain. Interestingly, our studies in cellular systems demonstrate that by co-transfection of cDNA encoding both the beta2 and the α7 subunit, the subunits form a heteromeric receptor structure that has profound consequences for the pharmacological effects. In addition, researchers at NRU have shown that nAChR bind to proteins of the Ly6 family. This family of proteins is structurally similar to each other and to the snake venom toxin, bungarotoxin that is known to bind strongly to the α7nAChR. We have studied the distribution and developmental regulation of Lynx proteins in rat brain (Thomsen [62]), and we are now interested to find out what consequences any involvement of these proteins on α7nAChR function may have. Our data strongly suggest that the human α7nAChR is different from both the receptor on which novel drugs have been screened as well as the α7nAChR in animals. This is further emphasized by the fact that a gene, *chrfam7a,* encodes a truncated version of the α7 nAChR subunit that when integrated into α7nAChR functions as a dominant negative subunit. 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. We have studied the expression of this gene and found that it is present in the human brain. Again the possible expression of this gene is important for drug action. It seems that agonists are less effective in the presence of the truncated α7nAChR subunit, whereas the effect of allosteric modulators does not seem affected.

Another important aim is to analyze the patient population in order to identify patients with cognitive disorders that would mostly benefit from treatment with cognitive enhancers. It is generally observed that the effect of neuropsychiatric treatment is highly variable, probably due to differences in the pathophysiology of the individual patients. In order to stratify patients that would benefit from treatment with α7nAChR modulators, we have investigated the correlation between variations in the gene encoding the α7nAChR subunit, *chrna7*, in schizophrenia and Tourette/ADHD patients (Melchior [46]), respectively. Further, we were able to determine variations in the number of alleles of the gene for *chrfam7a*, and we will follow up by investigating these gene variations in the NRU biobank material from normal individuals tested for cognitive abilities. Since this gene is found only in humans, it has major implications whether this protein is present or not and how much protein is incorporated in receptors.

Finally, we continued to evaluate and optimize work on 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.

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 finding that the presence of beta2 shows great influence on the effects of substances will set new standards for the identification of new drugs. Similarly, the presence of the duplicated α7 gene *chrfam7a* in the human brain has extensive interest for the industry.

The interdisciplinary cooperation has been keen in the project in 2013 culminating with a large annual meeting, where also international researchers and companies participated (see photo below). Also, a number of important collaborations with researchers in the field have been established, including the Pasteur Institute and Barrow Neurological Institute, Phoenix, and new project initiatives will be implemented in 2014.

Photo of the participants at the 2013 COGNITO annual meeting. The meeting took place in late September at Schæffergården in Gentofte.

The SPECT Laboratory

The SPECT laboratory (SPECT-lab) of NRU is located at the Department of Neurology on the 8th floor in the main complex of Rigshospitalet. The laboratory consists of two offices, a type B approved isotope laboratory, and a scanner room equipped with a 3-headed dedicated brain SPECT camera (Philips IRIX). The laboratory is staffed by three dedicated medical technologists; Gerda Thomsen (head), Glenna Skouboe, and Svitlana Olsen. In 2013, Glenna celebrated her impressive 40 years anniversary at Rigshospitalet, 36 of these in the SPECT-lab (see photo to the right).

Photo from the official cerenomy hosted by the Capital Region of Denmark where Glenna Skouboe received the Queen's medal (Dronningens Fortjenstmedalje) for her 40 years anniversary at Rigshospitalet.

Clinical work

Each year patients are referred to the SPECT-lab for diagnostic SPECT-scanning mostly from the Department of Neurology, Rigshospitalet, but also from Dianalund, Roskilde and other hospitals in Denmark. In 2013, the SPECT-lab conducted more than 250 clinical scans.

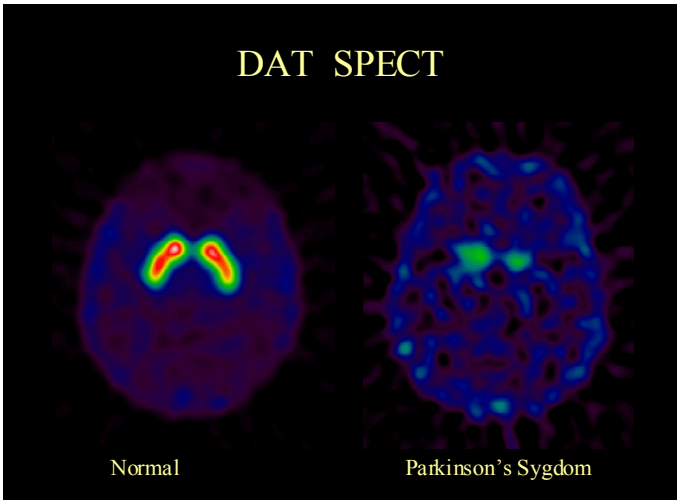


Figure 12: Dopamine transporter (DAT) binding of [123I]FP-CIT in a healthy person (left) and a patient with Parkinson's disease (right). There is a distinct loss of dopamine transporters in the patient.

Dopamine transporter (DAT) imaging

One type of the clinical SPECT scans conducted by the SPECT-lab is striatal dopamine transporter (DAT) imaging with the ligand [^{123}I]FP-CIT (**Figure 12**). 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. 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 2013, the SPECT-lab performed a total of 135 DAT investigations with [^{123}I]FP-CIT.

Blood flow imaging

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. Cerebral blood flow imaging has well-documented clinical applications particularly in dementia, cerebrovascular disease and epilepsy. At present, we mostly use the technique for brain perfusion by SPECT is the presurgical detection of the epileptic focus in patients with complex partial seizures refractory to medical treatment. In 2013, the SPECT-lab performed a total of 115 SPECT investigations with [^{99m}Tc]HMPAO.

Ictal-interictal SPECT imaging

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 magnetic resonance imaging (MRI). The technique is called SISCOM (Subtraction Ictal SPECT Coregistred to MRI) and it has proven to be a highly valuable diagnostic tool in non-invasive localization of the seizure-onset zone (**Figure 13**). The SISCOM technique also applies to investigations in children. In 2013, the SPECT-lab performed a total of 25 SISCOM-analyses.

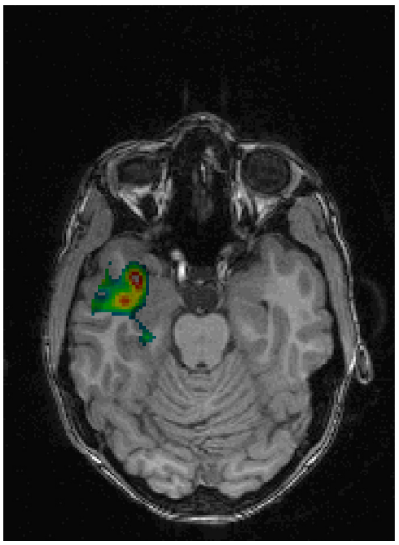


Figure 13: Example of a clearly visible active epileptic focus in the SISCOM image obtained by subtracting the ictal and interictal cerebral blood flow SPECT image, superimposed on the patient's own structural MRI.

Research in the SPECT-lab

Over the years, the SPECT-lab has consistently coupled research and development to ensure maximal clinical yield of the SPECT investigations. Previously we have, e.g., implemented a bolus-infusion protocol that allows for DAT imaging in patients with movement disorders, where the patients can stay shorter time in the scanner and still obtain a more accurate measure. We have also collected data from healthy individuals and together with EU researchers build 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. Further, we have developed and implemented software tools that allow for automatic delineation of brain regions to permit easy and reliable quantification. Finally, over a period of >5 years we have consistently examined our patients at the time of the SPECT-scanning and prospectively evaluated the clinical outcome to assess the predictive value of DAT imaging in 288 patients with subtle symptoms.

Again in 2013, the SPECT-lab has been actively engaged in several research projects and directly involved in several new peer-reviewed publications. The different research projects are described below.

Striatal DAT availability and body mass index (BMI)

The aim of this study was to test the hypothesis of lower DAT availability in obese healthy subjects using a selective DAT radiotracer in a sample of subjects with a wide range of BMI values. In a group of 33 subjects with BMI ranging from 21 to 49.5 kg/m^2, we could not demonstrate a statistically significant correlation between DAT and BMI, as measured with [^{123}I]PE2I SPECT. Thus, our study did not support that DAT is altered in obesity. Clinically, the results imply that [^{123}I]PE2I SPECT images can be interpreted without taking BMI into account (Thomsen [61]).

Striatal DAT availability and clinical severity in dementia with Lewy bodies (DLB)

In a sample of drug-naïve DLB patients (n=51), we demonstrated that striatal dopaminergic function, as assessed with DAT SPECT imaging and the highly DAT-selective radioligand [^{123}I]PE2I, did not correlate with MMSE score or with the clinical core features of DLB (parkinsonism, hallucinations, and fluctuations) (Ziebell [65]).

Striatal DAT availability and smoking

In 2013, we used data from the ENC-DAT database to show that there is no statistical significant difference in striatal DAT availability between active smokers, ex-smokers and non-smokers, as measures with [123I]FP-CIT SPECT (Thomsen [60]).

Possible new diagnostic marker of neuroinflammation

As part of the EU-funded INMiND project (described later), we have implemented [^{123}I]-CLINDE for human use. TSPO is a marker of neuroinflammation associated with microglial activation. In 2013, we have investigated regional SPECT [^{123}I]-CLINDE binding (**Figure 14**) in a group of patients expected to show microglial activation, and evaluated for the first time the quantification of [^{123}I]CLINDE for in vivo brain imaging in humans. The objective of this study is to demonstrate the usefulness of [^{123}I] CLINDE SPECT as a diagnostic marker of neuroinflammation in selected neurodegenerative diseases.

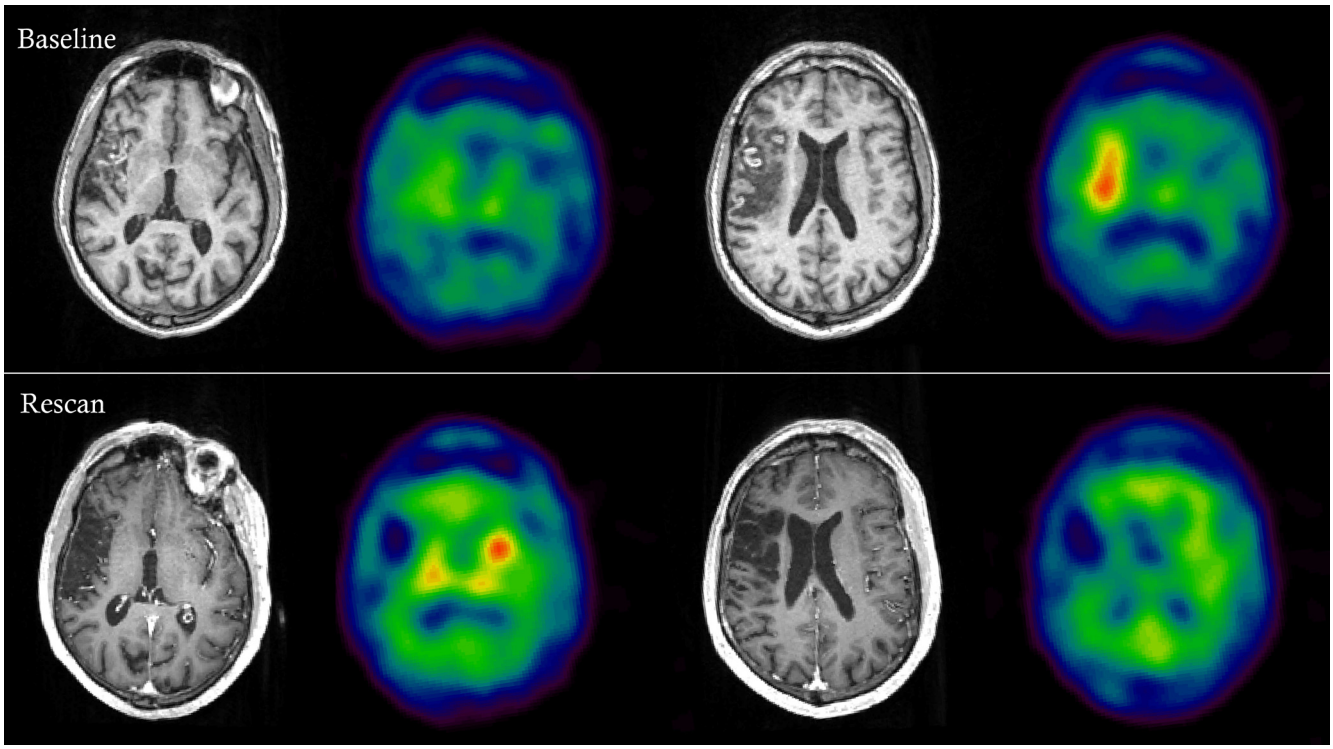


Figure 14: 65 years old male with an acute onset of severe left-side hemi-paresis. The initial scan revealed a large infarct in the supply-area of the right middle cerebral artery. The patient was scanned with (panels 2 and 4 from the left) [123I]CLINDE-SPECT and (panels 1 and 3) T1-MRI at baseline four weeks after onset (upper row) and rescanned again after another 7 months (lower row). At baseline the patient was still emitted at a hospital rehabilitation unit and presented clinically with fine motor disability of the left upper extremity. The baseline SPECT scan displays an increased binding of CLINDE to TSPO relating to the peri-infarct area. At rescan, clinically, the patient was well recovered and had only very discrete fine motor impairment of the left hand despite of the large loss of tissue displayed on the T1-MRI. The CLINDE binding to TSPO with regards to the peri-infarct area has decreased, however, binding of CLINDE to TSPO has increased in the insular area of the contra-lesional hemisphere. Increased contra-lesional TSPO binding could represent the role of activated microglia in neuromodulation and rehabilitation. Courtesy of Per Jensen, NRU.

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 2014 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 Bruce R. Rosen, MD, PhD, and the Center has been pioneering brain imaging with magnetic resonance (MR) imaging. 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 (KFJ) Prize at Rigshospitalet, and a fruitful collaboration was established between the two sites and further strengthened at a retreat meeting in Boston in 2012. Bilateral exchange of scientists has now taken place since 2011, to conduct scientific work within:

- Combined 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

Epilepsy

2013 marks the start of several epilepsy projects initiated at NRU in close collaborations with partners at Rigshospitalet, the Epilepsy Hospital Filadelfia in Dianalund, Glostrup Hospital and the University of New Mexico. In addition, NRU has contributed to a published project from the the Epilepsy Hospital and the University of Aarhus describing diagnostic accuracy using EEG source localisaton in epilepsy surgery candidates (Beniczky [3]).

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 approximately 100 patients are evaluated each year at Rigshospitalet and the Epilepsy Hospital Filadelfia in Dianalund.

In 2013 we have started a retrospective study on the outcome of the Danish epilepsy program from 1992-2009. Funding by 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. Outcome of epilepsy surgery is both measures of seizure freedom and quality of life.

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 extracranial EEG, MEG, MRI including fMRI, molecular imaging, and psychological and psychiatric data. The content of the database relates to the Swedish national database and the common data elements described by The National Institute of Neurological Disorders and Stroke (NINDS). NINDS is a part of the U.S. National Institute of Health (NIH). More than 25 members of the Danish Epilepsy Surgery Team organized in six working parties have contributed to the content of the database, and it will be launched in 2014. The design of the database will profit from the previous construction and implementation of the CIMBI database.

Functional imaging

In 2013 we started collaborations with Professor Carsten Thomsen from the Department of Diagnostic Radiology and with Professor Henrik Larsson from the Functional Imaging Unit at Glostrup Hospital. The aim is to implement EEG fMRI as a tool in the diagnostic work-up in epilepsy surgery candidates. 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 visited NRU for three months in 2013. Stefan Posse is a distinguished expert in MRI and will visit Copenhagen again in 2014 in order to further strengthen the collaboration between our institutions. The implementation and integration of multimodal functional imaging techniques in the diagnostic workup of epilepsy surgery candidates is of central importance 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 these years technology makes promises for us to take the natural step from thinking of focal epilepsy as disease of a single zone to a disease of epileptic networks.

Future treatment for patients with medically intractable epilepsy

In 2013 approximately 100 patients where referred to epilepsy surgery evaluation but only 35 patients were operated. It is essential to develop novel treatment alternatives to this group of medically refractory patients. In 2013 we have contributed

to projects initiated at the University of Lund (Professor Merab Kokaya) and the University of Copenhagen (Associate Professor David Voldbye) aiming at developing innovative treatment strategies for patients with medically intractable focal epilepsy. The optogenetic approach and the viral vector-induced overexpression of NPY are being explored in human tissue resected after neurosurgery and in dogs, respectively.

INMiND - Imaging of Neuroinflammation in Neurodegenerative Disease

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 May 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 making 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 in patients with neurological disease and from training activities to dissemination of knowledge.

In 2013 we focused on developing and testing a quantitative method for TSPO imaging with SPECT using the tracer [^{123}I]CLINDE. TSPO is present in increased numbers on activated microglia and used as a regional measure of neuroinflammation in the brain. Quantification of PET and SPECT data is easier if a reference region, e.g. cerebellum, devoid of specific binding exists. **Figure 15** shows TSPO binding in the cerebellum of 10 subjects categorized according to genotype. The rs6971 polymorphism determines a trimodal distribution in binding affinity of second-generation TSPO tracers. It is clearly shown that the distribution volumes (V_T) of [^{123}I]CLINDE in the cerebellum fall into three categories explained by the TSPO genotype. This indicates that specific binding in cerebellum is present, and it violates the assumption of reference tissue modelling. Hence, two-tissue compartment kinetic analysis of a 90-min dynamic scan with arterial blood sampling is recommended for the quantification of [^{123}I]CLINDE binding with SPECT.

We tested [^{123}I]CLINDE in stroke patients during recovery (in collaboraton with the Department of Neurology at Bispebjerg Hospital), in patients with glioblastoma multiforme (in collaboration with the Department of Neurosurgery at Rigshospitalet) in relation to experimental immunotherapy, and in multiple sclerosis patients (in collaboration with the Department of Neurology at Rigshospitalet). The stroke study is continuing in the PhD project of Per Jensen which has been supported by Rigshospitalet and the Danish Council for Research. **Figure 16** shows corresponding [^{123}I]CLINDE-SPECT, [^{18}F]FET-PET and MRI images in a patient with Glioblastoma Multiforme recently re-operated in the left frontal lobe. Extravasation of Gadolinium on MRI (MRI-Gd) is abundant in the area next to the surgical cavity and less noticeable in the right thalamus representing impairment of the blood-brain-barrier and likely tissue necrosis. The spatial distribution of [^{18}F]FET-PET signal changes is very similar to MRI-Gd signal changes. In contrast, [^{123}I]CLINDE-SPECT binding is mostly abundant in the brain stem and left thalamus. The patient unfortunately died shortly after, due to tumour growth in the brain stem area. TSPO-imaging may be a valuable measure of tumour growth rate.

In March 2013 NRU organised for the thirteenth time the yearly 5-days PhD course in Basic Kinetic Modeling in Molecular Imaging with seven participants from INMiND partners.

Figure 15: Mean distribution volumes (VT) of the reference region for each patient categorized by TSPO genotypes. Error bars show one standard deviation. TT: low affinity binder; AT: mixed affinity binder; and AA: high affinity binder. Courtesy of Ling Feng, NRU.

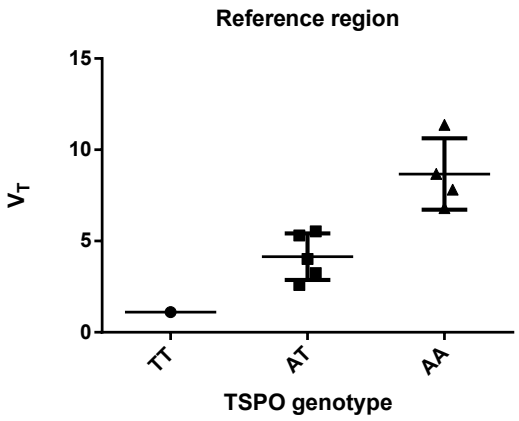
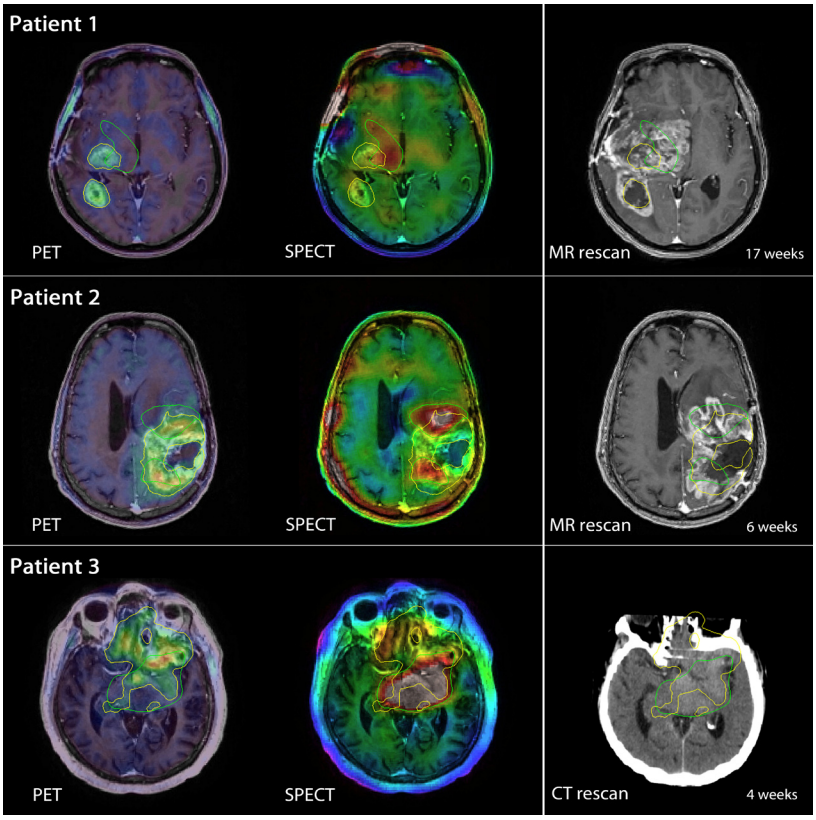


Figure 16: [18F]FET-PET (left panel) and [123I]CLINDE-SPECT (middle panel) images coregistered and overlayed to T1 post gd MRI for three diffrent patients (each row). Right panel shows structural images at follow-up. At follow-up the tumor appears to have proliferated primarily in the area of increased CLINDE binding at the baseline scan. TSPO imaging may be a marker of tumor cell proliferation and used for surgery or radiotherapy planning. Courtesy of Per Jensen, NRU.



Publications 2013

As is evident from the lists in this section, NRU has in 2013 published a total of 3 PhD dissertations, 1 course book, 8 book chapters, and 58 scientific peer-reviewed papers (1 is a collaborative multicenter study without NRU co-authorship). Another 7 NRU papers were accepted for publication in 2013 but not formally published last year.

In 2013, NRU collaborators in Cimbi published 1 book chapter and 10 papers without NRU affiliation, and they got another 3 papers without NRU affiliation accepted for publication. 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

- Mette Ewers Haahr, NRU. In Vivo PET Imaging of the Cerebral 5-HT4 Receptors in Healthy Volunteers - In Relation to Appetite, Memory and Pharmacological Intervention. University of Copenhagen, Faculty of Health Sciences. Defended Jan 11, 2013
- Martin Andreas Santini, NRU. The role of serotonin 2A receptors in prefrontal cortex function - Implications in schizophrenia. University of Copenhagen, Faculty of Health Sciences. Defended May 23, 2013
- Hanne Demant Hansen, NRU. Evaluation of PET radioligands for the cerebral 5-HT7 and 5-HT2A receptors. University of Copenhagen, Faculty of Health Sciences. Defended Oct 11, 2013

NRU Books

- Jensen CG. *Åben og Rolig*. [*Open and Calm*; a standardized 9-week course book on meditation-based stress-reduction], 120p. Copenhagen: Strandberg Publishing

NRU Book chapters

- Paulson OB, Henriksen HH, Parving H-H. Professor, Overlæge, dr.med. Niels A. Lassen – Klinisk fysiolog og hjerneforsker. In: Hansen SH, Permin H (eds.), *Bispebjerg Hospital 100 år. Mosaikker af et hospitals liv*. København, 2013:216-21
- Thage O, Paulson OB. Etablering og tidlig udvikling 1913-1975. In: Paulson OB, Thage O, Waldemar G (eds.), *Neurologi i 100 år - beretninger fra Rigshospitalets neurologiske afdeling 1913-2013*. Rigshospitalet, 2013:10-19
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NRU Papers in peer-reviewed journals

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Cimbi Papers beyond NRU

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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.

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