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Application R264-2017-2981

Name Walden Bjørn-Yoshimoto
Position Assistant Professor

Institution Bispebjerg and Frederiksberg Hospital
Institute Department of Neurology
Department Research Laboratory for Stereology and Neuroscience

Budget subtotals & total

Budget line	Year 1	Year 2	Year 3	Total
Salary - post doc	550.926,00 kr.	561.948,00 kr.	573.186,00 kr.	<u>1.686.060,00 kr.</u>
Other	10.000,00 kr.	10.000,00 kr.	10.000,00 kr.	<u>30.000,00 kr.</u>
Running costs	75.501,00 kr.	112.580,00 kr.	47.874,00 kr.	<u>235.955,00 kr.</u>
Equipment	25.000,00 kr.	0,00 kr.	0,00 kr.	<u>25.000,00 kr.</u>
Total	661.427,00 kr.	684.528,00 kr.	631.060,00 kr.	1.977.015,00 kr.

Project title

Levodopa-induced dyskinesias in Parkinson's disease

Summary of application

Parkinson's disease (PD) is a detrimental neurodegenerative disease that affects ~1% of the population above age 55. Levodopa is the preferred treatment, and while fairly effective, it comes with a serious side effect which is detrimental in its own right, namely levodopa-induced dyskinesias (LIDs). The occurrence of LIDs is higher with early onset PD, and the proportion of PD patients developing LIDs increases with time treated. After 5 years of levodopa treatment, approximately 80% of PD patients have developed LIDs.

The mechanism of LID development is not well understood, but involves changes in striatal-pallidal projections, which seem to be dependent on changes in dopamine D1 and D2 receptors on different subsets of neurons, as well as the serotonin 2A receptor. Besides dopamine innervation from the substantia nigra, these striatal-pallidal neurons are also

innervated by cortical-striatal glutamatergic projections, which can be regulated by an auto-receptor, mGluR2.

Our preliminary results show a tendency for decreased mGluR2 expression, and a tendency for increased dopamine D1 receptor expression in the striatum of PD patients.

We want to test the hypothesis that abnormal dopamine signaling to the striatum causes changes in cortical-striatal activity, which induces LIDs by dysregulation of the cortical-striatal pathway.

The approach is to investigate the expression of the glutamate, dopamine and serotonin receptors of interest. We have access to human brains from PD patients, which we will use to verify our hypothesis, while we will use rat models to dissect how and when during the disease development these changes occur. Both gene expression, epigenetic regulation and protein expression will be investigated. Finally, we will correlate the observations of receptor expression to functional observations in live rats, using the powerful and highly translational resting state functional magnetic resonance imaging (rsfMRI).

Project start date

Project end date

Personal information

First name	Walden
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Date of birth	1983-06-27
Address	Rødvig Alle 27
Postal code	2650
City	Hvidovre
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Academic degree	PhD
Position	Assistant Professor
Institution	Bispebjerg and Frederiksberg Hospital

The project**Project title**

Levodopa-induced dyskinesias in Parkinson's disease

Novelty & originality of the research

The novelty lies on the translational approach: we look in human brains to verify our hypothesis of levodopa-induced receptor changes and by using a preclinical animal model of dyskinesia we will investigate the cause-effect of these changes. Results will set the framework for a proof-of-concept fMRI approach. This will provide us with a preclinical tool with strong translational utility for future intervention studies. This will be the first animal model for LIDs in Denmark.

Relevance to health & biomedical science

An estimated 5-10,000,000 people worldwide live with Parkinson's disease (PD), ~80% of which develop LIDs after 5-10 years of L-DOPA treatment. The current project will shed light on the molecular mechanisms behind the development of this invalidating side-effect of the most effective PD treatment. At present, there is an unmet need for identifying new pharmacological targets that may prevent this progress, as well as biomarkers for LID susceptibility.

Date of PhD Degree or expected date of acquiring the PhD degree

15. august 2017

Own research results

My research has elucidated the endogenous trafficking pathway of the dopamine transporter in the first article i co-authored during my MSc. During my PhD, my work has focused on excitatory amino acid transporters (EAATs), where I have identified and characterized numerous novel tool compounds acting at the EAATs. During my PhD, I have contributing to four research articles and two review articles on the excitatory amino acid transporters, as well as an article identifying selective agonists at metabotropic glutamate receptor subtypes.

Since early 2017, my research efforts have helped elucidate selectivity properties and polypharmacology of the serotonin 2C receptor. The results of this has contributed to a study recently re-submitted to the journal Cell. This study shows two new crystal structures of the 2C receptor in complex with an agonist and an antagonist, respectively, and we provide mechanistic insights into the receptor recognition and opposing pharmacological actions of the two.

Further, I co-authored an article from my time in the private industry, improving production in yeast strains by identifying unwanted side-product production. Here I also co-authored a patent (submitted) for a method to identify compounds to target nonsense mutations, the basis of more than 1800 distinct genetic disorders.

Applicant's CV

Personal data

Walden Emil Bjørn-Yoshimoto
Sigynsgade 36, 4. TV, 2200 København N, Denmark

Education

2017-08-15 PhD in Pharmaceutical Sciences from the University of Copenhagen
2010-06-08 MSc in Biochemistry from the University of Copenhagen
2007-05-27 BSc in Biochemistry from the University of Copenhagen

Recent positions

2017– Postdoc at the University of Copenhagen, Department of Drug Design and Pharmacology.
2013–2017 PhD Student at the University of Copenhagen, Department of Drug Design and Pharmacology.
2017 Research assistant at the University of Copenhagen, Department of Drug Design and Pharmacology.
2011–2013 Research scientist (2012-2013) and research assistant (2011) at Evolva S/A

Scientific focus areas

So far my focus has mainly been on neurotransmitter transporters and receptors in relation to their basic biological functions. My hope is to expand my focus area with this postdoc project, in order to gain experience with larger biological systems. My long-term goal is to have my own research group, where we can attack problems in biology from both the whole organism and systems biology level, as well as the cell biology and molecular levels. But in order to achieve this, I need experience in the former.

International relations

2015: NIH, Bethesda, MD, USA

A 7-month scientific visit in the lab of Dr. Susan Amara, the Scientific director of the National institute of Mental Health at the NIH.

2014: Harvard Medical School, Boston, MA, USA

A 2-month scientific visit shared between the ICCB-Longwood and the lab of Professor Clifford Woolf at Boston Children's Hospital.

Supervision of students

In addition to teaching four different courses, ranging from bachelor-level to PhD courses, and ranging from lectures to laboratory exercises, I have tutored one student during her 6-month research project.

Management experience

Before my PhD, I worked at a small company (Evolva), where I managed a lab technician for two days a week, and a student helper full time on two research projects I was involved in. In addition, project management is part of what you learn during a PhD, and also when tutoring a student doing a research project (though I tried to tutor her to primarily manage her own project, keeping myself in the background, making sure it was on track).

Full publication list for applicant

Publications:

h-index: 3 (web of science), 83 citations (74 without self-citations).

2017 Lehka, B.J., Eichenberger, M., Bjørn-Yoshimoto, W.E., Vanegas, K.G., Buijs, N., Jensen, N.B., Dyekjaer, J.D., Jenssen, H., Simon, E., Naesby, M., 2017. Improving heterologous production of phenylpropanoids in *Saccharomyces cerevisiae* by tackling an unwanted side reaction of Tsc13, an endogenous double-bond reductase. *FEMS Yeast Res* 17, doi:10.1093/femsyr/fox004 (2017).

2016 Hansen, J.C., Bjørn-Yoshimoto, W.E., Bisballe, N., Nielsen, B., Jensen, A.A., Bunch, L., 2016a. beta-Sulfonamido Functionalized Aspartate Analogues as Excitatory Amino Acid Transporter Inhibitors: Distinct Subtype Selectivity Profiles Arising from Subtle Structural Differences. *J Med Chem* 59, 8771-8786.

2016 Hansen, S.W., Erichsen, M.N., Fu, B., Bjørn-Yoshimoto, W.E., Abrahamsen, B., Hansen, J.C., Jensen, A.A., Bunch, L., 2016b. Identification of a New Class of Selective Excitatory Amino Acid Transporter Subtype 1 (EAAT1) Inhibitors Followed by a Structure-Activity Relationship Study. *J Med Chem* 59, 8757-8770.

2016 Bjørn-Yoshimoto, W.E., and Underhill, S.M. (2016). The importance of the excitatory amino acid transporter 3 (EAAT3). *Neurochemistry international* 98, 4-18.

2016 Huynh, T.H., Erichsen, M.N., Tora, A.S., Goudet, C., Sagot, E., Assaf, Z., Thomsen, C., Brodbeck, R., Stensbol, T.B., Bjørn-Yoshimoto, W.E., Nielsen, B., Pin, J.P., Gefflaut, T., Bunch, L., 2016. New 4-Functionalized Glutamate Analogues Are Selective Agonists at Metabotropic Glutamate Receptor Subtype 2 or Selective Agonists at Metabotropic Glutamate Receptor Group III. *J Med Chem* 59, 914-924.

2016 Haym, I., Huynh, T.H., Hansen, S.W., Pedersen, M.H., Ruiz, J.A., Erichsen, M.N., Gynther, M., Bjørn-Yoshimoto, W.E., Abrahamsen, B., Bastlund, J.F., Bundgaard, C., Eriksen, A.L., Jensen, A.A., Bunch, L., 2016. Bioavailability Studies and in vitro Profiling of the Selective Excitatory Amino Acid Transporter Subtype 1 (EAAT1) Inhibitor UCPH-102. *ChemMedChem* 11, 403-419.

2016 Hansen, S.W., Erichsen, M.N., Huynh, T.H., Ruiz, J.A., Haym, I., Bjørn-Yoshimoto, W.E., Abrahamsen, B., Hansen, J., Storgaard, M., Eriksen, A.L., Jensen, A.A., Bunch, L., 2016c. New Insight into the Structure-Activity Relationships of the Selective Excitatory Amino Acid Transporter Subtype 1 (EAAT1) Inhibitors UCPH-101 and UCPH-102. *ChemMedChem* 11, 382-402.

2015 Jensen, A.A., Fahlke, C., Bjørn-Yoshimoto, W.E., Bunch, L., 2015. Excitatory amino acid transporters: recent insights into molecular mechanisms, novel modes of

modulation and new therapeutic possibilities. *Curr Opin Pharmacol* 20, 116-123.

2010 Eriksen, J., Bjørn-Yoshimoto, W.E., Jorgensen, T.N., Newman, A.H., Gether, U., 2010. Postendocytic sorting of constitutively internalized dopamine transporter in cell lines and dopaminergic neurons. *J Biol Chem* 285, 27289-27301.

Patent:

Submitted Heim, J., Jensen, S., Klein, J., Bjørn-Yoshimoto, W.E., 2015. Methods and materials for identifying compounds promoting translational read-through of nonsense mutations, Google Patents.

Brief description of the research environment

Members of Research Laboratory for Stereology and Neuroscience (RLfSN), Bispebjerg-Frederiksberg Hospital, are recognized as world experts in stereology. The laboratory includes a molecular neuroscience unit led by Dr. Susana Aznar. Dr. Aznar's group has extensive experience working with the 5-HT_{2A}R, and they have a proven track record working with several neurological disorders, including Parkinson's disease. They have been using human brain tissue, as well as animal models, both of which will be important for the proposed study.

This project is a collaboration between the RLfSN, the Gonz  les-Maeso lab in Virginia, USA, the Basal Ganglia Pathophysiology Laboratory at Lund University, Sweden, and Danish Research Centre for Magnetic Resonance (DRCMR) at Hvidovre Hospital. We will work closely with Ass. Professor Gonz  les-Maeso (Virginia, USA), Professor Angela Cenci (Lund, Sweden), as well as Ass. professor Tim Dyrby and Professor Hartwig Siebner (Hvidovre, Denmark) on the parts of the project where the project will improve from their expertise and facilities.

For Dr. Gonz  lez-Maeso, we will use his extensive experience with epigenetic profiling; for Dr. Angela Cenci, this will be her instrumental experience in setting up rat models; for Drs. Tim Dyrby and Hartwig Siebner, this will be the experience and facilities for doing resting state functional MRI in live rats, as well as Dr. Siebner's long experience in Parkinson's disease research.

Project description

The aim of this project is to investigate the effects of dopaminergic denervation on the presynaptic control of glutamate release in striatum and how this may contribute to the development of levodopa (L-DOPA) induced dyskinesias (LIDs). We want to dissect and establish a cause-effect pattern of dopamine depletion and L-DOPA administration on pre- and postsynaptic changes in striatum. This study will focus on metabotropic glutamate receptor 2 and 3 (mGluR2, mGluR3), dopamine D1 and D2 receptors (D₁R,

D₂R), as well as the serotonin 2A (5-HT_{2A}R) receptor.

Main objectives

1. Identify changes in expression and protein levels of the mGluR2/3 autoreceptors, as well as changes in D₁R, D₂R and 5-HT_{2A}R in striatum samples from brains from PD patients. The source of change will be investigated by looking for differences in binding and expression levels, as well as epigenetic differences in genes of interest compared to healthy controls and other non L-DOPA responsive parkinsonisms.
2. In an animal model of LID, investigate the cause-effect of the changes above. We will dissociate the effect of dopamine denervation, and the subsequent L-DOPA induced fluctuations of the pre- and postsynaptic receptors.
3. In an animal model of LID, use resting state functional magnetic resonance imaging (rsfMRI) to map whether LIDs are associated with enhanced cortical-striatal pathway activity, like seen in humans, and associate activity to the observations from study 2.

Background

Cortical-striatal pathway activity increase can trigger dyskinesia by an L-DOPA-mediated mechanism, as demonstrated by fMRI on humans ^{2,3}, and resting-state cortical-striatal connectivity predicts whether PD patients develop LIDs ⁴. This suggests a decreased inhibition of cortical-striatal glutamatergic afferents, which could be mediated by alterations in mGluR2 autoreceptors expressed on cortical-striatal projections ⁵. In a primate model of Parkinsonism, L-DOPA treatment reduced striatal binding of mGluR2/3, which correlated to increased dyskinetic scores ⁶. There is clinical support of a glutamatergic involvement in LIDs, as amantadine, a weak antagonist to NMDA receptors, has shown to have beneficial effects in the treatment of LIDs ⁷. However, amantadine causes many adverse effects, emphasizing the need for finding better targeted drugs, through a better understanding of the development of LIDs.

Studies in experimental models have associated the D₁R and D₂R to the pathophysiology of LIDs, but at which level imbalance occurs is still a matter of investigation ⁸. Increased plasma membrane expression of D₁R has been observed in animal models of PD ^{9,10}. More interestingly, there is a crosstalk between the D₁R and glutamatergic signaling that, when disrupted, may cause LIDs ¹¹. Both cortical-striatal projections as well as the D₁R are known to be important in synaptic plasticity in the striatum ¹²⁻¹⁴. Experimental studies on animals suggest that altered patterns of activity-dependent synaptic plasticity at striatal synapses is one of the main mechanisms behind LIDs ^{15,16}.

Expression levels of 5-HT_{2A}R are increased in rat models of PD, which are reversed by repeated L-DOPA injections¹⁷. Conversely, in MPTP-lesioned monkeys, 5-HT_{2A}R levels are increased by chronic L-DOPA treatment^{18,19}. Inhibition of NMDA-type glutamate receptors reverses the increased 5-HT_{2A}R binding observed, concomitantly with reducing LID. Like the mGluR2, the 5-HT_{2A}R is also expressed on cortical-striatal afferents²⁰, and a previous study shows that in a rat strain deficient in mGluR2, the *HTR2A* promoter is methylated, resulting in decreased striatal levels of 5-HT_{2A}R²¹. In MPTP-lesioned monkeys and PD patients, low doses of the 5-HT₂ receptor antagonist clozapine, or selective 5-HT_{2A}R inverse agonists alleviated dyskinesias²²⁻²⁴. Together, this suggests a complex functional interaction between serotonergic, dopaminergic and glutamatergic signaling in the striatum underlying LIDs¹⁸.

Hence, both pre- and postsynaptic maladaptation involving the mGluR2, DR1, DR2 and 5-HT_{2A}R are associated with LIDs. The exact causes and order of these adaptations, is still an open question.

Epigenetic regulation of gene expression is a long-term adaptive response. As shown in animal studies, LID is correlated with changes in the striatal methylome, including an increased methylation of the *GMR2* promoter²⁵. Epigenetic changes at *GMR2* are also induced by activation of the 5-HT_{2A}R and D₂R²⁶ indicating a regulatory role of the dopaminergic and serotonergic tonus on mGluR2 expression. Conversely, mGluR2 disruption correlates with methylation of the *HTR2A* promoter, and decreased 5-HT_{2A}R levels in striatum.

There are different levels at which mGluR2 and postsynaptic receptors are regulated, both at the transcriptional and membrane level through interaction with other receptors.

Our hypothesis (figure 1) is that the increase in cortical-striatal activity observed in relation to LIDs is due to alterations in presynaptic mGluR2 signaling resulting from dopamine denervation in the striatum and the aberrant dopamine signaling mediated by L-DOPA. This combination of pathological glutamatergic and dopaminergic signaling in the striatum of PD patients induces post-synaptic maladaptation in striatopallidal projection underlying the formation of LIDs.

Our own preliminary results show alterations in glutamate- and dopamine receptors in the striatum (figure 2). We want to further explore and dissect these tendencies in relation to LIDs.

Experimental approach

Objective 1 (figure 4A): We have access to a collection of human brains from the Bispebjerg Brain Bank (BBH-2010-06, I-Suite nr: 00971 and j.nr. 2007-58-0015) with accompanying medical records, see figure 3. We have information regarding disease duration, clinical diagnosis, pathological verification, L-DOPA exposure and additional medication for each brain. In this sample, we will follow up on observations from animal

studies, as well as our own preliminary observations about expression levels. Here, we will focus on the D1R, D2R, mGluR2/3 and 5-HT2AR. This will be done by two different experimental approaches, each one looking at different levels of regulation.

A: The relative receptor binding levels in the striatum will be assessed in the different patient groups. This will be done using radioligand binding, as previously described for mGluR2/3, D1R, D2R and 5-HT2AR, and as done in the main lab for this project ^{19,27-29}.

B: The RNA will be purified from the samples, and expression of genes of interest will be assessed using qPCR as previously described in the main lab for this project ³⁰. Changes in the methylation state of CpG islands and histones will be characterized for the GOIs. This will be in close collaboration with the group of González-Maeso, School of Medicine Virginia Commonwealth University, USA, where we will rely on their extensive expertise in epigenetic characterizations to expedite this part, which will be done essentially as previously described ^{21,31,32}.

Objective 2 (figure 4B): This objective will complement "objective 1" by allowing us to modulate the pathological state and pharmacological treatment of animals; thereby we can test at which state of PD and LID development receptor alterations occur. The post-mortem experiments in "objective 2" will be carried out in parallel with "objective 1". The iterative application of the observations from both studies will help direct the search for the targets of interest.

A: Rats (n=60) will be stereotactically lesioned with 6-OHDA or sham lesioned, and injected twice daily with either 10 mg/kg L-DOPA or saline for 21 days. This will yield four treatment groups, lesion+L-DOPA (n=20); lesion+vehicle (n=10); SHAM+L-DOPA (n=20); SHAM+vehicle (n=10). The L-DOPA treated rats will be separated into dyskinetic and non-dyskinetic groups by scoring abnormal involuntary movements (AIMs). From previous reports, we expect ~50% of animals to exhibit AIMs at this point ³³. This will be carried out in collaboration with Prof. Angela Cenci, Medicine Faculty, Lund University, Sweden. One hour after the last L-DOPA dose, rats will be sacrificed, brains excised and sliced on cryostat. In slices, binding levels for mGluR2/3, D1R, D2R and 5-HT2AR will be assessed in the different groups. This will be done using radioligand binding, similar to human samples in "objective 1A".

B: In a new group of rats (n=60) treated like above, striatum will be dissected, RNA or chromatin will be purified from the samples. Expression of genes of interest will be assessed using qPCR and epigenetic changes at the genes of interest will be investigated. Here, we will focus on the receptors for which we see a difference in expression and binding levels in "objective 2A". Samples will be analyzed for both methylation and histone acetylation analogously to human samples in "objective 1b".

Objective 3 (figure 4C): Rats (n=24) 6-OHDA-lesioned and L-DOPA treated, separated into two groups based on expression of AIMs (as described for "objective 2") will be subjected to isoflurane anesthesia and resting state functional MRI (rsfMRI) will be

measured to verify the predicted changes in activity patterns. Recently, it has been demonstrated that rsfMRI can be used in a meaningful and highly translational manner in a rat model of PD³⁴. The basic experimental setup will be as previously described³⁴, using the striatal convergence of nigrostriatal and cortical-striatal afferents as seed base, where we will use region of interest-based analysis of functional connectivity. This will be carried out in collaboration with Tim Dyrby at the Danish Research Center for Magnetic Resonance (DRCMR), Hvidovre Copenhagen University Hospital, Denmark.

Power considerations and statistical analysis

We aim for statistical power of at least 0.8. From previous gene expression and receptor binding studies we know that for the human samples, 15-20 individuals per group is sufficient for obtaining a medium effect size of at least 0.5. For the rat experiments, 10 animals per group are sufficient for obtaining a medium effect size of at least 0.5.

Training

I will be trained in methodology, as well as receive experience in training young scientists, as it is expected that 1-2 master's students will perform research under me. I will, in addition to the work performed at Bispebjerg, go to our collaborators laboratories to learn novel methodology to bring back to Denmark, as well as expand the scientific network and receive additional international experience.

Feasibility and contingency

While an ambitious project, we have an expert team of collaborators, which will greatly reduce the time spent on experimental optimization. Much of the project relies on the early findings, and since several groups have already reported interesting changes in our target receptors, we are confident that we will find interesting results in the initial experiments. The main question is not whether, but at what stage in the development of PD and LID, which is exactly what we aim to elucidate. While the three objectives are interconnected, they are not interdependent, ensuring the feasibility of the project, even if one part should fail.

Task allocation

The bulk of the project will be performed by me at the Research Laboratory for Stereology and Neuroscience (RLfSN), where they have implemented and documented experience with many of the experimental procedures^{21,29,30,35}. The epigenetic analysis will be performed by me during a short 2-month stay at the laboratory of Javier Gonzalez-Maeso. The preclinical dyskinesia model will be established at the RLfSN by me under supervision of Angela Cenci. The small animal rsfMRI and subsequent imaging analysis will be performed by me at the DRCMR, under supervision of Tim Dyrby. For timeline

and task overview see figure 4A-C.

Ethical approval

The Brain Bank is covered by BBH-2010-06, I-Suite nr: 00971 and j.nr. 2007-58-0015; the animal experimentation is similar to what has previously been granted in the group. It will be applied for shortly, and is expected to be granted.

Literature references

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32 Kurita, M. et al. Repressive Epigenetic Changes at the *mGlu2* Promoter in Frontal Cortex of 5-HT2A Knockout Mice. *Molecular Pharmacology* 83, 1166-1175, doi:10.1124/mol.112.084582 (2013).

33 Picconi, B. et al. Loss of bidirectional striatal synaptic plasticity in L-DOPA-induced dyskinesia. *Nature neuroscience* 6, 501 (2003).

34 Westphal, R. et al. Characterization of the resting-state brain network topology in the 6-hydroxydopamine rat model of Parkinson's disease. *PloS one* 12, e0172394 (2017).

35 Rydbirk, R. et al. Cytokine profiling in the prefrontal cortex of Parkinson's Disease and Multiple System Atrophy patients. *Neurobiology of Disease* 106, 269-278 (2017).

Short CV for collaborators

Ass. Prof. Tim Dyrby, Group Leader for Microstructure and Plasticity at DRCMR.

Tim Dyrbys research focuses on microstructure plasticity imaging in normal and diseased conditions. How to non-invasively map microstructural features of brain tissue i.e. to statistically model measures of axon diameter and density, as well as general anisotropic measures. To understand the architecture of the microstructural environment and its connectivity that impact microstructural imaging and modeling. Key imaging technology is diffusion MRI where we design and combine acquisitions to reflect specific microstructural features. Diffusion MRI is translational so Dr. Dyrby works with powerful preclinical MRI scanners to develop, test and validate new diffusion MRI acquisitions and apply them to human patient studies.

Positions since PhD:

2015- Associate Professor in multi-modal imaging, Image Analysis and Computer Graphics section, DTU compute

2014- Head of Preclinical research, DRCMR , Cph. Uni. Hosp.,Hvidovre, DK

2010- Senior Researcher, DRCMR, Cph. Uni. Hosp.,Hvidovre, DK

2009- Group leader of the Diffusion Imaging Group (DIG), DRCMR, Cph. Uni. Hosp.,Hvidovre, DK

2009-2010 Postdoc, DRCMR, Cph. Uni. Hosp.,Hvidovre, DK

h-index: 15 (web of science)

Ass. Prof Javier Gonzalez-Maeso, Department of Physiology and Biophysics, Virginia Commonwealth University.

Javier Gonzales-Maesos research explores the molecular mechanisms responsible for psychiatric disorders including schizophrenia, depression and alcoholism, as well as the basic signaling processes by which psychoactive drugs induce their therapeutic effects. Ongoing research projects employ the coordination of several interdisciplinary approaches ranging from computer structural modeling and biophysical methods in heterologous expression systems to mouse behavior assays and functional techniques in postmortem human brain samples.

Positions since PhD:

2016- Associate Professor (with Tenure), Department of Physiology and Biophysics. Virginia Commonwealth University.

2015-2016 Associate Professor, Department of Physiology and Biophysics. Virginia Commonwealth University.

2008-2015 Assistant Professor, Departments of Psychiatry and Neurology. Mount Sinai School of Medicine (New York).

2006-2008 Instructor, Department of Neurology. Mount Sinai School of Medicine (New

York).

2001-2005 Postdoctoral fellow, Department of Neurology. Mount Sinai School of Medicine (New York).

h-index: 26 (web of science)

Place(s) where the project will be carried out

Main laboratory - Susana Aznar lab:
Research Laboratory for Stereology and Neuroscience
Bispebjerg-Frederiksberg Hospital
Bispebjerg Bakke 23, door 11B, 2nd Floor
2400 Copenhagen NV

Epigenetics studies - Javier Gonz  les-Maeso lab:
Department of Physiology and Biophysics
Virginia Commonwealth University
School of Medicine
Molecular Medicine Research Building
Box 980551
Room 5-038
1220 East Broad Street
Richmond, Virginia 23298

Learning to establish animal model - Angela Cenci lab:
Department of
Experimental Medical Science
S  lvegatan 19,
BMC F10, Lund

Danish institution that will administer a grant

Institution: Bispebjerg Hospital
Institute: Research Laboratory for Stereology and Neuroscience
Department: Department of Neurology
Address: Bispebjerg Bakke 23, door 11B, 2nd Floor; 2400 Copenhagen NV; Denmark
Phone: 38635811

Project start

01. juni 2018

Project end

31. maj 2018

Explanation of budget

For salary, kits, reagents, MRI time, animals, and housing, I have used the exact cost as of October, 2017.

For other (conference participation, computer, etc.) I have estimated the cost.

Other applications from the research group submitted for the current application deadline?

Yes, running costs for a different project, that application is called:

New Pathways towards an effective immunotherapy for Parkinson's disease and Multiple System Atrophy

References / recommendations

Anders A. Jensen, Ph.D.
Department of Drug Design and Pharmacology
Faculty of Health and Medical Sciences
University of Copenhagen
Universitetsparken 2
DK-2100 Copenhagen Ø
Denmark
Phone: +45 23962434
Email: aaj@sund.ku.dk

It is a great pleasure for me to write this recommendation of Walden Emil Bjørn-Yoshimoto in connection with his post doc application "Levodopa-induced dyskinesias in Parkinson's disease: Deciphering the mechanism behind the underlying abnormal cortical-striatal network".

Walden performed his PhD project focused on the molecular pharmacology of excitatory amino acid transporters (EAATs) in my research group and obtained his PhD degree in August 2017. In the course of the project, Walden has been an important participant in the collaboration between my group and the medicinal chemistry group headed by Associate Professor Lennart Bunch on the development of various EAAT ligands, several of which displayed interesting selectivity profiles at the various EAAT subtypes. These projects have so far resulted in a total of five publications in international peer-reviewed scientific journals, and we are currently working on a couple of project based on novel leads for EAAT ligands identified by Walden in a screening. During a stay in the lab of Susan

Amara at the NIH (Bethesda, MD, USA), Walden worked on a study of the role of EAAT3 on the effects mediated by the psychostimulant amphetamine, and he has also studied the functional importance of a specific loop in EAAT2 for the substrate translocation of this transporter. We expect the results of both of these projects to be published in high-impact internal peer-reviewed scientific journals. Finally, Walden has co-authored two review articles on the EAATs.

In addition to his PhD project in my group, Walden has performed work on the basis of the polypharmacology of several antagonists of the serotonin 2C receptor in my lab. This work has contributed to a study that was recently re-submitted to the journal *Cell* after a positive initial review. Data which was not included in the cell paper is currently being drafted into an additional manuscript, which we expect to publish in "PLOS One" or "Neuropharmacology". I expect Walden to be first author on this paper, unless major revisions are required.

As outlined above, Walden has been very productive during his time in my group, where he has been a driven, dedicated and focused as PhD student that has worked very independently on his project. He worked with a constructive attitude solving the challenges that inevitably will arise in any research project, and he showed great fighter spirit and stamina in the efforts to solve these challenges and to get the project back on track again. Throughout his time in my group Walden displayed a high work ethic and responsibility, and he has taken great pride in producing scientific data of high quality. He also combined his practical lab work with a substantial theoretical insight and thus contributed considerably to the direction of the research in his project and to the scientific environment in my group.

In conclusion, I consider Walden a highly dedicated, skilled and promising scientist, and I am happy that he is planning to pursue a career in science. Thus, I whole-heartedly recommend him as a post doc candidate and his application for a post doc position, and I would be happy to answer any questions that this recommendation may give rise to.

Evolva S/A
Lersø Parkallé 42
2100 København Ø
Denmark
Phone +45 35 200 230
Email: info@evolva.com

It is a pleasure to write a letter of recommendation for Walden Emil Bjørn-Yoshimoto. Walden has worked as a research scientist in Evolva Biotech A/S since May 2011. He has worked under my supervision on Pharma-related drug discovery projects in the field of oncology and diabetes.

During his time, Walden has been instrumental in planning experiments for a recently initiated diabetes project. Here he has performed a variety of important cell-biological and biochemical research tasks including compound-induced differentiation of adipose-derived stem cells into white- and brown adipose tissue, biochemical characterization of the differentiation process and establishment of a real-time qPCR method to analyze gene activation in adipose cells. In addition, Walden has worked on oncology projects, where he has designed elegant luciferase reporter constructs and generated mammalian stable cell lines with functional reporter systems needed for compound screening. He has also conducted elaborate cell viability studies on cancer cell lines treated with compounds. Finally, Walden has established different yeast assay methods and performed growth-based analysis of yeast strains harboring human cancer targets. Part of this work is included in a manuscript currently in preparation.

Throughout his employment, Walden has conducted his work with an exceptional degree of enthusiasm, responsibility and maturity. He is extremely bright, independent and hard-working. He displays great skills in problem solving and has a creative approach to solve scientific problems. He thrives on challenges and has an exceptional grasp of science in general. He easily acquires new technical skills and his work is of very high standard. In addition, Walden is an extremely pleasant individual, always helpful, interacts well with others and is a pleasure to have in the laboratory.

I give Walden my highest recommendation without hesitation. I have no doubt that he will be highly successful in his further academic career.

Yours sincerely,

Sanne Jensen, Ph.D.
Senior Scientist and Project Manager

Molecular Neuropharmacology Group
Dept. Neuroscience and Pharmacology
The Panum Institute
3, Blegdamsvej
DK-2200 Copenhagen N
Denmark
Phone +45 35 32 75 48
E-mail: gether@sund.ku.dk

I am writing to express my strongest support for Walden Bjørn-Yoshimoto. Walden joined my research group first to perform his bachelor thesis work. His project was focused on

the dopamine transporter (DAT), which plays a key role in regulating dopamine signalling. Moreover, DAT is target for the action of widely abused psychotimulants such as cocaine and amphetamine. Walden's project was focused on cellular mechanisms controlling intracellular trafficking of DAT and this introduced him to several different methodologies including e.g. cloning procedures, cell culture, transfection, ELISA-based trafficking assays and confocal microscopy.

Because Walden performed very well during his bachelor project I was very glad that Walden decided to continue in the laboratory for his Master's thesis studies. He continued to focus on DAT trafficking and specifically he established a challenging quantitative internalization assay based on confocal microscopy and a double antibody labelling protocol using antibodies conjugated with different fluorophores. Using this method - as well as other techniques - he addressed to what degree constitutive internalization and postendocytoc sorting of DAT are regulated by ubiquitination. Convincingly, he demonstrated that ubiquitination is not critical for constitutive internalization but instead likely critical for internalization of DAT in response to activation of protein kinase C. His work was an important part of a paper that we published last year in J. Biol. Chem. (Walden was 2nd author).

Walden is an excellent student (among the 10% best at our university). He is dedicated, hard-working, intelligent and knowledgeable. He thinks carefully about his experiments and contributes with valuable new ideas and interpretations. He is very skilful in the lab and has a high success rate in his experiments. Moreover, he is has a very open and friendly personality.

Consequently, he is very good at interacting and collaborating with other people in the lab and always extremely helpful. Summarized I consider Walden a very talented young scientists and I can only give my very best and warmest recommendations.

Sincerely,
Ulrik Gether
M.D., D.M.Sc., Professor of Neuropharmacology

Abstract in Danish

Parkinsons syge (PD) er en sygdom, som rammer ~1% af befolkningen ældre end 55 år. Levodopa er den fortrukne behandlingsform, og er forholdsmæssig effektiv, men har betydelige bivirkninger i form af levodopa-induceret dyskinesi (LID). LID er mere almindelig ved tidlig sygdomsstart, og andelen af PD patienter som udvikler LID stiger med behandlingstiden. Efter 5 års levodopa behandling har ca. 80% af PD patienter udviklet LID.

Mekanismen bag er stadig en ukendt, selvom der er stærke indicier for at ændringer specifikke receptorer i striatum har indvirkning på LID. Dette område i striatum modtager også signaler fra cortex, og disse signaler ser ud til at være ændret i PD og LID. De bliver reguleret af en receptor, mGluR2, hvor vores præliminære data viser en tendens til nedsat udtryk i striatum af PD patienter.

Vi vil teste vores hypotese, at ændringerne i dopamin signalering som opstår i striatum af PD patienter er skyld i ændringer af aktiviteten af de glutamat neuroner fra cortex, som så igen medfører nye ændringer i striatum, som inducerer LID.

For at undersøge dette vil vi bruge menneskehjerne fra PD patienter til at undersøge vores hypotese om ændringer i disse receptorer. Desuden vil vi bruge rotte modeller, hvor vi kan manipulere sygdomsforløbet for at kunne se præcis på hvilket stadie de forskellige ændringer forekommer. Til sidst vil vi benytte en funktionel MRI af de levende rotter, til at korrelere vores observationer til ændret aktivitet i specifikke neuronbaner.

Budget

Year Type	Name	Amount of money	Details
1			
	Applied from the Lundbeck Foundation		
	Salary - post doc	550.926,00 kr.	
	Other	0,00 kr.	
	Running costs	0,00 kr.	
	Equipment	0,00 kr.	
	Running costs	3.475,00 kr.	RNeasy Lipid Tissue Mini Kit (50)
	Running costs	2.961,00 kr.	MagNA Lyser Green Beads (100)
	Running costs	5.265,00 kr.	Agilent RNA 6000 nano kit (300)
	Running costs	1.308,00 kr.	TURBO DNA-Free Kit (50)
	Running costs	18.910,00 kr.	qScript cDNA SuperMix (500)
	Running costs	0,00 kr.	
	Equipment	25.000,00 kr.	Computer and software
	Running costs	12.330,00 kr.	Purchase of rats (60)
	Running costs	31.252,00 kr.	Housing of rats (12 weeks).
	Other	10.000,00 kr.	Conference Participation
		<u>661.427,00 kr.</u>	
	Applied from others		
	Salary - post doc	0,00 kr.	
	Other	0,00 kr.	
	Running costs	0,00 kr.	
	Equipment	14.237,00 kr.	Automatic rotameter
	Running costs	0,00 kr.	
	Running costs	0,00 kr.	
	Running costs	0,00 kr.	
	Running costs	0,00 kr.	
	Running costs	0,00 kr.	
	Running costs	0,00 kr.	
	Equipment	0,00 kr.	

Year Type	Name	Amount of money	Details
1			
	Applied from others		
	Running costs	0,00 kr.	
	Running costs	0,00 kr.	
	Other	0,00 kr.	
		<u>14.237,00 kr.</u>	
	Provided by workplace		
	Salary - post doc	0,00 kr.	
	Other	37.675,00 kr.	SYBR Green qPCR Master Mix (for all qPCR), used both year 1 and year 2
	Running costs	3.424,00 kr.	L-DOPA and 6-OHDA
	Equipment	0,00 kr.	
	Running costs	0,00 kr.	
	Running costs	0,00 kr.	
	Running costs	0,00 kr.	
	Running costs	0,00 kr.	
	Running costs	0,00 kr.	
	Running costs	2.880,00 kr.	Primers (qPCR)
	Equipment	0,00 kr.	
	Running costs	0,00 kr.	
	Running costs	0,00 kr.	
	Other	0,00 kr.	
		<u>43.979,00 kr.</u>	
	Provided by others		
	Salary - post doc	0,00 kr.	
	Other	0,00 kr.	
	Running costs	0,00 kr.	
	Equipment	0,00 kr.	
	Running costs	0,00 kr.	
	Running costs	0,00 kr.	
	Running costs	0,00 kr.	
	Running costs	0,00 kr.	

Year Type	Name	Amount of money	Details
1			
	Provided by others		
	Running costs	0,00 kr.	
	Running costs	0,00 kr.	
	Equipment	0,00 kr.	
	Running costs	0,00 kr.	
	Running costs	0,00 kr.	
	Other	0,00 kr.	
		<u>0,00 kr.</u>	
2			
	Applied from the Lundbeck Foundation		
	Salary - post doc	561.948,00 kr.	
	Running costs	12.330,00 kr.	Purchase of rats (60 pieces)
	Running costs	31.252,00 kr.	Housing of rats, 12 weeks.
	Running costs	0,00 kr.	
	Running costs	4.069,00 kr.	Protease inhibitor cocktail, 5ML + Proteinase K
	Running costs	14.430,00 kr.	EZ-Magna ChIP, 22x (3 units)+ Human (Dnmt1), 250 units
	Running costs	0,00 kr.	
	Running costs	9.134,00 kr.	Antibodies epigenetics (Anti-acetyl-Histone H3 Antibody, Anti-trimethyl-Histone H3 (Lys27) Ab, Normal Rabbit IgG)
	Running costs	21.962,00 kr.	[3H]-LY341495 + glutamate
	Running costs	19.403,00 kr.	[3H]-SCH-23390 + (+)-butaclamol HCl
	Running costs	0,00 kr.	
	Running costs	0,00 kr.	
	Other	10.000,00 kr.	Conference Participation
		<u>684.528,00 kr.</u>	
	Applied from others		
	Salary - post doc	0,00 kr.	
	Running costs	0,00 kr.	
	Running costs	0,00 kr.	
	Running costs	0,00 kr.	

Year	Type	Name	Amount of money	Details
2				
		Applied from others		
		Running costs	0,00 kr.	
		Running costs	0,00 kr.	
		Running costs	0,00 kr.	
		Running costs	0,00 kr.	
		Running costs	0,00 kr.	
		Running costs	0,00 kr.	
		Running costs	0,00 kr.	
		Running costs	0,00 kr.	
		Other	0,00 kr.	
			<u>0,00 kr.</u>	
		Provided by workplace		
		Salary - post doc	0,00 kr.	
		Running costs	0,00 kr.	
		Running costs	0,00 kr.	
		Running costs	3.424,00 kr.	L-DOPA and 6-OHDA
		Running costs	0,00 kr.	
		Running costs	0,00 kr.	
		Running costs	2.880,00 kr.	Primers, epigenetics
		Running costs	0,00 kr.	
		Running costs	0,00 kr.	
		Running costs	0,00 kr.	
		Running costs	43.568,00 kr.	[3H]-MDL-100907 + ketaserin
		Running costs	30.239,00 kr.	[3H]-Raclopride + S-(-)-eticlopride HCL
		Other	0,00 kr.	
			<u>80.111,00 kr.</u>	
		Provided by others		
		Salary - post doc	0,00 kr.	
		Running costs	0,00 kr.	
		Running costs	0,00 kr.	
		Running costs	0,00 kr.	

Year Type	Name	Amount of money	Details
2			
	Provided by others		
	Running costs	0,00 kr.	
	Running costs	0,00 kr.	
	Running costs	0,00 kr.	
	Running costs	0,00 kr.	
	Running costs	0,00 kr.	
	Running costs	0,00 kr.	
	Running costs	0,00 kr.	
	Running costs	0,00 kr.	
	Other	0,00 kr.	
		<u>0,00 kr.</u>	
3			
	Applied from the Lundbeck Foundation		
	Salary - post doc	573.186,00 kr.	
	Other	10.000,00 kr.	Conference Participation
	Running costs	5.490,00 kr.	Purchase of rats (24)
	Running costs	12.384,00 kr.	Housing of rats for 12 weeks (43 per week per animal)
	Running costs	0,00 kr.	
	Running costs	30.000,00 kr.	fMRI time (pr. animal 2.500 kr)
		<u>631.060,00 kr.</u>	
	Applied from others		
	Salary - post doc	0,00 kr.	
	Other	0,00 kr.	
	Running costs	0,00 kr.	
	Running costs	0,00 kr.	
	Running costs	0,00 kr.	
	Running costs	0,00 kr.	
		<u>0,00 kr.</u>	
	Provided by workplace		
	Salary - post doc	0,00 kr.	

Year	Type	Name	Amount of money	Details
3				
		Provided by workplace		
		Other	0,00 kr.	
		Running costs	0,00 kr.	
		Running costs	0,00 kr.	
		Running costs	3.424,00 kr.	Purchase L-DOPA (25 gr) and 6-OHDA (500 mg)
		Running costs	0,00 kr.	
			<u>3.424,00 kr.</u>	
		Provided by others		
		Salary - post doc	0,00 kr.	
		Other	0,00 kr.	
		Running costs	0,00 kr.	
		Running costs	0,00 kr.	
		Running costs	0,00 kr.	
		Running costs	0,00 kr.	
			<u>0,00 kr.</u>	
			<u>1.977.015,00 kr.</u>	

Co-applicant(s) / Mentor

Name Susana Aznar
Email susana.aznar.kleijn@regionh.dk
CV

Personal data

Susana Aznar
 Work address: Bispebjerg Bakke 23, building 11B, 2nd floor, 2400 Copenhagen
 NV, Denmark

Education

1995: Ph.D in Neuroscience, Faculty of Biology, University Complutense of Madrid, Spain and Department of Anatomy, University of Odense, Denmark (European PhD program)

1991: M.Sci. in Cellular Biology, Faculty of Biology, University Complutense of Madrid, Spain

Recent positions

2012 - present: Senior Researcher/Head of Molecular Biology Section, Research Laboratory for Stereology and Neuroscience, Bispebjerg University Hospital, Denmark

2011- 2012: Fundraiser and Project Manager for large (up to 15 international partners) interdisciplinary EU biomedical research projects at the Institute for Micro- and Nanotechnology, Technical University of Denmark (*leave from research work*)

2006 - 2011: Senior Researcher at the Neurobiology Research Unit and Center for Integrated Molecular Brain Imaging (Cimbi), Copenhagen University Hospital, Copenhagen, Denmark

1998 - 2006: Postdoctoral Researcher at the Neurobiology Research Unit, Copenhagen University Hospital, Copenhagen, Denmark

1996 - 1998: Postdoctoral Researcher at the Center for Neuronal Survival, Montreal Neurological Institute, McGill University, Canada.

Scientific focus areas

In vitro and in vivo receptor research, neural pathways and structures sustaining behavior, genetics behind behavior, translational and preclinical approaches, neural plasticity,

biomarker-discovery approaches, human post-mortem studies

International relations

- Prof. Peter Gass and Ass.Prof. Barbra Vollmayr, Central Institute of Mental Health, Mannheim, Germany.
- Ass. Prof. Maribel Rios, Sackler School, Tufts University, Boston, USA.
- Prof. Pilar Flores-Cubos and Ass. Prof Margarita Moreno, Department of Psychology, University of Almeria, Spain
- Lab Director Alberto Fernandez Teruel, Department of Psychiatry and Legal Medicine, Autonomous University of Barcelona, Spain.
- Ass.Prof. Javier Gonzalez-Maeso, Department of Physiology and Biophysics, Virginia Commonwealth University School of Medicine, Richmond, Virginia, USA.
- Lab Director Jorg Tost, Centre National de Genotypage CEA, Institute de Génomique, Paris, France

Supervision

5 PhDs (+ 2 ongoing)

1 postdoc (+ 1 ongoing)

Management experience

Research group leader (2012-)

Productivity

43 publications (13 last- and 14 first-authorships). H-index 17 (Web of Science). 959 total citations, average citation pr item 21.8.

Publications

Select recent publications

Rydbirk R, Elfving B, Andersen MD, Langbøl MA, Folke J, Winge K, Pakkenberg B, Brudek T, **Aznar S**. Cytokine profiling in the prefrontal cortex of Parkinson's Disease and Multiple System Atrophy patients. *Neurobiol Dis*. 2017, 106:269-278.

Fomsgaard L, Moreno JL, de la Fuente Revenga M, Brudek T, Adamsen D, Rio-Alamos C, Saunders J, Klein AB, Oliveras I, Cañete T, Blazquez G, Tobeña A, Fernandez-Teruel A, Gonzalez-Maeso J, **Aznar S**. Differences in 5-HT2A and mGlu2 Receptor Expression Levels and Repressive Epigenetic Modifications at the 5-HT2A Promoter Region in the Roman Low- (RLA-I) and High- (RHA-I) Avoidance Rat Strains. *Mol Neurobiol*. 2017, doi: 10.1007/s12035-017-0457-y.

Hervig ME, Jensen NCH, Rasmussen NB, Rydbirk R, Olesen MV, Hay-Schmidt A, Pakkenberg B, Aznar S. Involvement of serotonin 2A receptor activation in modulating medial prefrontal cortex and amygdala neuronal activation during novelty-exposure. Behav Brain Res. 2017, 326:1-12.

Rydbirk R, Folke J, Winge K, Aznar S, Pakkenberg B, Brudek T. Assessment of brain reference genes for RT-qPCR studies in neurodegenerative diseases. Sci Rep. 2016,doi: 10.1038/srep37116.

Rasmussen NB, Olesen MV, Brudek T, Plenge P, Klein AB, Westin JE, Fog K, Wörtwein G, Aznar S. 5-HT_{2A} Receptor Binding in the Frontal Cortex of Parkinson's Disease Patients and Alpha-Synuclein Overexpressing Mice: A Postmortem Study. Parkinsons Dis. 2016, 2016:3682936. doi: 10.1155/2016/3682936.

Aznar S, Hervig Mel-S. The 5-HT_{2A} serotonin receptor in executive function: Implications for neuropsychiatric and neurodegenerative diseases. Neurosci Biobehav Rev. 2016, 64:63-82.

Klein AB, Ultved L, Adamsen D, Santini MA, Tobeña A, Fernandez-Teruel A, Flores P, Moreno M, Cardona D, Knudsen GM, Aznar S, Mikkelsen JD. 5-HT_{2A} and mGlu₂ receptor binding levels are related to differences in impulsive behavior in the Roman Low- (RLA) and High- (RHA) avoidance rat strains. Neuroscience. 2014, 263:36-45.

Aznar S, Klein AB. Regulating prefrontal cortex activation: an emerging role for the 5-HT_{2A} serotonin receptor in the modulation of emotion-based actions? Mol Neurobiol. 2013, 48(3):841-53.

Ziebell M, Khalid U, Klein AB, Aznar S, Thomsen G, Jensen P, Knudsen GM. Striatal dopamine transporter binding correlates with serum BDNF levels in patients with striatal dopaminergic neurodegeneration. Neurobiol Aging. 2012, 33(2):428.e1-5.

Name Hartwig Siebner
Email h.siebner@drcmr.dk
CV

Personal data

Hartwig Roman Siebner
 Work address: Kettegård Allé 30, 2650 Hvidovre, Denmark

Education

20.12.1991 Dissertation at Saarland University, Homburg (MD degree)
 08.07.2003 Venia Legendi - Privatdozent in Neurology, CAU, Kiel (D.M.Sc. - Doctor Medicinae)

Current positions

2013- Head of Movement Disorders Research Group, Dept. of Neurology, Copenhagen University Hospital Bispebjerg
 2011- Head of Research, FBE (includes DRCMR), HvH
 2009- Full professor for “Functional brain imaging“, Institute for Clinical Medicine, Faculty of Health & Medical Sciences, University of Copenhagen, Denmark

Academic Awards

Oppenheim Prize, German Dystonia Research Foundation (2003)
 Richard Jung Prize, German Society of Neurophysiology (2009)
 Global Excellence Award in Health Award (2014), Capital Region of Denmark

Scientific focus areas

Multimodal mapping of brain connectivity and plasticity
 Brain imaging of neurological and psychiatric diseases
 Non-invasive transcranial brain stimulation

International relations

Multiple ongoing collaborations and exchange with research groups in Berlin, Brisbane, Cambridge, Grenoble, Harvard, London, Messina, Munich, Naples, Tokyo

Supervision

38 Ph.D. students
 18 Post-doc students

Management experience

Research group leader (2002-)
 Leader of a large research centre (2011-)

Select current positions of trust

Lundbeck Foundation Center for Clinical Intervention and Neuropsychiatric Schizophrenia Research (CINS), Steering group member (2012-)
 National Danish 7T MRI project, Steering group member (2011-); Vice-chairperson, (2016-)
 Danish Society for Medical Magnetic Resonance, Steering group member (DSMMR) (2013-); Chairperson (2017-)

Danish Parkinson Association, Scientific Advisory Board member (2014-); Vice-chairperson (2016-)

The National Swedish 7T MRI project, Steering group member (2016-)

Centre for Brain, Behaviour, and Metabolism (CBBM), University of Lübeck, Germany, Scientific Advisory Board member (2016-)

National 7T MR network UK, Scientific Advisory Board member (2016-)

The Nordic Chapter of the International Society for Magnetic Resonance in Medicine (ISMRM), Steering group member (2017-)

Productivity

312 invited lectures & chairs; Organized 45 symposia, courses or workshops

400 peer-reviewed publications: 305 original papers, 47 reviews, 10 editorials, 36 book chapters, 2 proceedings; WEB of Science, 05.10.2017: H-Index: 60, Total citations: 14360 (no self-citations)

Publications

Select recent publications

1. Herz DM, Haagensen BN, Christensen MS, Madsen KH, Rowe JB, Løkkegaard A, Siebner HR (2015) Abnormal dopaminergic modulation of striato-cortical networks underlies levodopa-induced dyskinesias in humans. *Brain* **138**:1658-1666.
2. Løkkegaard A, Herz DM, Haagensen BN, Lorentzen AK, Eickhoff SB, Siebner HR (2016) Altered sensorimotor activation patterns in idiopathic dystonia - an activation likelihood estimation meta-analysis of functional brain imaging studies. *Hum Brain Mapp* **37**:547-557.
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6. Meder D, Haagensen BN, Hulme H, Morville T, Gelskov S, Herz DM, Diomsina B, Christensen MS, Madsen KH, Siebner HR (2016) Tuning the brake while raising the stake: Network dynamics during sequential decision-making. *J Neurosci* **36**:5417-5426.
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Name Angela Cenci Nilsson
Email angela.cenci_nilsson@med.lu.se
CV

Personal data

Angela Cenci Nilsson (author name: Maria Angela Cenci)
Work address: Basal Ganglia Pathophysiology Unit, BMC F11, 221 84 Lund, Sweden

Education

1994 Swedish clinical licenses ('legitimation'), medical doctor and neurologist specialist
1993 PhD degree in Neurobiology, Lund University
1990 Degree as Neurology Specialist (summa cum laude), University of Verona, Italy
1986 Medical Degree (summa cum laude), University of Verona, Italy

Recent positions

2008- Professor of Experimental Medical Research, Lund University (100% research)
2002-2008 Lektor (tenured lectureship), Dept. of Physiological Sciences, Lund University

Academic awards

2017 Elected as a member of AcademiaNet - Expert Database for Outstanding Female Academics (Robert Bosch Foundation in cooperation with Nature Publishing Group)
2017 Elected to the Council of the International Basal Ganglia Society (IBAGS)
2014 Elected as a member of the Lund Academy for Natural Sciences, Medicine and Technology ('Kungliga Fysiografiska Sällskapet i Lund')
2006 Medal of Honors for Parkinson's Research (Swedish Parkinson's Foundation)
2003 Erik K. Fernström Award for Young and Promising Investigators (Fernström Foundation, Sweden)
1987 Fellowship for International Research Training, Fidia Pharmaceuticals (Italy)

Scientific focus areas

Basal ganglia disorders, focusing on Parkinson's disease and levodopa-induced dyskinesias.

International relations

Ongoing collaborations with D James Surmeier, Northwestern University, Chicago IL (USA), Rui Costa, Champalimaud Foundation, Lisbon (Portugal), and David Sulzer, Columbia University, New York NY (USA)

Supervision

11 PhD students
22 postdocs

Management experience

2002- Group leader

Select current positions of trust

2017- Swedish National Microscopy Infrastructure, Steering Committee
2015- Swedish Brain Foundation, Scientific Committee
2014- SciLifeLab Bioimaging Platform (Stockholm), Steering Board
2014- Elected as a member of the FENS Committee for Higher Education and Training (CHET)
2014- External Scientific Advisory Board for “Cell Signaling in Chronic CNS Disorders” (Austria)
2014- World Parkinson Congress, Steering Committee
2013- International Parkinson and Movement Disorder Society, Chair
2012- International Brain Research Organization (IBRO), Paneuropean Committee
2010- M. J. Fox Foundation for Parkinson’s Research, Scientific Advisory Board
2003- Swedish Movement Disorder Society (SWEMODIS), Steering Board and founding member

Publications

Select recent publicaitons (out of 129 publications total with 7998 citations; H-index 52 as "Cenci, Maria Angela")

Alcacer C, Andreoli L, Sebastianutto I, Jakobsson J, Fieblinger T, and **Cenci MA** (2017) Chemogenetic stimulation of striatal projection neurons modulates responses to Parkinson’s therapy. *J Clin Investigation* 127:720-734

Shen W, Plotkin JL, Francardo V, Ko WK, Xie Z, Li Q, Fieblinger T, Wess J, Neubig RR, Lindsley CW, Conn PJ, Greengard P, Bezard E, **Cenci MA**, Surmeier DJ (2015) M4 Muscarinic Receptor Signaling Ameliorates Striatal Plasticity Deficits in Models of L-DOPA-Induced Dyskinesia. *Neuron* 88:762-73.

Francardo V, Bez F, Wieloch T, Nissbrandt H, Ruscher K, and **Cenci MA** (2014) Pharmacological stimulation of sigma-1 receptors has neurorestorative effects in experimental

parkinsonism. *Brain* 137:1998-2014.

Fieblinger T, Sebastianutto I, Alcacer C, Bimpisidis Z, Maslava N, Sandberg S, Engblom D, and **Cenci MA** (2014) Mechanisms of dopamine D1 receptor-mediated ERK1/2 activation in the parkinsonian striatum and their modulation by metabotropic glutamate receptor type 5. *J Neurosci.* 34:4728-40.

Fieblinger T, Graves SM, Sebel LE, Alcacer C, Plotkin JL, Gertler TS, Chan CS, Heiman M, Greengard P, **Cenci MA**, Surmeier DJ (2014) Cell type-specific plasticity of striatal projection neurons in parkinsonism and L-DOPA-induced dyskinesia. *Nature Commun.* 5(5316).

Ohlin KE, Sebastianutto I, Adkins CE, Lundblad C, Lockman PR, **Cenci MA** (2012) Impact of L-DOPA treatment on regional cerebral blood flow and metabolism in the basal ganglia in a rat model of Parkinson's disease. *Neuroimage* 61:228-239.

Ohlin KE, Francardo V, Lindgren HS, Sullivan SE, O'Sullivan SS, Luksik AS, Vassoler FM, Lees AJ, Konradi C, and **Cenci MA** (2011) Vascular endothelial growth factor is upregulated by L-dopa in the parkinsonian brain: implications for the development of dyskinesia. *Brain* 134:2339-57.

Rylander D, Parent M, O'Sullivan SS, Dovero S, Lees AJ, Bezard E, Descarries L, and **Cenci MA** (2010) Maladaptive plasticity of serotonin axon terminals in levodopa-induced dyskinesia. *Ann Neurol.* 68:619-28.

Fasano S, Bezard E, D'Antoni A, Francardo V, Indrigo M, Qin L, Dovero S, Cerovic M, **Cenci MA***, and Brambilla R* (2010) Inhibition of Ras-guanine nucleotide-releasing factor 1 (Ras-GRF1) signaling in the striatum reverts motor symptoms associated with L-dopa-induced dyskinesia. *Proc Natl Acad Sci U S A.* 107:21824-9. *shared senior authorship.

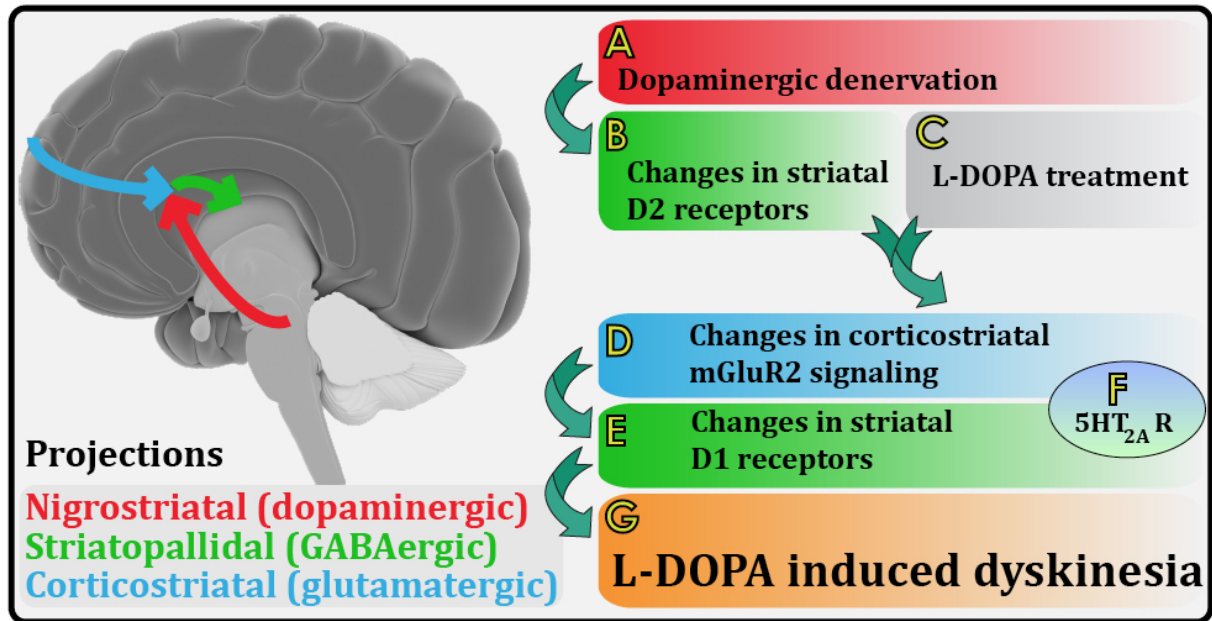
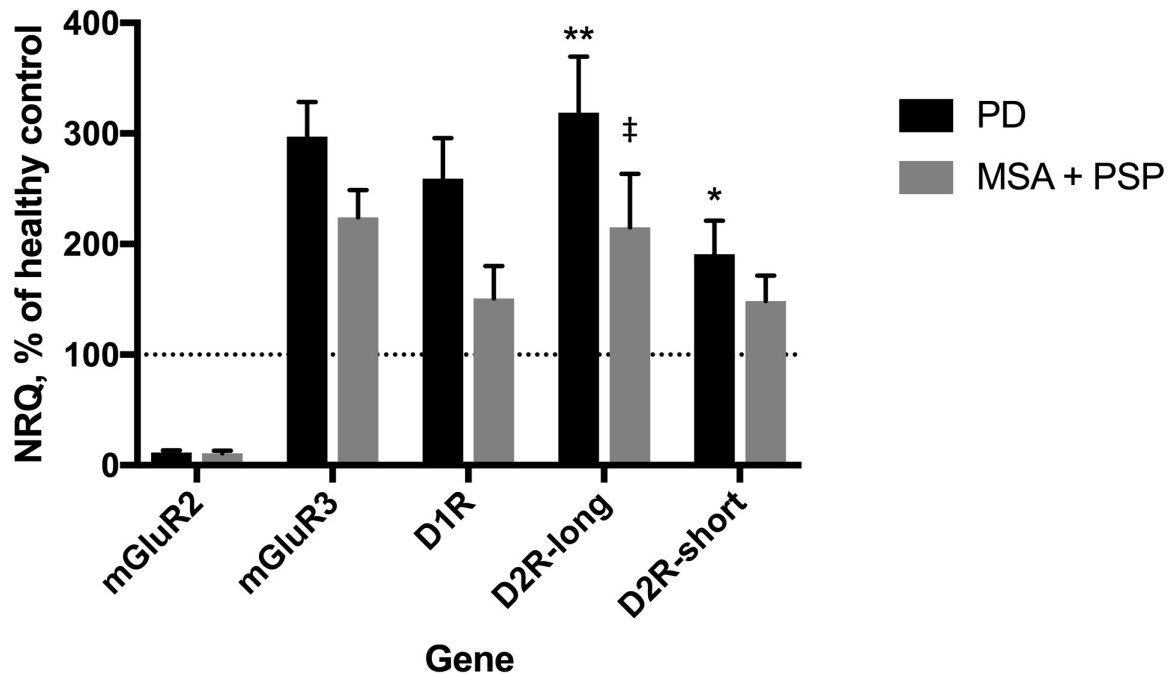


Figure 1: Working hypothesis:

Schematic overview of our working hypothesis. Left: Cross section of the human brain with the dopaminergic nigrostriatal pathway (red), GABAergic striatal-pallidal pathway (green) and glutamatergic cortical-striatal pathway (blue) indicated. (A) Nigro-striatal dopaminergic denervation causes changes in striatal dopamine D2 receptors (B). L-DOPA treatment (C), possibly exacerbated by the alterations in D2 receptors, leads to changes in presynaptic cortical-striatal mGluR2 signaling (D), thereby changing the activity of the cortical-striatal circuits. This in turn leads to maladaptations in striatal dopamine D1 receptors (E). Concomitant with either (D) or (E), serotonin 2A receptor changes occur (F). These mechanisms converge, leading to dyskinesias (G), by altering the relative activity of different striatal-pallidal projections.



n=1-7 (Healthy controls)

n=12-15 (PD)

n=11-20 (MSA-PSP)

* = $p < 0.05$ vs healthy control

** = $p < 0.01$ vs healthy control

‡ = $p < 0.05$ vs PD patients

Figure 2: Preliminary results:

Striatal mRNA expression of metabotropic glutamate receptors mGluR2 and mGluR3, dopamine D1 receptor (D1R), the long post-synaptic and short presynaptic dopamine D2 receptors (D2R-long and D2R-short, respectively) given as normalized relative quantity (NRQ) percentage of healthy controls. We see a tendency for decreased mGluR2 expression and increased mGluR3 expression in both Parkinson's disease (PD) patients, as well as patients suffering from multiple system atrophy (MSA) and progressive supranuclear palsy (PSP). Since MSA and PSP are not responsive to L-DOPA, these latter patients were used as a control for parkinsonian patients with no L-DOPA treatment. Likewise, there was a tendency for increased D1R expression in PD patients. The post-synaptic D2R-long expression was increased in both groups, though more so in the PD group, while the presynaptic D2R-short was only significantly increased in the PD group. We want to further characterize the alterations in these receptors, and the causal effect this may have on dopaminergic signaling in the striatum, particularly in respect to L-DOPA induced dyskinesia (LID). Each patient group consisted of 7-20 individuals, and experiments were done twice in duplicate. Statistical analysis was done using a two-way ANOVA with a Tukey post-test. Error bars represent SEM.

Patient group	n	Age (years)	Sex (M/F)	PMI (hours)
PD	15	76.8±9.3	13/2	36.04±22.12
MSA	19	64.9±6.5	6/13	44.2±22.8
PSP	16	73.5±8.2	12/4	31.25±17.09
NC	17	76.1±10.7	7/10	21.8±22.8

Figure 3: Patient data:

The brains currently available in the Bispebjerg Brain Bank for the project. The Brain bank is continuously being increased with new donations, thus the numbers may increase before or during the project. PD: Parkinson's disease; MSA: multiple system atrophy; PSP: progressive supranuclear palsy; NC: normal (healthy) control.

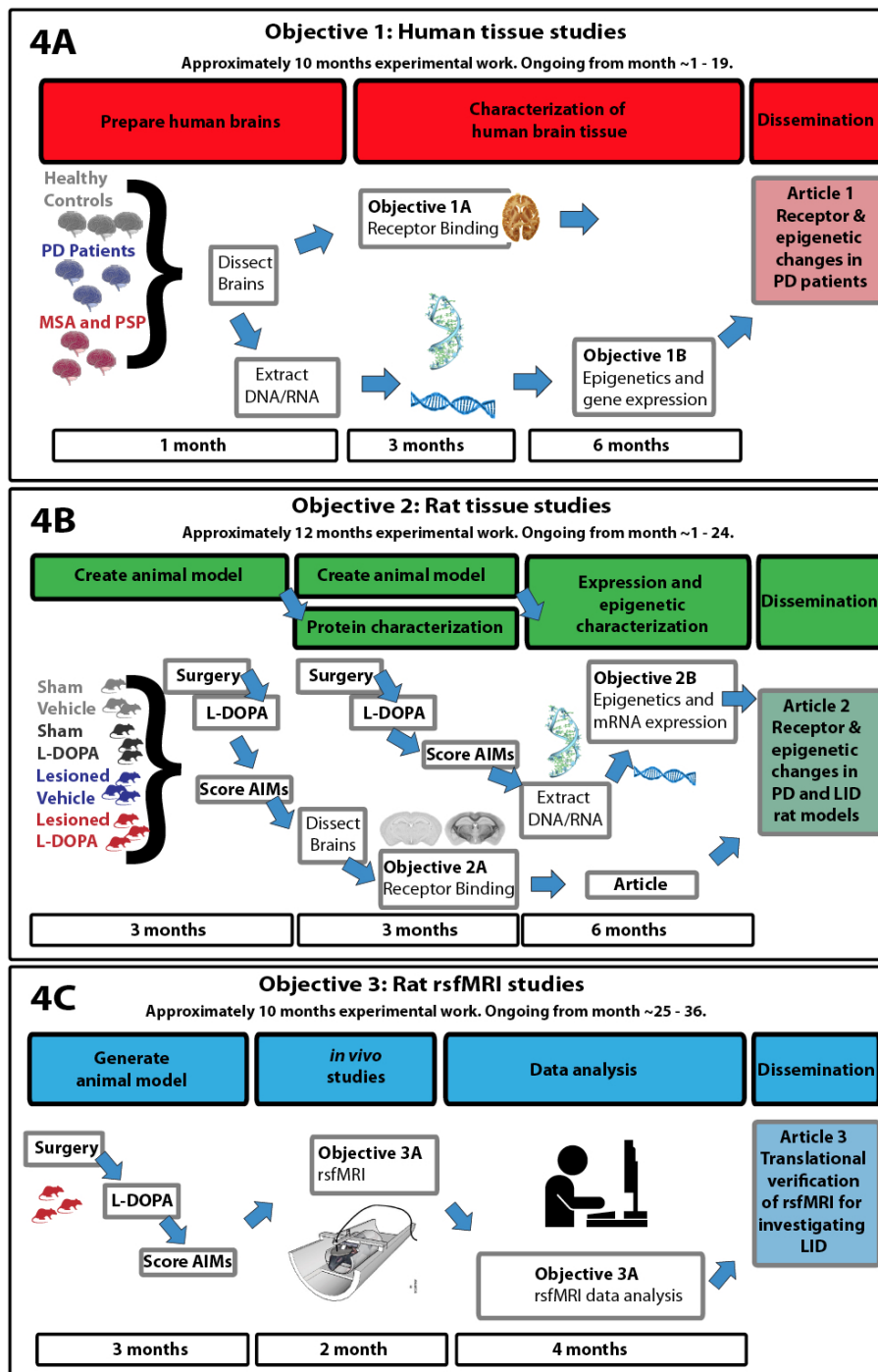


Figure 4: Objectives:

4A - Timeline and overview of objective 1. Human samples will be prepared for autoradiography studies to assess the expression and surface accessibility of functional receptors in the striatum. Parts of the striatal and cortical material will be used for mRNA expression and for epigenetic characterization of the receptors. Focus will be on the glutamate receptors, mGluR2 and mGluR3, the serotonin 2A receptor, as well as the dopamine D1 and D2 receptors. 4B - Timeline and overview of objective 2. Rat models of Parkinson's disease (6-hydroxy-dopamine lesioned, n=30), as well as control mice (sham-lesioned, n=30) will be subjected to levodopa (L-DOPA) or vehicle. The abnormal involuntary movements (AIMs) will be scored as a measurement of the degree of L-DOPA-induced dyskinesia (LID) symptoms induced. The rats will then be sacrificed, and receptor binding in the different groups will be assessed in a similar manner as described in objective 1. A new group of animals will be subjected to the same treatment (n = 30 + 30), and here the brain material will be used to assess the differences in mRNA levels and in epigenetic modifications in the different groups. Due to the size of the striatal sub-region to be characterized, the autoradiography and mRNA/epigenetic studies require two populations of animals. Due to the workload in characterizing the AIMs, as well as further post-mortem characterization, we have chosen not to make both groups simultaneously. 4C - Timeline and overview of objective 3. Rats subjected to the 6-hydroxydopamine lesions and levodopa (L-DOPA, n=24) treatment as described for objective 2 will be prepared, and abnormal involuntary movements will be scored. These animals will then be subjected to resting state functional magnetic resonance imaging (rsfMRI) to assess the functional implications of the development of levodopa-induced dyskinesia (LID) like symptoms on the cortical-striatal and striatal-pallidal projections.



LUND
UNIVERSITY

Department of Experimental Medical Science
Prof Angela Cenci Nilsson

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Lund, 14 November 2017

To Lundbeck Foundation Scientific committee

Letter of collaboration

With this letter I would like to confirm my support to the project entitled *"Decoding the molecular mechanism behind the underlying abnormal cortical-striatal network in levodopa-induced dyskinesias (LIDs)"*. I will contribute to the project with scientific and methodological guidance during the establishment and validation of a preclinical animal model for LID at the Research Laboratory for Stereology and Neuroscience, Bispebjerg-Frederiksberg Hospital, in collaboration with the Danish Research Center for Magnetic Resonance at Hvidovre Hospital.

The postdoc applicant, Walden Bjørn-Yoshimoto, will be introduced to the model during a short stay at my laboratory. I can see many future perspectives in this collaborative project as the validation of this preclinical LID model by fMRI will provide us with a strong translational tool for future intervention studies. Co-applicant, Professor Hartwig Siebner and I, are already successfully collaborating within the context of european consortia applications.

Sincerely,



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Figure 5: Letter of support, Angela Cenci:
Letter of support from Professor Angela Cenci.