

# Annual Report 2015







CENTER OF NEUROLOGY TÜBINGEN

# Annual Report 2015

## DIRECTORS

Prof. Dr. Thomas Gasser  
Prof. Dr. Mathias Jucker  
Prof. Dr. Holger Lerche  
Prof. Dr. Peter Thier  
Prof. Dr. Ulf Ziemann

EBERHARD KARLS  
UNIVERSITÄT  
TÜBINGEN



Hertie-Institut  
für klinische Hirnforschung



UNIVERSITÄTS  
KLINIKUM  
TÜBINGEN

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A photograph of a man in a white lab coat and glasses, looking intently at a test tube held by a gloved hand. The test tube contains a yellow liquid. The background is a blurred laboratory setting.

# The Center of Neurology



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## The Center of Neurology in 2015

**The Center for Neurology at the University of Tübingen was founded in 2001. It unites the Hertie Institute for Clinical Brain Research (HIH) and the University Hospital's Clinical Neurology Department. In research, teaching and patient care the center is dedicated to excellence in the study of the human brain and its disorders.**

The Center for Neurology presently consists of five departments, focussing on important areas of basic and clinical brain research and patient care, including Neurology and Stroke, Epilepsy, Neurodegenerative and Neurocognitive Disorders. All departments provide patient care within the University Hospital, while the clinical and basic research groups are part of the Hertie Institute.

The fact that all departments of the center actively participate, albeit to a different degree, in the clinical care of patients with neurologic diseases is central to the concept of successful clinical brain research at the Hertie Institute.

This applies most obviously to clinical trials, which are conducted, for example, in the treatment of Parkinson's disease, multiple sclerosis, epilepsy and brain tumors. However, the intimate interconnection of science and patient care is of eminent importance to all areas of disease-related neuroscientific research. It forms the very center of the Hertie concept and distinguishes the Center for Neurology from other neuroscience institutions.

In the year 2015 the German Council of Science and Humanities (Wissenschaftsrat) evaluated the Center of Neurology, focussing on its innovative structures. Based on a comprehensive report and an on-site evaluation, the evaluation was extremely positive. In particular, the close interaction between the basic science and patient care at the HIH and the University Hospital's Clinical Neurology Department was seen as a role model for clinical and translational research in Germany.

*Mit dem im Jahre 2001 unterzeichneten Vertrag zwischen der Gemeinnützigen Hertie-Stiftung (GHS) und dem Land Baden-Württemberg, der Universität Tübingen und ihrer medizinischen Fakultät sowie dem Universitätsklinikum Tübingen wurde das „Zentrum für Neurologie“ geschaffen. Damit entstand eines der größten Zentren für klinische und krankheitsorientierte Hirnforschung in Deutschland.*

*Das Zentrum besteht aus zwei eng verbundenen Institutionen, der Neurologischen Klinik und dem Hertie-Institut für klinische Hirnforschung (HIH). Die Aufgaben des Zentrums liegen sowohl in der Krankenversorgung durch die Neurologische Klinik als auch in der wissenschaftlichen Arbeit der im HIH zusammengeschlossenen Forscher. Die besonders enge Verknüpfung von Klinik und Grundlagenforschung innerhalb jeder einzelnen Abteilung und die Department-Struktur sind fundamentale Aspekte des Hertie-Konzeptes und ein Alleinstellungsmerkmal gegenüber anderen Institutionen der Hirnforschung in Deutschland. In der Department-Struktur sind die Professoren mit Leitungsfunktion akademisch und korporationsrechtlich gleichgestellt.*

*Das Zentrum besteht derzeit aus fünf Abteilungen: Der Abteilung Neurologie mit Schwerpunkt neurovaskuläre Erkrankungen (Prof. Dr. med. Ulf Ziemann), der Abteilung Neurologie mit Schwerpunkt neurodegenerative Erkrankungen (Prof. Dr. med. Thomas Gasser), der Abteilung Neurologie mit Schwerpunkt Epileptologie (Prof. Dr. med. Holger Lerche), der Abteilung Kognitive Neurologie (Prof. Dr. med. Hans-Peter Thier) und der Abteilung für Zellbiologie Neurologischer Erkrankungen (Prof. Dr. sc. nat. Mathias Jucker). Die ersten drei Genannten sind bettenführende Abteilungen in der Neurologischen Klinik, die beiden Letztgenannten sind an der Patientenversorgung im Rahmen von Spezialambulanzen beteiligt. Die klinischen Abteilungen sind für die Versorgung von Patienten mit der gesamten Breite neurologischer Erkrankungen gemeinsam verantwortlich. Die Einheit der Neurologischen Klinik in Lehre, Ausbildung und Krankenversorgung wird dabei durch eine gemeinsame Infrastruktur (Patientenaufnahme, Behandlungspfade, Poliklinik, diagnostische Labors, Bettenmanagement, Pflegedienst gesichert. Die Neurologische Klinik besteht daher nach innen und außen weiterhin als einheitliche Struktur. In den klinischen Abteilungen werden pro Jahr mehr als 5.000 Patienten stationär und rund 14.000 Patienten ambulant behandelt.*

*Das Zentrum für Neurologie wurde im Sommer 2015 auf Bitten des Landes Baden-Württemberg und der Gemeinnützigen Hertie-Stiftung durch den Wissenschaftsrat evaluiert. Grundlage der Evaluation war eine umfangreiche Bestandsaufnahme zu Forschung, Lehre und Krankenversorgung, wobei die Leistungsfähigkeit seiner innovativen Strukturen und seiner Organisationsform im Mittelpunkt standen. Der Wissenschaftsrat hat das Zentrum als modellhaft für die Universitätsmedizin in Deutschland gewürdigt. Besonders lobte der Wissenschaftsrat die vom HIH und der Tübinger Neurologischen Universitätsklinik gemeinsam etablierte Departmentstruktur. Das wichtigste wissenschaftspolitische Beratungsgremium von Bund und Ländern lobte in seiner Stellungnahme die praktizierte Verbindung von Grundlagenforschung und klinischer Praxis und bestärkt das Institut darin, diesen Weg fortzusetzen.*

# Facts & Figures

## CENTER OF NEUROLOGY

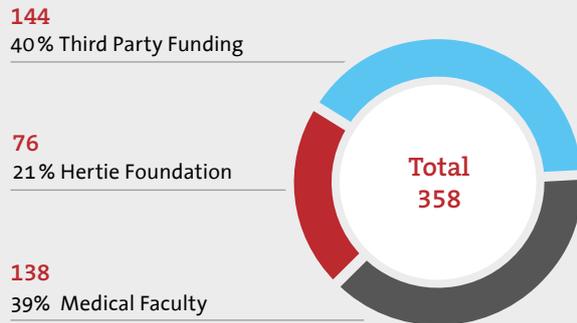


Forschung		Klinik	
Gemeinsame Poolmittel	Schlaganfall, Neuroprotektion & Plastizität, Experimentelle Neuroonkologie, Neuroimmunologie	<b>Abt. Neurologie mit Schwerpunkt neurovaskuläre Erkrankungen</b> <i>Prof. Dr. Ulf Ziemann</i>	<b>Stationär:</b> Stroke Unit und Allgemein-Neurologie <b>Spezialambulanzen</b>
	Parkinson, seltene neuro- degenerative Erkrankungen, Genetik, Biomarker	<b>Abt. Neurologie mit Schwerpunkt neurodegenerative Erkrankungen</b> <i>Prof. Dr. Thomas Gasser</i>	<b>Stationär:</b> Neurodegenerative Erkrän- kungen und Allgemein-Neurologie <b>Spezialambulanzen</b>
	Epilepsie, Migräne: Genetik, Mechanismen, Therapie, Bildgebung	<b>Abt. Neurologie mit Schwerpunkt Epileptologie</b> <i>Prof. Dr. Holger Lerche</i>	<b>Stationär:</b> Epilepsien & prächirurgi- sche Epilepsie-Diagnostik und Allgemein-Neurologie <b>Spezialambulanzen</b>
	Wahrnehmung und Handlungs- kontrolle, soziale und exekutive Funktionen und ihre Störungen	<b>Abt. Kognitive Neurologie</b> <i>Prof. Dr. Hans-Peter Thier</i>	<b>Spezialambulanzen</b>
	Alzheimer, Amyloid Angiopathie, Hirnalterung	<b>Abt. Zellbiologie neurologischer Erkrankungen</b> <i>Prof. Dr. Mathias Jucker</i>	<b>Spezialambulanzen</b>
	Neuroregeneration, Lernen und Gedächtnis	<b>Unabhängige Nachwuchsgruppen</b>	
<b>Gemeinsame Infrastruktur</b>			

Gemeinsame Poliklinik

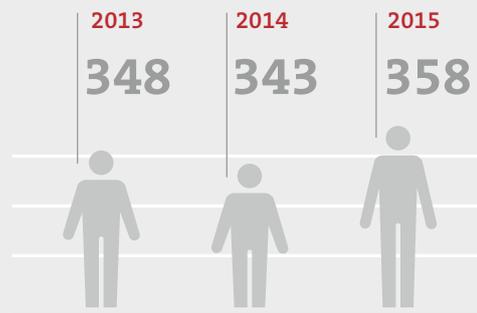
**NUMBER OF STAFF IN 2015**

Center of Neurology without nursing services (by headcount)



**DEVELOPMENT OF STAFF**

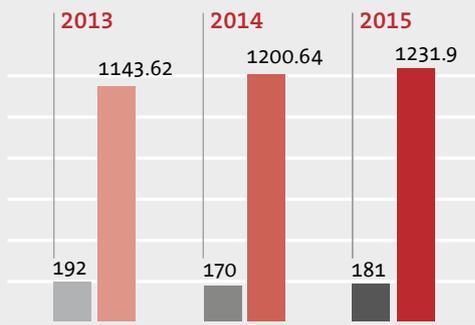
Center of Neurology (by headcount)



**NUMBER OF PUBLICATIONS**

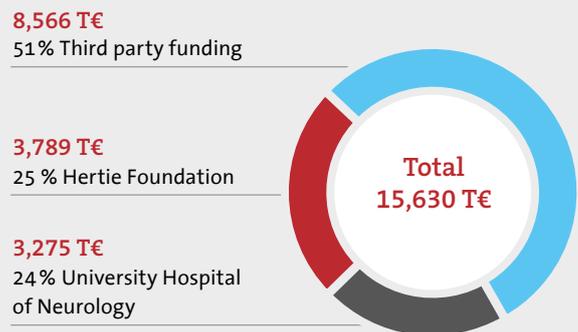
**IMPACT FACTORS**

Center of Neurology (SCIE and SSCI / in 100 %)



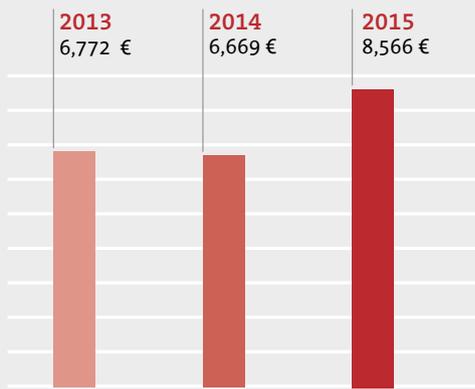
**TOTAL FUNDINGS IN 2015**

Center of Neurology



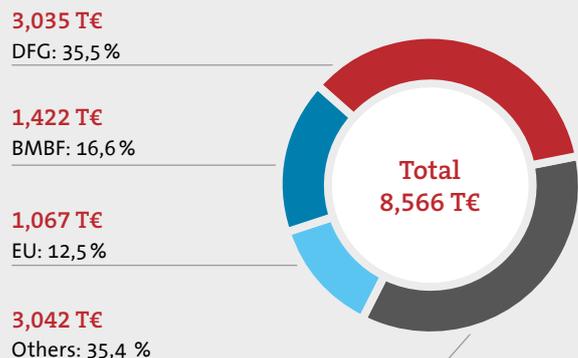
**THIRD PARTY FUNDING**

Center of Neurology (T€)



**THIRD PARTY FUNDING IN 2015**

Center of Neurology



# University Hospital of Neurology

12:02





<b>UNIVERSITY HOSPITAL OF NEUROLOGY</b>	<b>12</b>
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## University Hospital of Neurology

### CLINICAL CARE

The University Hospital's Clinic of Neurology treats inpatients with the complete spectrum of neurologic diseases on three general wards. Patients with acute strokes are treated on a specialized certified stroke-unit, which allows 24-hour surveillance and treatment. Neurointensive-care patients are treated in a cooperative model on the intensive care unit of the Clinic of Neurosurgery. A specialized EEG-monitoring unit allows continuous long-term EEG recordings for patients with intractable epilepsies.

In the outpatient unit of the clinic around 14,000 (including diagnostic procedures) patients are examined and treated every year, many of them in specialty clinics which are directed by recognized specialists in their respective fields.

### PATIENTENVERSORGUNG

*Die Neurologische Klinik am Universitätsklinikum Tübingen behandelt Patienten mit dem gesamten Spektrum neurologischer Erkrankungen auf drei Allgemeinstationen. Patienten mit akuten Schlaganfällen werden auf einer zertifizierten Schlaganfall-Spezialstation („Stroke-Unit“) behandelt, die rund um die Uhr die erforderlichen Überwachungs- und Therapiemaßnahmen erlaubt. Neurointensiv-Patienten werden in einem kooperativen Modell hauptsächlich auf der neurochirurgischen Intensivstation behandelt. Daneben gibt es eine spezielle Einheit zur kontinuierlichen Langzeit-EEG-Ableitung (EEG-Monitoring) für Patienten mit schwer behandelbaren Epilepsien.*

*In der neurologischen Poliklinik werden jährlich rund 14.000 Patienten (inkl. diagnostischer Prozeduren) ambulant betreut, viele davon in Spezialambulanzen, die von ausgewiesenen Experten für die jeweiligen Erkrankungen geleitet werden.*

## Clinical Performance Data



Close monitoring of patients at the intensive care unit.

### INPATIENT CARE

The inpatient units of the University Hospital of Neurology treated more than 5,000 patients in 2015.

#### NUMBER OF ADMISSIONS

5,059

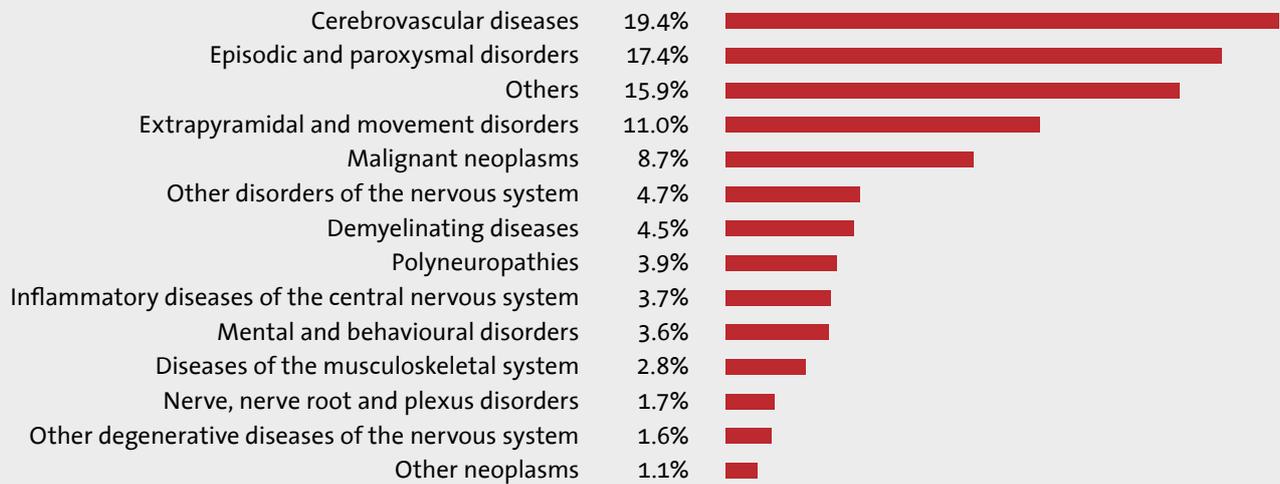
#### LENGTH OF STAY (IN DAYS)

5.1

#### CASE-MIX-INDEX 2015

1.46

### INPATIENT DIAGNOSIS GROUPS



### OUTPATIENT CARE

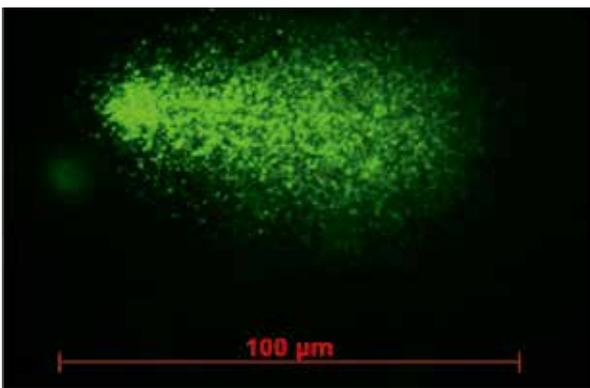
NUMBER OF CONSULTATIONS  
(including diagnostic procedures)

13,990

## Outpatient Clinics



Deep brain stimulation for Parkinson's disease: X-Ray image of an electrode inserted to the brain.



Comet assay indicating impaired DNA repair in lymphoblastoids of patients with recessive ataxias. Comet of DNA fragments in a lymphoblast with increased numbers of double strand breaks.

### ATAXIA

The ataxia clinic provides state-of-the-art tools to discover the molecular causes of ataxia, thereby working in close cooperation with the Department of Neuroradiology (MRT, MR-Spectroscopy) and the Institute of Medical Genetics. Here we developed new tools to investigate the genetic basis of ataxias. To address the increasing number of genes causing ataxia we use not only most recent next generation-sequencing gene panels (allowing parallel sequencing of all known ataxia genes), but now also whole exome-sequencing (WES) and even whole genome sequencing (WGS). Therapeutic options depend largely on the underlying cause of ataxia, the genetic defect, and concomitant symptoms.

In cooperation with Dr. Winfried Ilg and Prof. Giese from the Center for Integrative Neuroscience (CIN), the experts developed special videogame-based exercise programs (“exergames”) for ataxia and evaluate therapeutic effects by ataxia scores, gait analysis, and quantitative tests for fine motor skills.

Within the European Ataxia Study Group ([www.ataxia-study-group.net](http://www.ataxia-study-group.net)) we participate in a natural history study and biomarkers study of sporadic late-onset ataxias (SPOR-TAX). Moreover, we are part of a worldwide consortium (EUROSCA) to aggregate and follow up patients with dominant spinocerebellar ataxias (SCA), which is an inevitable prerequisite for interventional trials in the future. This work now also focusses on presymptomatic SCA subjects, where the clinical disease has not yet started, aiming to detect motor, imaging and biosample biomarkers that allow to trace the disease trajectory even before its clinical beginning (RISCA). This might allow to develop interventions in stages where neuronal resources are not yet exhausted and subjects' way of living is not yet severely incapacitated. In addition, our ataxia clinic is the national leading clinic for the international consortium on aggregating and deep-phenotyping early onset ataxias (EOA). This network is a rich resource for our main research focus of discovering new ataxia genes. The clinic is run by Dr. M. Synofzik, Dr. S.Hayer and Dr.C. Wilke and is supervised by Prof. Dr. L. Schöls.

### DEEP BRAIN STIMULATION

Also known as “brain pacemaker”, deep brain stimulation (DBS) is considered the most significant progress in the treatment of neurodegenerative movement disorders over the last decades. As a novel treatment option DBS has been implemented in Tübingen in cooperation with the Department of Neurosurgery already in 1999. The concept of treatment and medical attendance developed by the network for deep brain stimulation of the University Clinic of Tübingen (BrainStimNet; [www.brainstimnet.de](http://www.brainstimnet.de)) involves close interaction between neurologists, neurosurgeons, psychiatrists and physiotherapists. Patients are referred from outside neurologists as well as our own outpatient clinics for movement disorders and psychiatric diseases.

In 2013, the relevance of Tübingen as a specialized center for deep brain stimulation was underscored by its contribution to the European multicentre EARLYSTIM-study that proved for the first time an improved quality of life in patients undergoing DBS in early disease stages (Schuepbach et al., NEJM, 2013). Moreover, based on own basic research in the identification of novel targets for DBS in Parkinson's disease, two independent randomized controlled trials for unmet axial symptoms like 'freezing of gait' and 'imbalance and falls' in Parkinson's disease were initiated. Here, the first study on high frequency stimulation of the substantia nigra pars reticulata (SNr) as an add-on to the conventional subthalamic nucleus stimulation was successfully accomplished and proved an effect on freezing of gait (Weiss et al., BRAIN, 2013). The work on nigral stimulation for resistant freezing of gait now translates into a large multicentre randomized controlled trial initiated and coordinated by the Tübingen Centre (ClinTrials.gov: NCT02588144). The trial is currently active and recruiting.

Patients who are likely to benefit from DBS undergo a detailed program of standardized neurological, neuropsychological, neuroradiological, and cardiological examinations on our ward for neurodegenerative diseases. Patients treated with DBS are closely followed by our outpatient clinic to ensure optimal adjustment of stimulation parameters. The outpatient clinic for DBS is focused on patient selection and counselling of patients eligible for DBS based on neurological examination and medical history. Moreover, the BrainStimNet Tübingen organizes regular conferences for patients and relatives in cooperation with the German Parkinson's disease Association (dPV). Appointments are scheduled two days per week in the outpatient clinic for DBS. Patients are seen by Dr. L. Roncoroni, I. Hanci, and Dr. D. Weiss.

## DIZZINESS SERVICE

The dizziness outpatient service offers state-of-the-art diagnostic evaluation, treatment and follow-up for patients suffering from acute or chronic dizziness. As the limited resources of the unit should be primarily devoted to the assessment of patients suffering from specific forms of dizziness, admitting institutions are requested to filter out patients whose complaints are an unspecific reflection of a more general problem. The dizziness service is available



for outpatients on Wednesday mornings. The diagnostic work-up starts with a precise assessment of the history and character of the complaints. It is followed by a thorough clinical examination with special emphasis on visual, vestibular, and oculomotor functions complemented by video-oculography, measurement of the subjective vertical, electroencephalography, and ultrasound examination of the major blood vessel supplying the brain. If needed, high-resolution 3D eye movement measurements based on cutting-edge video or search coil techniques are added. As a result of this work-up, functional alterations compromising spatial vision and orientation may be disclosed, which in many cases do not have a morphological basis ascertainable by brain imaging techniques.

Revealing specific forms of dizziness leads to the application of specific therapeutic measures such as exercises customized to treat benign paroxysmal positional vertigo. Most of the patients seen in the unit suffer from dysfunction of the organ of equilibrium, the vestibular labyrinth, or a disturbance of the brain mechanisms processing vestibular information. In others, the dizziness can be understood as a specific form of phobia or related psychological maladjustment. Currently, attempts are being made to establish improved therapeutic offers also for this latter group of patients not suffering from a primary neurological or otological condition. The dizziness service is run by Dr. J. Pomper.

## Outpatient Clinics

### DYSTONIA AND BOTULINUM TOXIN TREATMENT

The outpatient clinic offers a comprehensive diagnostic work-up and the full range of treatment options for patients with different forms of dystonia, spasticity, and hyperkinetic movement disorders. In cooperation with the headache clinic (PD Dr. T. Freilinger), treatment with botulinum toxin injections for patient with chronic migraine is provided.

Approximately 450 to 500 patients are treated regularly with botulinum toxin (BoNT) injections in intervals of 3 to 6 months. Of those nearly 60 percent are treated for dystonia (including Blepharospasm and Meige-Syndrom as well as cervical, segmental, multifocal,

generalized and task-specific dystonia) and facial hemispasm, 30% for spasticity and 10% for other indications (including migraine, hyperhidrosis, and hypersalivation). For patients with dystonia or spasticity BoNT treatment is often optimized using EMG-, electro stimulation-, or ultrasound-guided injection techniques e.g. for the treatment of deep cervical muscles in cervical dystonia. The clinic also participates in several multicenter trials to evaluate new preparations as well as new indications for BoNT treatment.

Dystonia patients with insufficient response to standard treatments can be treated with deep brain stimulation (DBS) of the internal pallidum in collaboration with the clinic for deep brain stimulation and the BrainStimNet ([www.brainstimnet.de](http://www.brainstimnet.de)).

Besides pharmacologic and surgical treatment, a wide range of physical and ergotherapeutic therapies are offered. Over the last years, an increasing number of outpatients with spasticity have been managed with a combined treatment of BoNT injections and physiotherapy at the local MTR (Medical Training and Rehabilitation Center, University of Tübingen).

Appointments are scheduled every week on Wednesday and Thursday in the Outpatient Clinic of the Center of Neurology. The medical staff of this unit includes G. Beck (technical assistant), Dr. F. Bernhard and Prof. L. Schöls.



## EPILEPSY

The Department of Neurology and Epileptology started its operations in November 2009. Since then, a large inpatient and outpatient clinic has been built offering the whole spectrum of modern diagnostic procedures and therapy of the epilepsies and all differential diagnoses of paroxysmal neurological disorders, such as syncope, dissociative disorders with pseudoseizures, migraine, transient ischemia, and also rare disorders, as episodic ataxias and paroxysmal movement disorders.

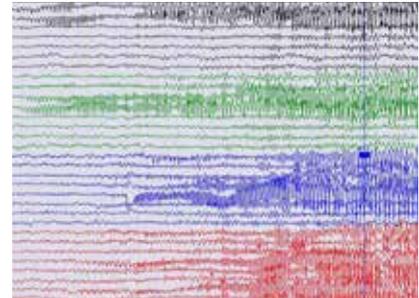
The epilepsy outpatient clinic offers consulting and treatment in particular for newly diagnosed, difficult-to-diagnose and difficult-to-treat cases, and for specific questions including women with epilepsy, pregnancy under antiepileptic treatment, and genetic aspects. The study center offers medical and other clinical trials to explore novel treatment options. The inpatient unit with 28 beds (Wards 42/45), running under the supervision of Prof. Dr. Y. Weber, PD Dr. N. Focke and PD Dr. T. Freilinger, includes acute care for epileptic seizures and status epilepticus, longterm complex treatment for difficult cases, and a Video-EEG-Monitoring Unit which is operated in cooperation with the Department of Neurosurgery. Within this unit, inpatients are continuously and simultaneously monitored with video and electroencephalography (EEG) for differential diagnostic and presurgical evaluations.

Epilepsy surgery, an effective treatment for patients resistant to anti-convulsive medication, deep brain stimulation of the thalamus and vagal nerve stimulation are provided in close cooperation with the Department of Neurosurgery (Dr. S. Rona, Prof. Dr. J. Honegger, Prof. Dr. A. Garabaghi). The epilepsy outpatient clinic (Prof. Dr. H. Lerche, Prof. Dr. Y. Weber and PD Dr. N. Focke) offers consulting and treatment in particular for difficult cases and specific questions including pregnancy under antiepileptic treatment and genetic aspects. Altogether we treat about 2,000 adult patients per year.

## FRONTOTEMPORAL DEMENTIA AND EARLY-ONSET DEMENTIAS

Frontotemporal Dementias (FTD) are a heterogeneous group of neurodegenerative diseases characterized by progressive changes in personality and behavior and/or progressive language disturbances. FTD often starts not in very late life, but between 50–60 years, yielding it one of the most common early-onset dementias (onset < 65 years).

The disease spectrum of FTD and possible differential diagnosis is complex, reaching from Progressive Supranuclear Gaze Palsy (PSP) to Alzheimer's disease (AD), and often extending phenotypes complicated by additional parkinsonian syndromes or Amyotrophic Lateral Sclerosis (ALS). Our experts from the FTD clinic are specialists on these differential diagnoses, including rare neurometabolic dementias like Niemann Pick Type-C. A specific focus is given on an extensive clinico-neuropsychological work-up complemented by latest cerebrospinal



Start and spread of an epileptic seizure in the EEG over 10 seconds

fluid biomarkers and next-generation genetics. Given the large share of genetic causes of FTD – up to 30–50% seem to be inherited – such next-generation-sequencing procedures like panel sequencing and whole exome sequencing offer a new window not only towards exact molecular diagnosis but also towards individualized counselling and therapy. We are part of the FTLDc consortium, which is establishing a large nationwide cohort of patients with FTD-spectrum diseases, comprehensively characterized on a clinical, neuropsychological, imaging and biomarker level. Moreover, we participate in the international multi-center GENFI consortium, aggregating and characterizing symptomatic and asymptomatic carriers with mutations in FTD genes in a longitudinal fashion. This ambitious endeavor will allow to unravel the neuropsychological, imaging and molecular changes in FTD even before its clinical onset, thus offering a novel window for therapy in the future. The clinic is run by Dr. C. Wilke and supervised by Dr. M. Synofzik.

## Outpatient Clinics



Neuro-geriatric patients receive physiotherapy for mobility training.

### GERIATRICS

Geriatric patients are a special group of elderly people, usually over 70 years of age, who present with multiple and complex medical problems. In these patients, disabilities ranging from cerebrovascular to neurodegenerative diseases are most prevalent in combination with cardiovascular, respiratory, and metabolic disorders. Approximately 30% of the patients admitted to the Neurology department are older than 70 years and most of them fulfill the criteria of being a “geriatric patient”. Geriatric patients are often handicapped by a number of additional symptoms, such as incontinence, cognitive decline or dementia, and susceptibility to falls. These additional symptoms do not only complicate the convalescence process but also interfere, together with the primary disease, with functional outcome, daily activities and quality of life. It is thus our primary aim to identify quality of life-relevant functional deficits associated with the disease and comorbidities,

using established geriatric assessment batteries. Affected patients receive goal-oriented physiotherapy for mobility training, neuropsychological training, speech therapy, and occupational therapy. Patients, spouses as well as family members receive specific information about community services and organization of geriatric rehabilitation. Staff directly involved in the different services includes Prof. W. Maetzler, Prof. R. Krüger, Markus Hobert and Dipl. Soz. Päd. FH A. Steinhauser.

Scientific projects on the evaluation of geriatric topics are performed, e. g. with the Department of Geriatric Medicine at the Robert-Bosch-Hospital in Stuttgart (Prof. Clemens Becker) and with the Department of Psychiatry and Psychotherapy (Prof. Eschweiler).

The Neurology Department is a member of the Center of Geriatric Medicine. This Center was established at the University Medical Center of Tübingen in 1994 to improve the care for geriatric patients in this region. The activities of the University Clinics for Medicine IV, Neurology, and Psychiatry are currently coordinated by the University Clinic for Psychiatry and Psychotherapy. External partners are the Paul-Lechler-Krankenhaus in Tübingen, the community hospital in Rottenburg and the rehabilitation clinic in Bad Sebstiansweiler near Tübingen. The Neurology Department provides a regular consult service for these institutions, and takes an active part in seminars, teaching, and training activities of the Center of Geriatric Medicine.

## HEADACHE AND NEUROPATHIC PAIN

The outpatient unit is dedicated to neurological pain syndromes, including headache and facial pain as well as neuropathic pain syndromes. Patients should be referred preferably by neurologists or pain management specialists. Appointments are available from Tuesday through Friday, and patients will be provided with mailed headache/pain diaries and questionnaires well before their scheduled appointment.

One major clinical focus is the diagnostic work-up and multimodal treatment of chronic headache disorders like chronic migraine (CM), medication-overuse headache or chronic tension-type headache. The unit further specializes in the diagnosis and treatment of rare primary headache syndromes like trigeminal autonomic cephalalgias (TACs; e.g. cluster headache, paroxysmal hemicrania or SUNCT syndrome) as well as rare monogenic migraine variants such as hemiplegic migraine. Finally, patients with neuropathic pain syndromes are diagnosed and treated in close collaboration with the Department of Anesthesiology, which organizes monthly interdisciplinary pain conferences.

The outpatient clinic organizes local patient education events and serves as a platform to provide access to ongoing clinical studies including both multi-center trials as well investigator-initiated pilot trials. Selected patients with otherwise refractory chronic headache disorders are offered access to new treatment modalities including botulinum toxin for CM or neurostimulation techniques (e.g. non-invasive vagal nerve stimulation), which are currently under evaluation. Inpatient treatment will be available in special cases (e.g. exacerbations of cluster headache, difficult cases of medication withdrawal). To address psychiatric comorbidities, which are highly prevalent and clinically relevant in chronic pain disorders, the unit is in close collaboration with both the Department of Psychosomatic Medicine and the Department of Psychiatry. The outpatient clinic is run by PD Dr. T. Freilinger together with a team of colleagues (one board-certified neurologist, four neurology residents).



# Outpatient Clinics

## LEUKODYSTROPHIES IN ADULTHOOD

Leukodystrophies are usually regarded as diseases that occur in infancy and childhood. However, for most leukodystrophies adult-onset forms have been identified but still frequently escape detection. In cooperation with the Department of Neuropediatrics in the Children's Hospital we analyze the natural course of the diseases and especially of adult-onset variants of leukodystrophies as an essential prerequisite for therapeutic trials. Nerve conduction studies and evoked potentials are currently investigated as potential progression markers. Genotype-phenotype studies help to recognize unusual disease manifestations and to identify factors modifying the course of leukodystrophies. For an increasing number of these neurometabolic disorders treatment by enzyme replacement, substrate inhibition or stem cell transplantation become available. Patients are seen by Dr. J. Just and Prof. Dr. L. Schöls.

## MOTONEURON DISEASE

Motoneuron diseases are caused by the degeneration of motor neurons in the cerebral cortex (upper motor neuron) and/or the ventral horns of the spinal cord (lower motor neurons). In the most common form of motoneuron disease – amyotrophic lateral sclerosis (ALS) – both upper and lower motor neurons are affected.

In most cases ALS is a sporadic disease, but in about 10% of patients there is a familial background. Our specific focus is concentrated on the genetic work-up of both apparently sporadic as well as familial cases, aiming to explore the frequencies of ALS genes, discovering new ALS genes and unravelling the molecular pathways underlying genetic ALS. Detailed neurological examination provides essential diagnostic information. Paraclinical tests include nerve conduction studies, electromyography, and evoked potentials. Additional diagnostic procedures (e. g. blood tests, lumbar puncture, and imaging of the brain and spinal cord) are necessary to exclude rare diseases mimicking ALS. Therefore, in most cases an inpatient treatment is required to confirm the diagnosis of ALS. Treatment of respiratory problems is provided in close cooperation with the pulmonologists.

Follow-up of patients as well as management of symptoms and complications are provided by the clinic. The clinic is run by Dr. J. Just and supervised by Dr. M. Synofzik and Prof. Dr. L. Schöls.

## NEUROIMMUNOLOGICAL DISORDERS

Patients with multiple sclerosis (MS), neuromyelitis optica (NMO), immune-mediated neuropathies, and other neuroimmunological disorders are regularly seen in the outpatient-clinic for neuroimmunological diseases. Complex cases are discussed in interdisciplinary conferences with colleagues from rheumatology, neuroophthalmology, neuroradiology, and neuropathology. The Center of Neurology is certified as an MS priority center by the German Multiple Sclerosis Society (DMSG) and is a member of the Clinical Competence Network for Multiple Sclerosis (KKNMS), the Neuromyelitis Optica Study Group (NEMOS) and European Susac Consortium (EUSAC).

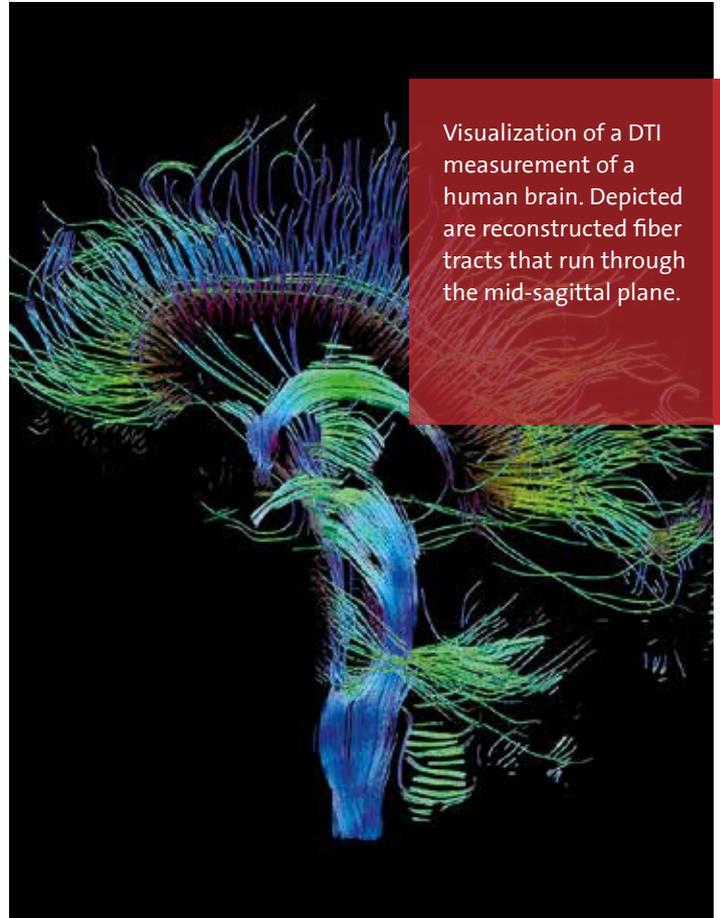
Patients with MS are referred from other institutions for diagnosis, follow up, or second opinion. Counselling about immunomodulatory and immunosuppressive therapy follows the guidelines by the German "Multiple Sclerosis Therapy Consensus Group". Standardized examination of patients is performed according to the Expanded Disability Status Score (EDSS) and the Multiple Sclerosis Functional Composite Score (MFSC). M. Dengler and M. Jeric (study nurses) organize appointments and offer training for injection of interferons and glatiramer acetate. A large number of patients participate in currently approximately 15 different clinical trials, which explore safety and efficacy of new treatments in relapsing-remitting MS, progressive MS and NMO. Clinical trials are managed by a team of study nurses (C. Delik, U. Küstner, K. Strauß-Oppitz). In 2015, the outpatient clinic was run by Dr. C. Ruschil (resident) and supervised by Dr. F. Bischof (until 07/2015), Dr. M. Krumbholz (since 07/2015, special expertise in MS), Dr. A. Grimm (since 05/2015, special expertise in immune-mediated neuropathies) and Prof. U. Ziemann.

## NEUROMUSCULAR DISORDERS

For the diagnosis of neuromuscular diseases the correct collection of medical history, including family history, is particularly important. In addition, the patients are examined neurologically and possibly electrophysiologically. In the clinic the indication to further necessary investigations such as MRI or muscle biopsy is provided. The therapy is tailored to the individual patient and his particular type of the disease and usually includes a medicated as well as physiotherapy regimen. The neuromuscular clinic is run by Dr. C. Freilinger.

## NEUROLOGIC MEMORY OUTPATIENT CLINIC

Dementia is one of the most frequent problems of the elderly population and a major cause of disability and mortality. The most common forms of dementia are Alzheimer's disease, vascular dementia, and dementia associated with Parkinsonian syndromes (including idiopathic Parkinson's disease, diffuse Lewy body disease, progressive supranuclear palsy). The latter syndromes also represent the clinical and scientific focus of our memory clinic. Some of the dementia syndromes are treatable, and a minority of them (e.g. inflammation-associated dementias) are potentially curable. A thorough investigation with clinical, neuropsychological, biochemical and imaging methods of progressive cognitive deficits is therefore essential. In a weekly memory outpatient clinic such a program is offered. In addition, multimodal therapeutic strategies including medication, memory training, and social counseling are provided, in co-operation with the memory clinic of the Department of Psychiatry. A particular aim of the clinical and imaging studies are a better understanding of the differences/similarities between Alzheimer's disease and dementias associated with parkinsonism. Furthermore, the work focuses on the time course of disease progression and the efficacy of existing and new treatment options. The Neurologic Memory Clinic is run by Prof. W. Maetzler, M. Hobert, Dr. I. Liepelt-Scarfone and Dr. S. Graeber-Sultan.



Visualization of a DTI measurement of a human brain. Depicted are reconstructed fiber tracts that run through the mid-sagittal plane.

# Outpatient Clinics

## NEURO-ONCOLOGY

### (i) Clinical Neuro-Oncology

The management of neuro-oncological patients is coordinated in the Interdisciplinary Division of Neuro-Oncology, that was established in July 2014. The unique feature of this Division is (i) its affiliation to two departments, i.e. to the Department of Neurology & Stroke (Prof. Ziemann) and to the Department of Neurosurgery (Prof. Tatagiba), and (ii) the appointment of the head of the Division (Prof. Tabatabai) as a full (W3) professor of Neuro-Oncology on 18 July 2014.

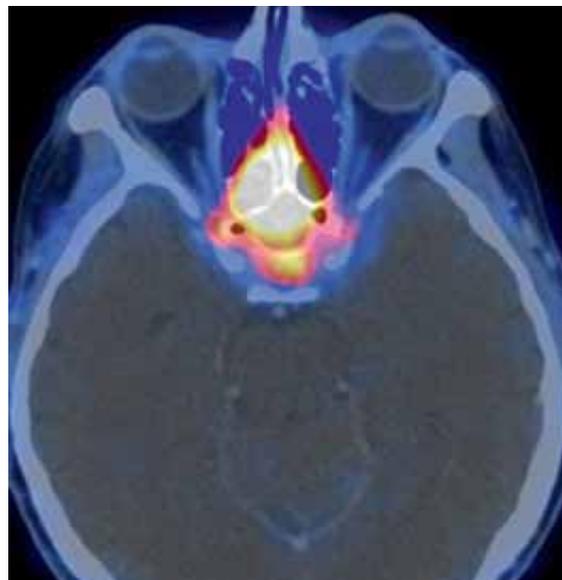
As a consequence, the outpatient clinic is organized as an interdisciplinary outpatient clinic with neurological and neurosurgical appointments, and the reports use a header with both Departments reflecting a bridging between both Departments in the field of Neuro-Oncology.

In addition, the Interdisciplinary Division of Neuro-Oncology is part of the Center of CNS tumors under the roof of the Comprehensive Cancer Center Tübingen-Stuttgart and very closely cooperates with the Departments of Radiation Oncology, Radiology & Neuroradiology & Nuclear Medicine, Pathology & Neuropathology. As Prof. Tabatabai is also the elected Chair of the Center of CNS Tumors, strategies of the CCC can be easily and readily implemented into the strategical plan of the Division of Neuro-Oncology. The center has recently received the certificate of the German Cancer Society (DKG).

Patients who need treatments or procedures will be mainly admitted to in the ward (Station 45) in the Department of Neurology & Stroke and will be supervised by the Neuro-Oncology team in both departments.

The main objectives of the Division of Neuro-Oncology are:

- To offer cutting-edge innovative treatments in clinical trials
- To participate in national and international consortia and trial groups (e.g. NOA, EORTC, RTOG)
- To diagnose, treat and monitor patients with neuro-oncology tumors at each stage of their disease
- To provide guidance for supportive care and palliative treatment
- To provide a second opinion for patients seeking for advice

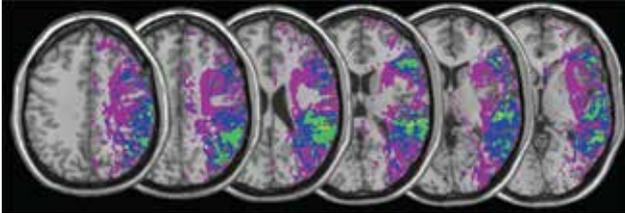


Meningioma of a 70 year old patient, visualized by PET/CT, a combination of positron emission tomography and computer tomography.

The team for patient treatment and/or clinical trials is composed of Prof. Ghazaleh Tabatabai, Dr. Felix Behling (neurosurgery resident), Dr. Marilyn Koch (neurology resident), Dr. Lars Füllbier (board-certified neurosurgeon, resident), Dr. Constantin Roder (neurosurgery resident and coordinator of the Center of CNS Tumors), Dr. Cristiana Roggia (board-certified in Internal Medicine; senior physician, head of study coordination), Dr. Marco Skardelly (neurosurgery attending).

### (ii) Laboratory of Clinical and Experimental Neuro-Oncology

Curative approaches in Neuro-Oncology are very rare, thus continuous research is inevitable and urgently warranted. The Laboratory of Clinical and Experimental Neuro-Oncology at the Hertie Institute for Clinical Brain Research (head: Prof. Tabatabai) has been established since June 2014 and conducts basic research projects for identifying new targets for therapy and optimizing the delivery of therapeutic molecules to experimental tumors.



In patients with stroke lesions, we use Normalized perfusion-weighted Imaging (PWI) to identify the abnormally perfused brain area(s) that receive enough blood supply to remain structurally intact, but not enough to function normally. In order to recognize these common areas in groups of patients, we analyse the increase of time-to-peak (or TTP) lesion-induced delays by using spatial normalization of PWI maps as well as symmetric voxel-wise inter-hemispheric comparisons. These new techniques allow comparison of the structurally intact but abnormally perfused areas of different individuals in the same stereotaxic space, and at the same time avoid problems due to regional perfusion differences and to possible observer-dependent biases.

## NEUROPSYCHOLOGY

Strokes not only lead to motor and sensory impairment, but often also cause disorders of higher brain functions such as speech, attention, perception, memory, intelligence, problem solving or spatial orientation. The prerequisite for designing a treatment strategy, which is effective and tailored to the patient's particular needs, is a careful neuropsychological evaluation of the specific pattern of disorders. The Neuropsychology Outpatient Clinic determines, for example, whether a patient exhibits an abnormal degree of forgetfulness or whether signs of dementia emerge. It is also considered whether a patient is capable of planning appropriate actions to perform given tasks, whether speech is impaired, or which kinds of attention-related functions may have been damaged and need to be treated. These and other examinations are carried out in the Neuropsychology Outpatient Clinic of the Neuropsychology Section (head Prof. Dr. Dr. H.-O. Karnath).

## NEUROPSYCHOLOGICAL TESTING

In addition to motor and sensory impairment, stroke often leads to cognitive or affective disorders. These disorders affect attention, perception, memory, language, intelligence, planning and action, problem solving, spatial orientation, or sensorimotor coordination. Effective treatment of these impairments requires a careful neuropsychological examination of the impairment. Neuropsychological examinations for the Center of Neurology are conducted at the Neuropsychology Section (head Prof. Dr. Dr. H.-O. Karnath).

## NEUROVASCULAR DISEASES

The neurovascular outpatient clinic provides services for patients with neurovascular diseases including ischemic and hemorrhagic stroke, cerebral and cervical artery stenosis, microvessel disease, cerebral vein thrombosis, vascular malformations, and rare diseases such as cerebral vasculitis, endovascular lymphoma or arterial dissection. Its focus is on diagnosis, discussion and decision about treatments, secondary prevention, and neurorehabilitation strategies and schedules. Diagnostic tests performed as part of an outpatient visit include: Doppler and duplex ultrasound of cervical and intracranial vessels, transthoracic and transesophageal echocardiography, contrast echocardiography, 24-hour Holter ECG and blood pressure monitoring, implantation of an event-recorder for long-term ECG monitoring in selected ischemic stroke patients with suspected atrial fibrillation, and evaluation by a physiotherapist focusing on rehabilitation potentials. Computed tomography and magnetic resonance imaging are carried out in cooperation with the Department of Neuroradiology at the University of Tübingen. Echocardiograms are performed by PD Dr. C. Meyer-Zürn (cardiologist and internist, shared appointment by the Department of Neurology and Stroke and the Clinic of Cardiology). The outpatient clinic was run in 2015 by a team of residents (Dr. F. Härtig, Dr. M. Ribitsch, Dr. H. Richter) and supervised by Dr. S. Poli and Prof. U. Ziemann of Tübingen. In cooperation with the department of cardiology, eventrecorders are implanted in selected ischemic stroke patients with suspected atrial fibrillation.

# Outpatient Clinics

## PARKINSON'S DISEASE

The Center of Neurology at the University of Tübingen runs the largest outpatient clinic for patients with Parkinson syndromes in Southern Germany. More than 120 patients are seen every month. A major focus of the clinic is the early differential diagnosis of different Parkinson syndromes. The development of transcranial sonography by members of the department draws patients from all over Germany to confirm their diagnosis which, if necessary, is substantiated by other tests, like the smelling test or neuroimaging investigations with SPECT and/or PET. Genetic testing is offered to patients and relatives with familial Parkinson syndromes who may obtain genetic counselling in cooperation with the Department of Medical Genetics. The Department of Neurodegeneration is one of two German centers that participate in the international Parkinson Progression Marker Initiative (PPMI), a 5-year follow-up of de novo Parkinson patients to better understand aetiology and disease progression and the P-PPMI-(prodromal-PPMI) study, which follows individuals at high risk for PD to better understand the early phase of neurodegeneration. Both studies are supported by the Michael J Fox Foundation. Additionally, large scale longitudinal studies are being performed to better understand the different phases of neurodegeneration as well as symptom development and progression to finally enable more specific, individualized therapies. Another focus is the treatment of patients in later stages of the disease with severe non-motor symptoms or drug-related side effects. In some of these patients, treatment may be optimized in co-operation with the Ward for Neurodegenerative Diseases by intermittent or continuous apomorphine or duodopa application, which is supervised by a specially trained nurse. Other patients are referred for deep brain stimulation (DBS). Various

multicenter drug trials, for patients in different stages of Parkinson's disease but also atypical Parkinsonian syndromes offer the possibility to participate in new medical developments. Moreover, close cooperation with the outpatient rehabilitation center and the establishment of a Parkinson choir guarantee the involvement of additional therapeutic approaches.

With the improvement of motor control by new treatment strategies, non-motor symptoms in patients with advanced Parkinsonian syndromes are an area of increasing importance. In particular, dementia and depression have been recognized to be frequent in these patients. Standards in diagnosis of these symptoms are being established and optimized treatment is offered. Seminars and courses on specific topics related to diagnosis and treatment for neurologists, in cooperation with the lay group for Parkinson's patients (Deutsche Parkinson Vereinigung, dPV) are organized. Moreover, visitors from all over the world are trained in the technique of transcranial sonography in regular teaching courses.

The outpatient unit cooperates with German Center for Neurodegenerative Diseases (DZNE) under a common roof, called the "Integrated Care and Research Center" (ICRU). Appointments are scheduled daily in the outpatient clinic of the Center of Neurology.

## SPASTIC PARAPLEGIAS

The outpatient clinic for hereditary spastic paraplegias (HSP) offers a specialist setting for the differential diagnostic workup and genetic characterization of patients with spastic paraplegia using the facilities of the Hertie Institute for Clinical Brain Research and cooperations with the Institute of Medical Genetics and the Department of Neuroradiology. Therapeutic options depend essentially on the underlying cause of the disease. Symptomatic treatment includes antispastic drugs, intrathecal application of Baclofen, local injections of Botulinum toxin and functional electrical stimulation. Tübingen is the disease coordinator for HSP in the NEUROMICS project funded by the EU that aims to discover new genes, gene modifiers as well as metabolic factors that cause or modify hereditary neurodegenerative diseases taking advantage of a broad spectrum of OMICS techniques like genomics, transcriptomics and lipidomics. The clinic is run by Dr. R. Schüle, Dr. T. Rattay and Prof. Dr. L. Schöls.

## TREMOR-SYNDROMES

Although essential tremor is with a prevalence of 1 to 5 % the most frequent movement disorders, diagnosis and especially differential diagnosis is often challenging. In the outpatient clinic for tremor a thorough standardized assessment battery has been established to facilitate diagnosis and decision for therapeutic strategies. Close cooperation with the clinic for DBS (deep brain stimulation, headed by Dr. D. Weiss) ensures the inclusion of this highly effective treatment option into decision making.

## POLYNEUROPATHIES

The outpatient clinic for patients with polyneuropathies handles about 350 patients per year with several kinds of neuropathy, e.g. mononeuropathies (immune-mediated, traumatologic) as well as polyneuropathies (immune-mediated, e.g. CIDP, MMN, GBS, vasculitic or inherited as the Charcot-Marie-Tooth type 1,2, X) as well as orphan diseases, e.g. M. Refsum or amyloidosis. In cooperation with the department of Neurophysiology – including neurography, EMG or ultrasound – the diagnostic work out is well established. The clinic is run by Dr. A. Grimm and Ms. D. Vittore.

# Clinical Laboratories

## CLINICAL CHEMISTRY LABORATORY

The Clinical Chemistry Laboratory collects more than 1,700 samples of cerebrospinal fluid (CSF) per year throughout the University Medical Center. Routine parameters include cell count, glucose, lactate and protein analysis, i. e., albumin and IgG in serum and CSF. Oligoclonal bands in CSF and serum are detected by isoelectric focusing and silver staining. Cytology of CSF is analysed on cytopsins after Giemsa or Pappenheim staining. Junior staff are routinely trained to perform basic CSF examination techniques and the interpretation of results as part of their speciality training. The laboratory takes part in quality management activities of CSF parameters. Immunopathology includes the determination of a set of autoantibodies for the diagnosis of certain neuroimmunological syndromes: autoantibodies to acetylcholine receptors, muscle specific tyrosine kinase (MuSK), titin, aquaporin-4, autoantibodies associated with neurological paraneoplastic syndromes (Anti-Hu, Anti-Yo, Anti-Ri, and subspecifications), and autoantibodies to gangliosides for immunoneuropathies. Cell populations in CSF and blood samples are examined by flow cytometry using a FACScalibur cytometer. These include determination of CD20+ cells in patients under B cell depleting therapies, CSF CD4/CD8 ratio in patients suspected to have neurosarcoidosis, assessment of CD4/CD62L cells in patients treated with natalizumab as well as detailed immunophenotyping in patients with complex inflammatory diseases of the nervous system. In addition, CSF-levels of amyloid beta42, tau, and phospho-tau are measured to differentiate various forms of dementia. In case samples that have to be sent to external reference laboratories (e.g. CSF JCV testing for natalizumab-associated PML), the neurochemical laboratory takes care of preparing and sending the samples, as well as organizing the reports. The laboratory is supervised by Prof. W. Maetzler, PD. F. Bischof (until 07/2015), and Dr. M. Krumbholz (since 07/2015).



Electroencephalography (EEG) is used to record the spontaneous electrical activity of the brain by multiple electrodes placed on the scalp in a standardized manner. Abnormalities in EEG due to epileptiform brain activity represent the major diagnostic feature of epilepsy.

## EEG LABORATORY

The electroencephalography (EEG) laboratory is equipped with four mobile digital and two stationary recording places (IT-Med). For analysis, six additional PC-based EEG terminals are available. The recording and analysis workstations are connected via an internal network, and digital EEG data are stored on local servers making all previous and current EEGs available 24 hours a day, 7 days a week. At the neurological intermediate care and stroke unit, a digital 4-channel EEG unit is available and is used to continuously monitor patients with severe brain dysfunction such as status epilepticus, or various forms of coma. Each year, approximately 3,000 EEGs are recorded in outpatients and inpatients. The typical indications are epilepsy, coma or milder forms of altered consciousness, and the differential diagnoses of brain tumors, stroke, brain injury, and neurodegenerative disorders. EEG training is conducted according to the guidelines of the German Society for Clinical Neurophysiology and Functional Imaging (DGKN). The EEG training course lasts for 6 months and is provided for 4 neurological residents at a time. Laboratory staff: Ms Wörner, Ms Mahle, Ms Vohrer (staff technicians), Prof. Dr. Y. Weber (head of the laboratory) and PD N. Focke.



Transcranial magnetic stimulation for testing integrity of the central motor system.

## EMG LABORATORY AND NERVE/MUSCLE ULTRASOUND

The EMG Laboratory offers all the standard electromyography and neurography procedures for the electrodiagnosis of neuromuscular diseases including polyneuropathies, entrapment neuropathies, traumatic nerve lesions, myopathies, myasthenic syndromes, and motor neuron diseases. In selected cases, polygraphic recordings for tremor registration, registration of brainstem reflexes, exteroceptive reflexes and reflexes of the autonomous nervous system are performed. In addition, the new diagnostic tool of high resolution ultrasound (Phillips Epiq 7, 18 MHz Probe and Mindray T7, 14 MHz Probe) was introduced in 2015. With the ultrasound of the peripheral nervous system as well as of the muscles, several neuromuscular disorders, e.g. polyneuropathies, motoneuron diseases, myopathies, nerve tumours, entrapment syndromes and traumata can be visualized.

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The laboratory is equipped with two digital systems (Dantec Keypoint G4). A portable system (Nicolet Viking Quest) is available for bedside examinations. In 2015, more than 2,500 patients were seen and more than 15,000 recordings were done. In most cases (approximate 70%), a combination of neurography and electromyography is requested. In addition, a Neurosoft Evidence 9000MS stimulator is available for transcranial magnetic stimulation and recording of motor evoked potentials in approximately 800 patients per year. Further, 450 patients were examined by neuromuscular ultrasound since 05/2015.

In 2015, the EMG Laboratory was run by a team of technical assistants (D. Tünnerhoff-Barth, D. Joswik, V. Servotka) and residents (Dres. M. Hobert, J. Müller vom Hagen, S. Schiemann, D. Vittore-Welliong, C. Wilke, S. Wolking, C. Zipser, P. Martin) under the supervision of Dr. A. Grimm (since 05/2015), Dr. F. Müller-Dahlhaus and Prof. L. Schöls.

## ENG LABORATORY

Approximately 250 patients suffering from otoneurological or neuro-ophthalmological problems are examined each year using electronystagmography (ENG) and a variety of complementary techniques. Most patients examined present specific vestibular syndromes (also see Dizziness Service). For diagnosis, eye movements are recorded binocularly using DC oculography, are digitally stored and analysed offline. Eye movements are induced by single diodes to test saccades or gaze holding, by a laser system eliciting smooth pursuit eye movements, and by whole field visual stimuli to evoke optokinetic nystagmus in all directions. Besides testing of visually guided eye movements, which provide information on cerebellar and brainstem functions, emphasis is placed on the examination of the vestibular system including the search for spontaneous nystagmus, head shaking nystagmus, positioning/positional nystagmus, and the assessment of the vestibuloocular (VOR) reflex (caloric and rotation tests). The recordings are performed by Y. Schütze and analyzed by Dr. J. Pomper. For more complex questions, e.g., isolated testing of single canals, movements of the eyes and head, as a function of head rotation and visual stimulation, are measured in three dimensions using magnetic search coils. The laboratory also offers non-invasive eye movements recording using video techniques (Chronos) and performs otolith testing such as the measurement of the subjective visual vertical and vestibular evoked myogenic potentials (VEMP). The laboratory is supervised by Dr. J. Pomper.

# Clinical Laboratories

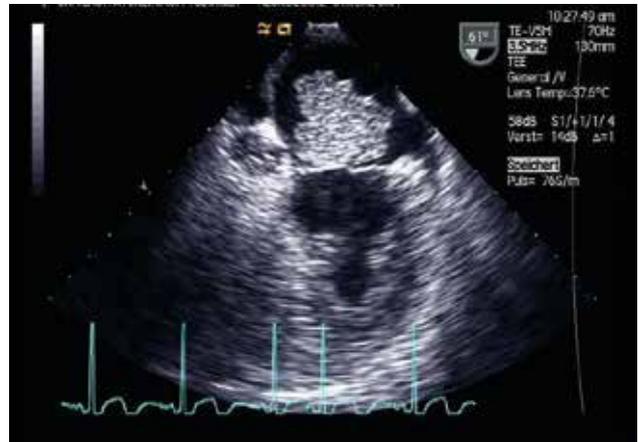
## EVOKED POTENTIALS (EP) LABORATORY

The EP (evoked potentials) laboratory provides a full range of evoked potential procedures for both inpatient and outpatient testing. All recordings are performed using a 4-channel system and can be conducted in the laboratory as well as in patient rooms, intensive care units and operating rooms. Procedures include visual evoked potentials, brain stem auditory evoked potentials, short latency somatosensory evoked potentials of the upper and lower extremities, and spinal evoked potentials.

Around 2,500 examinations are performed every year on more than 1,600 patients. The recordings are conducted by A. Deutsch, K. Fuhrer and Y. Schütze and are supervised by Dr. A. Grimm (since 05/2015), Dr. F. Müller-Dahlhaus, and Prof. L. Schöls. The EP recordings are analyzed and interpreted during daily conferences according to the guidelines of the German Society for Clinical Neurophysiology (DGKN), and visited by up to six interns.

## OPTICAL COHERENCE TOMOGRAPHY (OCT) LABORATORY

Optical coherence tomography (OCT) allows for non-invasive assessment of the structure of the retina and the thickness of the retinal nerve fiber layer. OCT is not only useful in various ophthalmologic diseases, but also in CNS diseases, such as multiple sclerosis, Parkinson's disease, Alzheimer's disease, intracranial hypertension, and even Friedreich's ataxia. The OCT's advantages include a harmless, fast and cheap analysis at high resolution in the micrometer range that can be repeated as necessary. At present, this technique is used in a study for multiple sclerosis to analyse the retinal nerve fiber layer thickness since these nerve fibers are part of the CNS and affected by multiple sclerosis, offering an independent analysis tool for neuronal damage by this disease and for monitoring neuroprotection by treatment. The OCT laboratory is run by Dr. C. Ruschil and D. Celik under the supervision of Dr. M. Krumbholz.



Transesophageal echocardiogram (TEE) showing a left atrial myxoma protruding through the mitral valves in a young patient with multiple embolic strokes.

## NEUROCARDIOLOGY LABORATORY

As diseases of the heart are responsible for up to 30% of all strokes and usually cause territorial embolic ischemic infarcts, cardiac investigations are urgently required in stroke patients to find potential cardiac causes and in order to reduce the risk of stroke recurrence. Therefore, all stroke patients undergo a detailed cardiac investigation which is performed by the neurocardiology laboratory.

The neurocardiology laboratory, headed by the cardiologist and internist PD Dr. C. Meyer-Zürn, provides the full spectrum of non-invasive cardiac work-up, such as transthoracic and transesophageal echocardiography including M-Mode, 2-D mode, pulse wave, continuous-wave and color Doppler investigations as well as contrast-enhanced echocardiography for the detection of intracardiac shunts or intracardiac thrombi. A close rhythm monitoring using 24-hour Holter ECG and implantation of eventrecorder for the detection of atrial fibrillation is performed in selected stroke patients. Other diagnostic tools include 24-hour blood pressure monitoring, and selection of patients for cardiac MRI or CT in cooperation with the department of radiology. For invasive diagnostic and/or treatment, patients are referred to the department of cardiology.

Other patients of the neurology department, which are frequently examined in the neurocardiology laboratory, are patients with suspected heart failure, chest pain, Parkinson patients with planned deep brain stimulation and patients with unexplained syncope.

Yearly, we conduct approximately 1,700 echocardiographic examinations, over 1,200 Holter ECGs, and about 800 24-hour blood pressure measurements. All investigations are done according to the guidelines of the German and European Societies of Cardiology.

### NEUROSONOLOGY LABORATORY

The neurosonological laboratory is equipped with two color-coded duplex sonography systems: a Toshiba Aplio and a Philips Epiq7. Additionally, two portable CW/PW Doppler systems – a DWL Multi-Dop pro and a DWL Multi-Dop T digital – are available. Neurosonological examinations are performed by the ultrasound assistants Ms Nathalie Vetter and Ms Yvonne Schütz as well as a resident physician under the supervision of Dr. Sven Poli, consultant stroke neurologist and neurointensivist.

The laboratory itself is situated within the outpatient department of the Dept. of Neurology and is mainly used for non-acute or elective ultrasound examinations of in- and outpatients. The mobile Doppler and duplex units are used for examinations of acutely ill patients on our Stroke Unit allowing for the full range of neurosonological assessment at the bedside immediately after admission.



**Transcranial B-mode sonography procedure:**  
The probe is placed at the temporal bone window in a patient in supine position to assess the brain in standardized scanning planes.

Routine diagnostic tests include duplex imaging of extracranial carotid, vertebral, and subclavian arteries, as well as the transcranial Doppler and duplex sonography of the vertebrobasilar circulation and the Circle of Willis (with and without contrast). Functional testing for vertebral steal, bubble tests for assessment of right to left shunts (e.g. persistent foramen ovale), and continuous Doppler monitoring of the cerebral blood flow (e.g. before, during and after neuroradiological interventions) or for detection of cerebral microembolisms (high-intensity transient signals) are also routinely performed.

Each year, the total number of Doppler/duplex examinations conducted at our laboratory amounts to approximately 4,000 of extracranial arteries and approximately 3,000 of intracranial arteries.

# Occupational, Physical and Speech Therapy

## OCCUPATIONAL THERAPY

The treatment program is focused on patients with handicaps from acute strokes, brain tumors, inflammatory diseases, movement disorders, neurodegenerative diseases and disabilities from disorders of the peripheral nervous system. In 2015, 1,837 patients were treated.

Occupational therapy provides the following training programs: training in motor function to improve patient's ability to perform daily activities, training in sensorimotor perception, adaptive equipment recommendations and usage training, cognitive training, and counselling of spouses and relatives. Currently eight occupational therapists are working within the "Therapie Zentrum" responsible for the neurological wards under the supervision of Anke Nölck.



## PHYSIOTHERAPY

All neurological inpatients with sensory or motor deficits, movement disorders, pain syndromes, and degenerative spinal column disease are allocated to individualized physiotherapy. Currently twelve physiotherapists under the supervision of MSc Marion Himmelbach are working within the "Therapie Zentrum" responsible for the neurological wards. The physiotherapist treatment is based on guidelines, which had been worked out for special disease groups according to the current knowledge. This includes for example lumbar disc prolaps, stroke, ataxia, Parkinson's disease. Within the year 2015, 2,601 patients were treated.



Fiberoptic endoscopic evaluation of swallowing (FEES) of a patient with dysphagia.

## SPEECH THERAPY

Neurological patients with swallowing and speech-/language disorders receive speech therapy while staying in hospital. The emphasis within the team of eight speech therapists under the supervision of MSc Natalie Rommel is the assessment and treatment of patients with dysphagia.

Every acute stroke patient receives a bedside and, if necessary, a video-endoscopic or video-fluoroscopic swallowing examination. Therefore dysphagia can be recognized at an early stage, an aspiration pneumonia can be prevented and a specific therapy can be planned for every individual patient. Every acute stroke patient also receives a bedside speech- and language examination. The aim of the speech therapy with these patients is to improve their communication ability. In 2015, 1,660 patients with dysphagia, aphasia and dysarthria received speech therapy.



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**Hertie-Institut**  
für klinische Hirnforschung

**Since its founding 14 years ago, the Hertie Institute has grown to more than 350 employees at all levels, from technicians to PhD students to full professors. The institute's achievements include discoveries related to the molecular, genetic and physiological basis of a number of major neurologic diseases.**

The institute presently consists of five departments. They combine basic and clinical research with patient care, albeit to different degrees and with variable emphasis: the departments of Neurology and Stroke, Epileptology, and Neurodegenerative Disorders treat outpatients in specialty clinics, but also inpatients with the whole spectrum of neurological diseases, while the Departments of Cognitive

Neurology and Cellular Neurology provide specialized diagnostic services and care in an outpatient setting only, focusing on neurocognitive impairments and Alzheimer's disease, respectively.

The institute is home to a total of 18 professors, 350 members and 34 research groups. 32 belong to the aforementioned departments. Two exist as independent research groups, which were established in 2006. In 2014 an international committee evaluated the junior research group "Physiology of Learning and Memory" and recommended tenure. In line with this together with the "Institute for Medical Psychology and Behavioural Neurobiology" (headed by Prof. Jan Born) a joint research group was established.



In 2015, scientists at the Center for Neurology obtained more than 8.5 million Euros in third party funding and published 181 papers in peer reviewed journals.

Finally, the construction of the new building at the Tübingen site of the German Center for Neurodegenerative Diseases (DZNE) was finished. In the long term, this building will accommodate up to 150 scientists conducting research on nervous system diseases such as Alzheimer's or Parkinson's to develop new preventative, diagnostic and therapeutic strategies.

To foster the interaction between the CIN (Werner Reichardt Centre for Integrative Neuroscience), DZNE and HIH the first Neuroscience Campus Get together was jointly set up in the year 2015.

All these developments will ensure the long-term success of the neuroscience community in Tübingen.

Prof. Dr. Thomas Gasser  
 Prof. Dr. Mathias Jucker  
 Prof. Dr. Holger Lerche  
 Prof. Dr. Peter Thier  
 Prof. Dr. Ulf Ziemann

## Das Hertie-Institut für klinische Hirnforschung (HIH) in 2015

*14 Jahre nach seiner Gründung durch die Gemeinnützige Hertie-Stiftung, die Universität Tübingen und das Universitätsklinikum Tübingen gehört das HIH auf dem Gebiet der klinischen Hirnforschung zum Spitzenfeld europäischer Forschungseinrichtungen. Herausragende Forschungsergebnisse haben das Institut auch über die Grenzen Europas hinaus bekannt gemacht.*

*Das HIH besteht derzeit aus fünf Abteilungen: der Abteilung Neurologie mit Schwerpunkt neurovaskuläre Erkrankungen (Prof. Dr. med. Ulf Ziemann), der Abteilung Neurologie mit Schwerpunkt neurodegenerative Erkrankungen (Prof. Dr. med. Thomas Gasser), der Abteilung Neurologie mit Schwerpunkt Epileptologie (Prof. Dr. med. Holger Lerche, der Abteilung Kognitive Neurologie (Prof. Dr. med. Hans-Peter Thier) und der Abteilung für Zellbiologie Neurologischer Erkrankungen (Prof. Dr. sc. nat. Mathias Jucker). Die ersten drei Genannten sind bettenführende Abteilungen in der Neurologischen Klinik, die beiden Letztgenannten sind an der Patientenversorgung im Rahmen von Spezialambulanzen beteiligt. Die klinischen Abteilungen sind für die Versorgung von Patienten mit der gesamten Breite neurologischer Erkrankungen gemeinsam verantwortlich.*

*In den Abteilungen sind zurzeit 18 Professoren und etwa 350 Mitarbeiter in 32 Arbeitsgruppen tätig. Hinzu kommen noch zwei unabhängige Forschungsgruppen.*

*Die Arbeitsschwerpunkte des HIH liegen im Bereich neurodegenerativer und entzündlicher Hirnerkrankungen, der Schlaganfallforschung, Epilepsien und der Erforschung der Grundlagen und Störungen von Wahrnehmung, Motorik und Lernen. Zu den bedeutenden Forschungserfolgen des HIH*

*zählen die Entdeckung wichtiger genetischer und molekularer Grundlagen der Entstehung und Progression neurologischer Erkrankungen. Das HIH, ein Modellprojekt für Public Private Partnership, hat auch im Jahr 2015 mehr als 8,5 Millionen Euro an Drittmitteln eingeworben und 181 Veröffentlichungen in wissenschaftlichen Fachzeitschriften publiziert. Diese Zahlen belegen unter anderem die wissenschaftliche Leistungsfähigkeit des Zentrums. Die Gemeinnützige Hertie-Stiftung wendete bisher rund 33 Millionen Euro für das HIH auf und wird ihre Förderung fortsetzen.*

*Auch strukturell geht das HIH neue Wege. Die Reformansätze gelten vor allem drei Schwerpunkten: Die Einrichtung einer Department-Struktur, die Einrichtung eines Pools von flexibel und kurzfristig einsetzbaren Fördermitteln und der Aufbau eines Modells für eine leistungsabhängige Prämie für alle Mitarbeiter.*

*Ein weiterer innovativer Aspekt des HIH ist die Einrichtung von abteilungsunabhängigen Junior-Arbeitsgruppen im „Tenure Track-Verfahren“. Die erste dieser Arbeitsgruppen, die sich schwerpunktmäßig mit neuroregenerativen Prozessen des Rückenmarks beschäftigt, wurde im Frühjahr 2006 eingerichtet und 2010 aufbauend auf einer erfolgreichen internationalen Evaluierung in eine selbständige Arbeitsgruppe umgewandelt.*



*In den Abteilungen sind zurzeit 18 Professoren und etwa 350 Mitarbeiter in 28 Arbeitsgruppen tätig. Die Gemeinnützige Hertie-Stiftung wendete bisher rund 30 Millionen Euro für das HIH auf und wird ihre Förderung fortsetzen.*

*Im Jahr 2013 nahm der Leiter dieser unabhängigen Forschungsgruppe die Professur „Restorative Neurowissenschaften“ am Imperial College in London an. Zusätzlich hierzu leitet er seine Gruppe weiterhin am HIH: Die zweite Gruppe „Lernen und Gedächtnis“ wurde Ende 2014 international evaluiert und gemeinsam mit dem Institut für Medizinische Psychologie und Verhaltensneurobiologie unter Leitung von Prof. Born im Jahr 2015 verstetigt.*

*Um die Interaktion zwischen den neurowissenschaftlichen Insituten am Standort Tübingen zu stärken, wurde in diesem Jahr das erste Neuroscience Campus Get together gemeinsam mit unseren Nachbarn, dem Deutschen Zentrum für Neurodegenerative Erkrankungen (DZNE) und dem Werner Reichardt Centre for Integrated Neuroscience (CIN) initiiert.*

*Eine besondere Bedeutung für die Zukunft des Zentrums kommt auch seiner Beteiligung an der erfolgreichen Bewerbung von Tübingen als Partnerstandort des „Deutschen Zentrums für Neurodegenerative Erkrankungen, DZNE“ zu. Die Etablierung dieses Partnerstandortes führt zu einer erheblichen Stärkung des neurowissenschaftlichen Standorts.*

*Im April 2015 wurde der Neubau des DZNE bezogen. In dem Gebäude sollen langfristig bis zu 150 Wissenschaftlerinnen und Wissenschaftler Erkrankungen des Nervensystems wie Alzheimer oder Parkinson erforschen und neue Strategien für die Prävention, Diagnose und Therapie entwickeln.*

*Prof. Dr. Thomas Gasser  
Prof. Dr. Mathias Jucker  
Prof. Dr. Holger Lerche  
Prof. Dr. Peter Thier  
Prof. Dr. Ulf Ziemann*



Department  
of Neurology  
and Stroke



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## Departmental Structure

**The Department of Neurology and Stroke covers a wide spectrum of neurological diseases. It is part of the University Medical Center and also provides primary care in the district of Tübingen for patients with neurological disorders.**

The clinical and scientific expertise of the Department of Neurology & Stroke (Director: Prof. Ulf Ziemann) covers complex neurovascular diseases (ischemic stroke, intracranial hemorrhage, cerebral vasculitis, vascular malformations, rare neurovascular diseases such as endovascular lymphoma, paraneoplastic coagulopathy, or reversible cerebral vasoconstriction syndrome), neuroimmunology (multiple sclerosis, neuromyelitis optica, myasthenia gravis, autoimmune neuropathies and others), and brain tumors and brain metastases. Specialized teams in stroke medicine (intensive care and stroke unit, rehabilitation), neuroimmunology and neurooncology provide expert multidisciplinary care for patient with these disorders. As an integral part of the Comprehensive Cancer Center (CCC), the Departments of Neurology, Neurosurgery, Radiooncology, Neuroradiology and Neuropathology form the Center of Neurooncology. The newly established interdisciplinary Section



Prof. Dr. Ulf Ziemann is head of the Department of Neurology and Stroke Neurology.

of Neurooncology (Head: Prof. Ghazaleh Tabatabai) is a unique section associated with this Department and the Clinic of Neurosurgery to coordinate clinical service and research in Neurooncology. Specialized outpatient clinics in Neurovascular Diseases, Neuroimmunology and Neurooncology offer the best available therapy and provide the infrastructure for clinical trials and investigator-initiated research.

The Department of Neurology and Stroke provides the clinical basis for its Research Groups at the Hertie Institute for Clinical Brain Research. All Research Groups have strong interest in bridging neuroscience and health care in translational research concepts. Currently, six Research Groups exist that are active in brain networks & plasticity (Prof. Dr. Ulf Ziemann), stroke and neuroprotection (Dr. Sven Poli), neuroimmunology (PD Dr. Felix Bischof/Dr. Markus Krumbholz), clinical and experimental neuro-oncology (Prof. Dr. Ghazaleh Tabatabai), molecular neuro-oncology (Prof. Dr. Ulrike Naumann) and speech disorders (Prof. Dr. Hermann Ackermann). The Research Groups are located in immediate proximity of the clinical services in the CRONA hospital building, or in the Hertie Institute for Clinical Brain Research.

Close collaborations exist with the other departments and research groups at the Hertie Institute. The department also cooperates closely with the physiotherapy department at the University Medical Center (Zentrum für ambulante Rehabilitation), which has a focus on physiotherapy for stroke rehabilitation.

The Department of Neurology and Stroke offers lectures for medical students, physicians in training, nursing staff, physiotherapists and speech therapists. The grand round series welcomes internationally renowned clinical scientists giving state of the art lectures. The neurology therapy seminar gives up-to-date overviews on recent advances in neurology, internal medicine, neurosurgery, neuro-ophthalmology, neuroradiology and other areas relevant to the treatment of patients with neurological diseases. The lectures of basic and clinical neurology, the seminar on neurology, and the training course on neurological examination skills are integral parts of the Medical School curriculum and are usually honored by the evaluation of the students.



The Department offers lectures for medical students, physicians in training, nursing staff, physiotherapists and speech therapists.

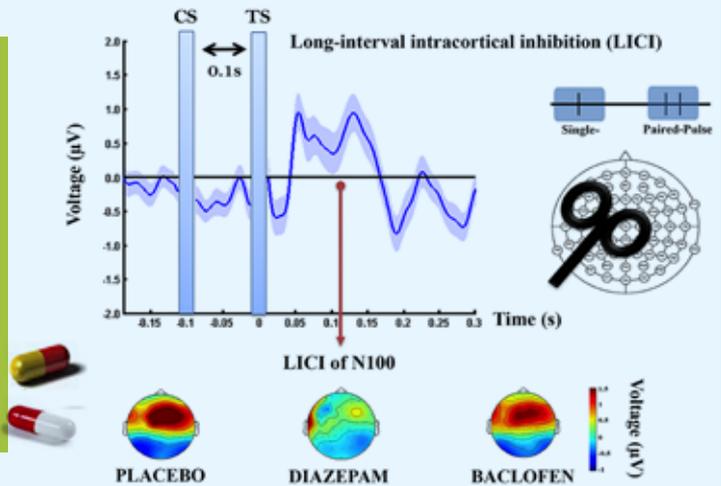
# Neuroplasticity

Brain Networks & Plasticity (BNP) Laboratory

Head: Prof. Dr. Ulf Ziemann

Team: 18 members

Key words: human motor cortex / motor learning / plasticity / cortical connectivity / stroke rehabilitation / non-invasive brain stimulation / closed-loop stimulation / EEG / MEG / MRI / fMRI / TMS-EEG / neuropharmacology



Pharmaco-TMS-EEG: a novel technique to measure pharmacological effects in the human brain

The human brain has an amazing capacity to reorganize, which ensures functional adaptation in an ever-changing environment. This capacity for neural plasticity becomes even more important for rehabilitation after cerebral injury such as stroke. Our group focuses on understanding principles of neural plasticity in the human cortex, and on using novel techniques of non-invasive brain stimulation, in particular closed-loop stimulation using information of instantaneous brain states based on real-time EEG analysis to modify highly efficiently neuronal networks. In particular, we are interested in how mechanisms of neural plasticity underlie learning in the healthy brain and re-learning in the injured brain. What rules govern learning? How do brain networks change after brain injury to compensate for and regain lost functionality? And can this knowledge be used to predict and facilitate rehabilitation after brain injury? We address these questions by imaging (fMRI, DTI) and electrophysiological (EMG, EEG, MEG) methods in combination with non-invasive brain stimulation (TMS, tDCS) and pharmacology. Our goal is to develop new rehabilitative strategies and make meaningful advances in the clinical practice of patients with brain diseases.

Das menschliche Gehirn besitzt eine erstaunliche Fähigkeit zur Reorganisation, die Voraussetzung für die Anpassung an sich ständig ändernde Umweltbedingungen ist. Diese Fähigkeit zur Plastizität ist von herausragender Bedeutung für Erholungsprozesse nach Schädigungen des Gehirns, wie einem Schlaganfall. Unsere Arbeitsgruppe fokussiert auf der Untersuchung von Plastizität der motorischen Hirnrinde auf systemneurowissenschaftlicher Ebene und der Entwicklung innovativer Methoden der nicht-invasiven Hirnstimulation, insbesondere Closed-Loop Stimulation unter Nutzung der EEG-Echtzeitanalyse von instantanen elektrophysiologischen Zuständen des Gehirns, um neuronale Netzwerke zielgerichtet und hoch-effizient zu modifizieren. Im Besonderen sind wir darin interessiert zu verstehen, welche Mechanismen von Plastizität Lernen im gesunden Gehirn, und Wiedererlernen verlorengegangener Fertigkeiten im geschädigten Gehirn nach Schlaganfall unterliegen. Wie verändern sich Netzwerke des Gehirns nach Schädigung um Funktionsdefizite auszugleichen? Kann der Erkenntniszuwachs über diese Zusammenhänge genutzt werden, um den Rehabilitationserfolg eines einzelnen Patienten vorherzusagen und/oder durch gezielte Intervention zu verbessern? Wir adressieren diese Fragen mit moderner Bildgebung (funktionelle Magnetresonanztomographie, Diffusionstensor-Bildgebung), elektrophysiologischen Methoden (EMG, EEG, MEG) in Kombination mit nicht-invasiver Hirnstimulation (transkranielle Magnetstimulation, transkranielle Gleichstromstimulation) und Neuropharmakologie. Unser Ziel ist, innovative und effektive neurorehabilitative Strategien zu entwickeln, die einen relevanten Fortschritt bei der Rehabilitationsbehandlung von Patienten mit neurologischen, insbesondere neurovaskulären Erkrankungen bedeuten.

**Progress report:** In 2013 the Brain Networks & Plasticity (BNP) lab was founded.

## Pharmaco-TMS-EEG

Several projects are aiming at improving our understanding of the physiological underpinnings of TMS-evoked EEG potentials: Combining transcranial magnetic stimulation (TMS) and electroence-

phalography (EEG) constitutes a powerful tool to directly assess human cortical excitability and connectivity. TMS of the primary motor cortex elicits a sequence of TMS-evoked EEG potentials (TEPs). By using several different GABA-Aergic (alprazolam,

diazepam, zolpidem) and GABA-Bergic drugs (baclofen), we have now provided evidence that inhibitory neurotransmission through GABA-A receptors (GABAAR) contributes to early TEPs (< 50ms after TMS), whereas GABA-B receptors (GABABR) play a role for later TEPs (at around 100ms after TMS). Currently, we are systematically extending this pharmaco-TMS-EEG research by investigating the effects of glutamatergic drugs and voltage-gated ion channel blockers on TEPs.

This research opens a novel window of opportunity to study alteration of GABAA-/GABAB-related inhibition, and potentially in the future other neurotransmitter systems in disorders such as epilepsy, schizophrenia, or after ischemic stroke.

#### Enhancing effect size of non-invasive brain stimulation on plasticity and learning by brain polarization

We are investigating the possibility to increase the size effect of non-invasive brain stimulation (cortico-cortical paired associative TMS) to induce long-term potentiation (LTP)-like plasticity by concurrent brain polarization using transcranial direct current stimulation (tDCS), according to the following rationale and project plan: A novel MR-navigated paired associative transcranial magnetic stimulation (PAS) technique in combination with transcranial direct current stimulation (tDCS) to induce cooperative spike-timing dependent plasticity (STDP)-like. We will specifically target the supplementary motor area (SMA) – primary motor cortex (M1) connection to strengthen effective connectivity of this pathway. We expect that strengthening of the SMA-M1 pathway will enhance motor performances and motor learning processes. In a first step, the experiments will be performed in young healthy subjects with a high potential for plastic change to explore the effects of combined SMA-M1 PAS and M1-tDCS on motor performance and learning.

This research is important because voluntary movements of the hand are associated with coordinated neuronal

activity in a distributed large-scale cortical motor network. Task-dependent increases in effective connectivity, in particular of the connection between SMA and M1, funnel driving activity into the voluntarily active M1. Stroke patients with hand paresis typically show impairments of this task-dependent increase of SMA-M1 connectivity and activation of the ipsilesional M1. The degree of these abnormalities correlates with motor clinical deficits of the paretic hand, so that strengthening of SMA-M1 connectivity may support motor recovery in stroke patients.

#### CLOSED-LOOP Brain Stimulation

Our group is pioneering the development of measuring instantaneous

brain states by real-time high-density EEG analysis. We focus on alpha oscillations in motor cortex and are now capable of triggering TMS on exact phases of the endogenous oscillations at millisecond precision. This is a major advancement for closed-loop stimulation, i.e. to trigger TMS at and only at pre-specified brain states. First experiments are ongoing to induce LTP-like plasticity changes using the new closed-loop approach. We expect that this will radically change therapeutic brain stimulation in the near future by shifting from the currently used open-loop protocols to closed-loop stimulation. This will enable individualized and highly time-resolved therapeutic interference with neuronal networks of the human brain.

## SELECTED PUBLICATIONS

Cash RF, Murakami T, Chen R, Thickbroom GW, **Ziemann U** (2016) Augmenting Plasticity Induction in Human Motor Cortex by Disinhibition Stimulation. *Cereb Cortex* 26: 58-69.

Lenz M, Galanis C, **Müller-Dahlhaus F**, Opitz A, Wierenga CJ, Szabo G, **Ziemann U**, Deller T, Funke K, Vlachos A (2016) Repetitive magnetic stimulation induces plasticity of inhibitory synapses. *Nat Commun* 7: 10020.

Triesch J, **Zrenner C**, **Ziemann U** (2015) Modeling TMS-induced I-waves in human motor cortex. *Prog Brain Res* 222: 105-124.

Goldsworthy MR, **Müller-Dahlhaus F**, Ridding MC, **Ziemann U** (2015) Resistant Against De-depression: LTD-Like Plasticity in the Human Motor Cortex Induced by Spaced cTBS. *Cereb Cortex* 25: 1724-1734.

**Müller-Dahlhaus F**, Lücke C, Lu MK, Arai N, Fuhl A, Herrmann E, **Ziemann U** (2015) Augmenting LTP-Like Plasticity in Human Motor Cortex by Spaced Paired Associative Stimulation. *PLoS One* 10: e0131020.

Fuhl A, **Müller-Dahlhaus F**, Lücke C, Toennes SW, **Ziemann U** (2015) Low doses of Ethanol Enhance LTD-Like Plasticity in Human Motor Cortex. *Neuropsychopharmacology* 40: 2969-2980.

**Premoli I**, Castellanos N, Rivolta D, **Belardinelli P**, Bajo R, **Zipser C**, **Espenhahn S**, Heidegger T, **Müller-Dahlhaus F**, **Ziemann U** (2014) TMS-EEG signatures of GABAergic neurotransmission in the human cortex. *J Neurosci* 34: 5603-5612.

**Premoli I**, Rivolta D, **Espenhahn S**, Castellanos N, **Belardinelli P**, **Ziemann U**, **Müller-Dahlhaus F** (2014) Characterization of GABAB-receptor mediated neurotransmission in the human cortex by paired-pulse TMS-EEG. *Neuroimage* 103C: 152-162.

Goldsworthy MR, **Müller-Dahlhaus F**, Ridding MC, **Ziemann U** (2014) Inter-subject Variability of LTD-like Plasticity in Human Motor Cortex: A Matter of Preceding Motor Activation. *Brain Stimul* 7: 864-870.

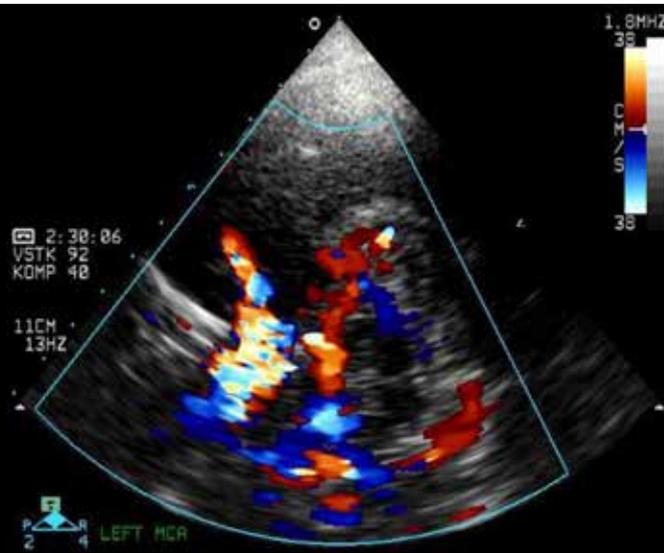
Gharabaghi A, Kraus D, Leao MT, Spuler M, Walter A, Bogdan M, Rosenstiel W, Naros G, **Ziemann U** (2014) Coupling brain-machine interfaces with cortical stimulation for brain-state dependent stimulation: enhancing motor cortex excitability for neurorehabilitation. *Front Hum Neurosci* 8: 122.

# Stroke and Neuroprotection Laboratory

Head: Dr. Sven Poli

Team: 11 members

Key words: stroke / neuroprotection / cerebral ischemia / intracerebral hemorrhage, ICH / hypothermia / normobaric oxygen therapy, NBO / hyperbaric oxygen therapy, HBO



**The research focus of our Stroke & Neuroprotection Laboratory is to find new and to optimize existing neuroprotective strategies that can help to minimize brain damage after stroke. Furthermore, we aim to study and characterize molecular mechanisms involved in ischemic-hypoxic damage and reperfusion-reoxygenation-induced neuronal death. Our current projects evaluate the effects of selective brain hypothermia combined with normobaric and hyperbaric hyperoxygenation. Our goal is to provide translational research with a close link to clinical application.**

*Forschungsschwerpunkt unseres Stroke & Neuroprotection Labors ist die Entwicklung neuer und die Optimierung existierender neuroprotektiver Strategien um den Hirnschaden nach einem Schlaganfall zu reduzieren. Darüber hinaus ist es unser Ziel die molekularen Mechanismen zu charakterisieren, welche der ischämisch-hypoxischen aber auch der reperfusions-reoxygenierungs-induzierten neuronalen Schädigung zugrunde liegen. Unsere aktuellen Projekte evaluieren die Effekte der kombinierten Anwendung von selektiver Hirnkühlung und normobarer oder hyperbarer Hyperoxygenierung. Unsere Forschung ist translational mit einem engen Bezug zur klinischen Anwendung.*

## **Neuroprotective effects of selective brain cooling with intra-arterial cold infusions (IACI) in acute ischemic stroke**

Due to a significantly smaller target volume, selective brain cooling allows for higher brain cooling rates with only minor body core temperature reductions. “Hijacking” the brain-supplying blood flow, intra-arterial cold infusions (IACI) could be a promising strategy for rapid induction of brain hypothermia and may easily be performed during endovascular intervention.

We applied IACI (0°C) in a filament middle cerebral artery occlusion rat model through the internal carotid artery via a specifically designed infusion port allowing for continuous pre- and post-reperfusion brain cooling. So far, we have systematically studied the brain temperature change under different infusion rates and infusate temperatures. We are investigating the dose- and time-effect of IACI induced neuroprotection in MCAO stroke model, as well as associated mechanisms and potential complications.

### **Hypothermia & hyperoxygenation: Co-stars in neuroprotection after acute ischemic stroke**

To study the synergistic neuroprotective effects of hypothermia and hyperoxygenation is another research interest of ours. As even selective and more so whole body cooling may cause shivering and therefore increased oxygen consumption, hypothermia without additional oxygen supply might worsen hypoxic conditions in penumbral tissue. Besides, hypothermia causes a left-shift in the hemoglobin (Hb)-O<sub>2</sub> binding curve which leads to an increased affinity of O<sub>2</sub> towards hemoglobin and thus to a decreased release of O<sub>2</sub> from the blood into the surrounding tissue.

By combining hyperoxygenation and hypothermia, the potential hypoxic condition brought by hyperthermia can be minimized. Since hypothermia not only increases the concentration of Hb-bound O<sub>2</sub> in the blood, but also that of physically dissolved O<sub>2</sub> in the plasma which diffuses out of capillaries along a concentration gradient and independent of the Hb- O<sub>2</sub> binding curve. Similarly, hypothermia may also attenuate potentially neurotoxic reactive oxygen species (ROS) produced during hyperoxygenation. In our previous work, the neuroprotective capacity of whole body hypothermia (HT) combined with / without normal baric oxygenation (NBO) was preliminarily evaluated. Significantly lower infarct volume was observed in HT + NBO compared to HT. Currently, the synergistic neuroprotective effects of intra-arterial cold infusions (IACI) + NBO are under investigation.

### **SELECTED PUBLICATIONS**

Kollmar R, **Poli S**. Hypothermie und targeted temperature management (TTM) als Therapiekonzept. In: Schwab S, Schellinger P, Unterberg A, Werner C, Hacke W, eds. *Neurointensiv. Springer; 2015: 227-239.*

**Esposito E, Ebner M, Ziemann U, Poli S**. In cold blood: Intraarterial cold infusions for selective brain cooling in stroke. *J Cereb Blood Flow Metab.* 2014; 34: 743-752.

**Poli S, Purrucker J, Priglinger M, Sykora M, Diedler J, Rupp A, et al.** Safety evaluation of nasopharyngeal cooling (rhinochill(r)) in stroke patients: An observational study. *Neurocrit Care.* 2014; 20: 98-105.

**Poli S, Purrucker J, Priglinger M, Ebner M, Sykora M, Diedler J, et al.** Rapid induction of cooling in stroke patients (icool1): A randomised pilot study comparing cold infusions with nasopharyngeal cooling. *Crit Care.* 2014; 18: 582.

**Poli S, Purrucker J, Priglinger M, Diedler J, Sykora M, Popp E, et al.** Induction of cooling with a passive head and neck cooling device: Effects on brain temperature after stroke. *Stroke.* 2013; 44: 708-713.

**Poli S, Veltkamp R.** Oxygen therapy in acute ischemic stroke – experimental efficacy and molecular mechanisms. *Curr Mol Med.* 2009; 9: 227-241.

Schubert GA, **Poli S, Schilling L, Heiland S, Thome C.** Hypothermia reduces cytotoxic edema and metabolic alterations during the acute phase of massive sah: A diffusion-weighted imaging and spectroscopy study in rats. *J Neurotrauma.* 2008; 25: 841-852.

Schubert GA, **Poli S, Mendelowitsch A, Schilling L, Thome C.** Hypothermia reduces early hypoperfusion and metabolic alterations during the acute phase of massive subarachnoid hemorrhage: A laser-doppler-flowmetry and microdialysis study in rats. *J Neurotrauma.* 2008; 25: 539-548.

Thomé CFC, Schubert GA, **Poli S, Mendelowitsch A, Heiland S, Schilling L, et al.** Hypothermia reduces the metabolic alterations caused by acute vasospasm following SAH: A microdialysis and magnetic resonance spectroscopy study in rats. In: Macdonald RL, ed. *Cerebral vasospasm – advances in research and treatment. Thieme (New York); 2004: 146-152.*

# Neuroimmunology

Head: PD Dr. Felix Bischof

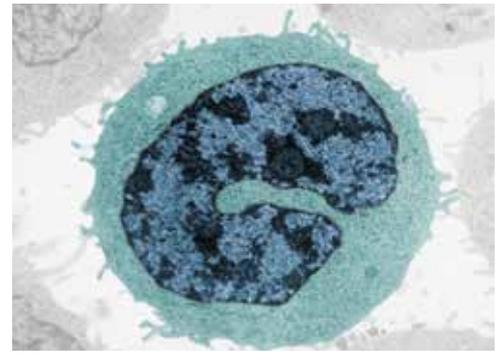
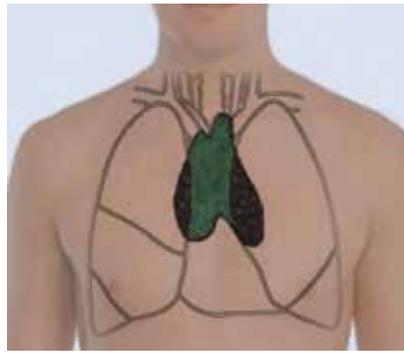
Team: 5 members

Key words: multiple sclerosis / neuromyelitis optica / progressive multifocal leukoencephalopathy / glycosylation / T cell differentiation / terminally differentiated B cells / T cell – neuronal interaction

**Work in this group is focused on the role of the immune system in inflammatory diseases of the central nervous system (CNS) and stroke and covers the whole spectrum from basic science to clinical science and translation into innovative treatments. Biochemical and cellular techniques as well as animal models are employed to assess how cells of the immune system interact with each other and with cells of the CNS. To this end, biotechnological tools were established to directly investigate and modulate the activity of CNS-specific CD4 T cells. In addition, we assess the function of immune cells in humans with inflammatory CNS diseases and develop and apply novel treatments to these patients and assess their efficacy and immunological consequences.**

*Die AG Neuroimmunologie beschäftigt sich mit der Rolle des Immunsystems bei entzündlichen Erkrankungen des Zentralen Nervensystems (ZNS) und beim Schlaganfall und deckt das gesamte Spektrum von der Grundlagenwissenschaft über die klinische Forschung bis hin zu neuen Therapieansätzen ab. Biochemische, zelluläre und tierexperimentelle Methoden werden eingesetzt, um die Funktion und Aktivität von Immunzellen und ihre Interaktion mit Zellen des ZNS zu untersuchen. Zu diesem Zweck wurden neue biotechnologische Methoden entwickelt, mit denen die Aktivität ZNS-spezifischer T-Helfer Zellen direkt untersucht und experimentell verändert werden kann.*

Anatomic position of the human thymus (black/green). Self tolerance and autoimmunity are dependent on a complex selection process in this organ.



Human T lymphocytes.

We established techniques to isolate, cultivate and analyze recently identified lymphocyte populations including murine and human T helper type nine (Th9) cells and terminally differentiated B lymphocyte populations. The interaction of Th9 cells and neurons was assessed using primary neuronal cultures, immunofluorescence microscopy, transcriptome analysis, calcium imaging and experimental autoimmune encephalomyelitis, the murine model of the human disease multiple sclerosis.

In addition, we assessed whether carbohydrate residues on the surface of immune cells are involved in regulating autoimmunity within the CNS. We demonstrated that during the development of experimental autoimmune encephalomyelitis (EAE), the animal model of multiple sclerosis, immune cells alter surface expression of N-linked carbohydrate residues. Specifically, we established a role of N-linked glycosylation in the development of induced regulatory T cells, a specific subset of lymphocytes which display a pivotal role in the regulation of self-directed immune responses.

In addition, we established alterations in peripheral lymphocyte populations that coincide with the development of the devastating human disease progressive multifocal leukoencephalopathy (PML).

## SELECTED PUBLICATIONS

Maricic I, Halder R, **Bischof F**, Kumar V.

Dendritic cells and anergic type I NKT cells play a crucial role in sulfatide-mediated immune regulation in experimental autoimmune encephalomyelitis.

*J Immunol.* 2014 Aug 1; 193(3): 1035-46.

Abbasi A, Forsberg K, **Bischof F**.

The role of the ubiquitin-editing enzyme A20 in diseases of the central nervous system and other pathological processes.

*Front Mol Neurosci.* 2015 Jun 15; 8: 21.

Dubois E, Ruschil C, **Bischof F**.

Low frequencies of central memory CD4 T cells in progressive multifocal leukoencephalopathy.

*Neurol Neuroimmunol Neuroinflamm.* 2015 Oct 29; 2(6): e177.

Hoffmann FS, Hofereiter J, Rübsamen H, Melms J, Schwarz S, Faber H, Weber P, Pütz B, Loleit V, Weber F, Hohlfeld R, Meinl E, **Krumbholz M**.

Fingolimod induces neuroprotective factors in human astrocytes.

*J Neuroinflammation.* 2015 Sep 30; 12(1): 184.

Wuhrer M, Selman MHJ, McDonnell LA, Kümpfel T, Derfuß T, Khademi M, Olsson T, Hohlfeld R, Meinl E, **Krumbholz M**.

Proinflammatory pattern of IgG1 Fc glycosylation in multiple sclerosis cerebrospinal fluid.

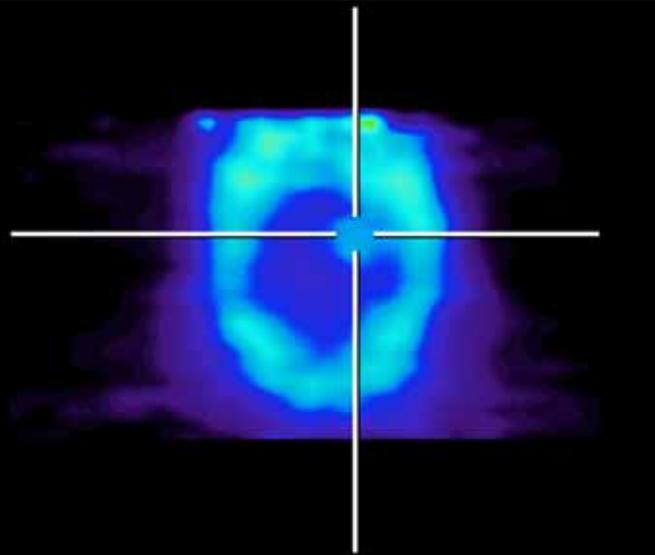
*J Neuroinflammation.* 2015 Dec 18; 12(1): 235. doi: 10.1186/s12974-015-0450-1.

# Clinical and Experimental Neuro-Oncology

Head: Prof. Dr. Ghazaleh Tabatabai

Team: 14 members

Key words: neuro-oncology / primary brain tumor / brain metastasis / cancer stem cells / cellular therapy / therapy resistance / oncolytic virus / innovative clinical trials



The central nervous system (CNS) can be affected by primary or by metastatic tumors. The majority of meningiomas, vestibular schwannomas and pituitary gland adenomas can be efficiently treated with neurosurgical intervention alone. Yet, recurrent or progressive disease occurs in these diseases, too. For most other histological entities, including astrocytoma, oligodendroglioma or ependymoma, even multimodality treatments only lead to a transient window of stable disease, depending on the additional molecular features that are present in the tumor, for example: presence or absence of mutations in the isocytate dehydrogenase (IDH), presence or absence of methylation of the O6-methylguaninmethyltransferase (MGMT), presence or absence of deletions of chromosomal regions 1p and/or 19q, presence or loss of alpha-thalassemia/mental retardation X-linked (ATRX), presence or absence and location of mutations in the human telomerase reverse transcriptase (TERT). Moreover, clinical evidence-based standards for metastatic CNS tumors are rare, because these patients have been mainly excluded from clinical trial enrollment until recently. Taken together, basic and translational research is a necessity to better understand molecular mechanisms of tumor initiation and acquired therapy resistance.

The scientific objectives of our group (students, technicians, postdoctoral researchers and physicians) are

- (i) To understand molecular principles of tumor initiation and recurrence, particularly by studying cancer stem cell biology and mechanisms of acquired therapy resistance.
- (ii) To generate novel and precise treatment strategies, particularly by using cell-based vehicles, oncolytic viruses, immunotherapeutic strategies
- (iii) To identify novel biomarkers, particularly by integrating longitudinal clinical information, imaging features and molecular alterations for facilitating the design of therapeutic strategies
- (iv) To conduct innovative clinical trials

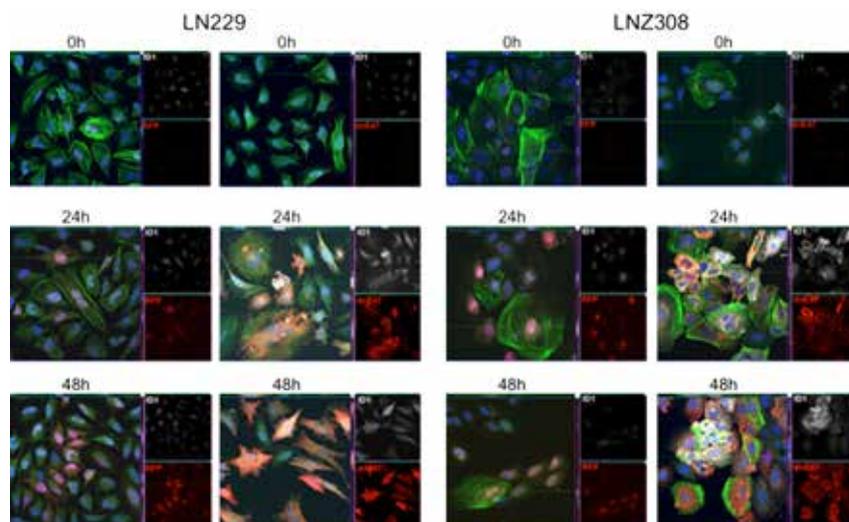
*Im Zentralen Nervensystem (ZNS) entstehen diverse primäre oder metastatische Tumoren. Zwar führt bei den meisten Patienten mit Meningeomen, Schwannomen und Hypophysenadenomen bereits die alleinige neurochirurgische Resektion zu einer Heilung. Jedoch gibt es auch hier wiederkehrende Tumoren. Bei den meisten anderen histologischen Entitäten, z.B. die Gruppe der Astrozytome, Oligodendrogliome oder Ependymome, führen hingegen sogar kombinierte multimodale Therapiestrategien nur zu einer vorübergehenden Stabilisierung, deren Dauer von weiteren molekularen Charakteristika in diesen Tumoren abhängt. Für Patienten mit ZNS-Metastasen, also Absiedlungen von Tumoren außerhalb des ZNS, sind klinische evidenzgesicherte Therapiestrategien selten, weil diese Patienten bis vor kurzem von einer Teilnahme in klinischen Studien ausgeschlossen wurden.*

*Folglich sind grundlagenwissenschaftliche und translationale Forschungsprojekte eine conditio sine qua non, um die molekularen Grundlagen der Tumorbiologie besser zu verstehen und darauf basierend neue therapeutische Zielstrukturen zu definieren.*

*Die wissenschaftlichen Ziele unserer Forschung sind*

- (i) *Analyse molekularer Grundlagen der Tumorinitiierung und –progression (Tumorstammzellen, Therapieresistenz)*
- (ii) *Entwicklung neuer Behandlungsstrategien und ihrer Kombination (Zellbasierte Therapie, onkolytische Viren, Immuntherapie)*
- (iii) *Identifikation neuer Biomarker (Integrative Analyse von Informationen aus klinischen Daten, Bildgebung und molekularen Alterationen) für die Tumor-spezifische Therapie und für Begleittherapien von Tumorpatienten*
- (iv) *Konzeption und Durchführung innovativer klinischer Studien*

Overexpression of dnE47 prevents nuclear translocation of ID-1. LN229 (column 1 and 2) and LNZ308 (column 3 and 4) were either lentivirally transduced with control (columns 1 and 3) or with dnE47 (columns 2 and 4) and subcellular ID-1 location (upper right insert) was observed for 48 hours. After initial nuclear localization, ID-1 was partly retained in the cytoplasm in dnE47-overexpressed cells.



### Targeting the transcriptional bHLH network

*Collaboration partner: Olivier Raineteau, PhD (Stem Cell and Brain Research Institute Lyon, France)*

Helix-loop-helix (HLH, ID proteins) and basic HLH (bHLH, e.g., Olig2) proteins are transcription factors and are well-characterized in the context of neural stem cell proliferation and maintenance. Glioblastoma express (b)HLH, too, and their overexpression correlates with poor clinical outcome. HLH/bHLH proteins need dimerization partners form either homo- or heterodimers with E proteins in the cytoplasm and translocate only then from the cytoplasm to the nuclear for DNA binding and transcriptional initiation. We overexpressed a dominant negative form of E47 (dnE47) that lacks its nuclear localization signal in long-term glioma cells and in glioma stem-like cells and thereby prevented nuclear translocation of bHLH proteins (Fig. 1). Our current experiments focus on dissecting the molecular network upon dnE47 overexpression for identifying potential therapeutic targets that can be applied in the clinical setting.

### Tumor-associated epilepsy

*Skardelly M, Brendle E, Noell S, Behling F, Wuttke T, Schittenhelm J, Bisdas S, Meisner C, Rona S, Tatagiba M, Tabatabai G; Predictors of preoperative and early postoperative seizures in patients with intra-axial primary and metastatic brain tumors: A retrospective*

*observational single center study.*

*Ann Neurol 2015;78:917-928.*

Antiepileptic treatment of brain tumor is rather ill-defined and depends on the individual physicians' choice. We aimed at developing a score for estimating risk seizures in subpopulations of brain tumor patients. To this end, we enclosed 650 patients > 18 years of age who underwent brain tumor surgery. Logistic regressions were performed to determine the effect sizes of seizure-related risk factors and to develop prognostic scores for the occurrence of preoperative and early postoperative seizures.

Age  $\geq$  60 years (OR = 3.32,  $p = 0.041$ ), total tumor/edema volume  $\leq$  64cm<sup>3</sup> (OR = 3.17,  $p = 0.034$ ), complete resection (OR = 15.50,  $p = 0.0009$ ), diencephalic location (OR = 12.2,  $p = 0.013$ ), and high-grade tumors (OR = 5.67,  $p = 0.013$ ) were significant risk factors for surgery-related seizures. Antiepileptics (OR = 1.20,  $p = 0.60$ ) did not affect seizure occurrence. For seizure occurrence, patients were stratified into 3 prognostic preoperative and into 2 prognostic early postoperative groups. Based on the developed prognostic scores, patient stratification for prospective studies may be feasible in the future.

### SELECTED PUBLICATIONS

Skardelly M, Brendle E, Noell S, Behling F, Wuttke TV, Schittenhelm J, Bisdas S, Meisner C, Rona S, Tatagiba MS, **Tabatabai G** (2015). Predictors of preoperative and early postoperative seizures in patients with intra-axial primary and metastatic brain tumors: A retrospective observational single center study. *Ann Neurol 78(6): 917-28.*

Weller M\*, **Tabatabai G\***, Kästner B, Felsberg J, Steinbach JP, Wick A, Schnell O, Hau P, Herrlinger U, Sabel MC, Wirsching HG, Ketter R, Bähr O, Platten M, Tonn JC, Schlegel U, Marosi C, Goldbrunner R, Stupp R, Homicsko K, Pichler J, Nikkhah G, Meixensberger J, Vajkoczy P, Kollias S, Hüsing J, Reifenberger G\*\*, Wick W\*\*, DIRECTOR Study Group (2015). MGMT Promoter Methylation Is a Strong Prognostic Biomarker for Benefit from Dose-Intensified Temozolomide Rechallenge in Progressive Glioblastoma: The DIRECTOR Trial. *Clin Cancer Res 2015 21(9): 2057-64* (\* co-shared first authorships, \*\* co-shared senior authorships).

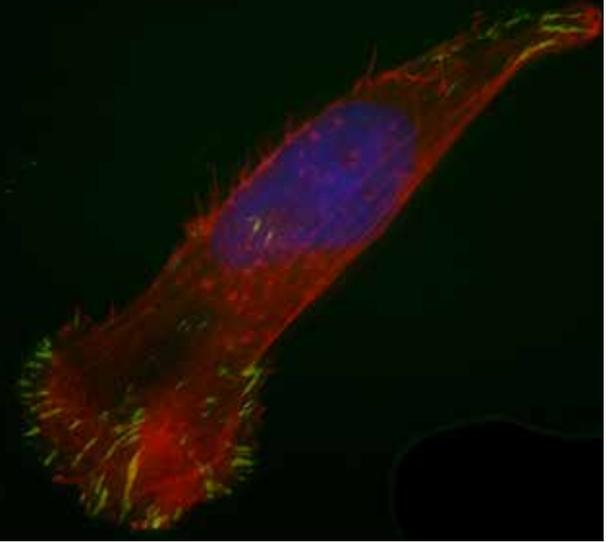
Wirsching HG, Krishnan S, Florea AM, Frei K, Hasenbach K, Reifenberger G, Krayenbuehl N, Weller M, **Tabatabai G** (2014). Thymosin  $\beta$ 4 gene silencing decreases stemness and invasiveness in glioblastoma. *Brain 137: 433-48.*

# Molecular Neuro-Oncology

Head: Prof. Dr. Ulrike Naumann

Team: 5 members

Key words: brain tumor / glioblastoma /  
virotherapy / gene therapy



Migrating glioblastoma cell

**The Research Group Molecular Neuro-Oncology investigates various aspects of the biology of glioblastomas (GBM), the most frequent and lethal human brain tumor. Characteristics of this tumor are its rapid and invasive growth into the healthy brain, its capability to suppress immune cells to attack the tumor as well as its resistance to chemotherapeutic drugs and radiation therapy. To know the biology of GBM in detail is important for the development of novel therapeutic strategies. We examine the therapeutic effects of natural compounds and so called oncolytic viruses and examine the function of cancer associated transcription factors and signalling cascades as well as that of secreted cellular proteins like cytokines or growth factor regulators to identify new targets for novel cancer therapy approaches.**

*Die Arbeitsgruppe für Molekulare Neuro-Onkologie befasst sich mit Fragestellungen zur Tumorbilogie des Glioblastoms (GBM), dem häufigsten und bösartigsten Hirntumor des Menschen mit einer, selbst bei optimaler Therapie, mittleren Überlebenszeit von nur 12 bis 15 Monaten. Die Bösartigkeit dieses Tumors basiert darauf, dass GBM schnell und invasiv in gesundes Hirngewebe einwachsen. Gliomzellen hindern zudem Immunzellen daran, sie zu attackieren und sind größtenteils resistent gegenüber Standardtherapien wie Bestrahlung oder Chemotherapie. Die Biologie des GBM zu kennen ist deshalb die Grundvoraussetzung, um neue Behandlungsverfahren entwickeln zu können. Wir arbeiten unter Verwendung verschiedener Strategien daran, das invasive Wachstum von GBM-Zellen zu vermindern und versuchen Tumorzellen wieder für Standard-Therapieansätze zu sensibilisieren. Zudem beschäftigen wir uns mit der Wirkung „onkolytischer“ Adenoviren, die für die GBM-Therapie eingesetzt werden können.*

Glioblastoma (GBM) is the most common and lethal brain neoplasm with a median patient survival after standard therapy of 12 to 15 month. Only few therapeutic regimens provide a short increase in survival. The failure of effective therapy regimens is based on its malignant characteristics: glioma cells are mainly resistant to chemotherapeutic drugs and radiation, they are highly motile, this way invading the healthy brain, and actively suppress the function of tumorspecific immune cells. In our research projects we are interested to receive information concerning the tumor immunology, to identify

factors that regulate the capability of a glioma cells to move, and to analyse how glioma cells manipulate their surrounding micromilieu to optimize survival and growth.

A glioma cell either migrates or proliferates, but never does both at the same time. This is known as the GO OR GROW hypothesis of glioma. Drivers of GBM motility are bad growth conditions like starvation or hypoxia. The induction of migration needs cytokines, protein modifiers altering the extracellular matrix, cytoskeleton members and regulators of adhesion. Contrarily, drivers of growth, which

means proliferation, are optimal living conditions and induction of growth of glioma cells involves dyregulated signaling pathways as well overexpression of growth factors. Until today, less is known about the factors that in glioma cells regulate the switch from GROW to GO or from GO to GROW. In collaboration with Prof. Mittelbronn (Neuropathology, Frankfurt/Main) we identified the neuropeptide processor carboxypeptidase E (CPE) as a switch factor involved in the decision of a cell to stay and grow or to move away if conditions are suboptimal. Reduced CPE expression levels in a cohort of GBM samples compared to healthy

brain prompted us to analyze the function of CPE as a putative tumor suppressor. Indeed, CPE loss in glioma patients was associated with worse prognosis. In glioma cells, CPE over-expression reduced, whereas its inhibition or knockdown enhanced GBM cell motility. Vice versa, enhanced CPE expression induced cell growth whereas cells with low CPE expression proliferate slower. Our findings indicate an anti-migratory role of CPE in GBM with prognostic impact for patient survival. In our recent project we investigate the role of CPE in cell signaling pathways associated with cell motility and cell proliferation as well as its capability to reduce the invasive growth of GBM cells in vivo using a mouse GBM model.

In Europe, cancer patients widely use mistletoe preparations for complementary cancer therapy. We could demonstrate that mistle lectins (MLs) enforce immune cells to attack and to kill GBM cells. Beside its immune stimulatory effect, mistletoe extracts as well as recombinant mistle lectin I mitigated GBM cell motility, paralleled by decreased expression of genes known to push and by enhanced expression of genes known to delimitate cancer progression. Besides this, mistletoe preparations raise the effects of the glioma standard therapy. Treatment of GBM with mistletoe extracts also delayed tumor growth in mice. MLs, showing multiple positive effects in the treatment of GBM, may therefore hold promise for concomitant treatment of human GBM. In our present experiments we are interested if MLs are able to inhibit GBM-induced neovascularization.

The tumor suppressor p53 is inactive in more than 50% of all human tumors, including GBM. We have explored the therapeutic potency of a synthetic, p53-based chimeric protein named CTS-1. CTS-1 expression induced growth arrest and cell death in cancer cell lines. Modulation of gene expression is responsible for the antitumor properties of CTS-1.

Interestingly, NFkB activation was mandatory for Ad-CTS-1 induced cell death. In a recent project we found that IkbZ, an atypical member of the inhibitor of NFkB family, being a modulator of NFkB transcriptional activity and harboring transcription factor activity, is differentially expressed in CTS-1-resistant versus CTS-1-sensitive glioma cells. IkbZ expression is furthermore induced by irradiation of glioma cells and induces the expression of inflammatory cytokines such as interleukin-6 or CXCL1. High levels of IkbZ correlate with poor prognosis of glioma patients. Therefore, IkbZ has been identified as a putative novel target for glioma therapy.

Oncolytic adenoviruses (OAV) that replicate selectively in tumor, but not in normal cells are used as potent and safe agents to fight cancer. These viruses have displayed potential to efficiently kill not only cancer cells, but also cancer stem cells. In collaboration with Dr. Holm (TU Munich) we have analyzed the antitumoral effects of an OAV. We have demonstrated that in vitro OAV works synergistically with the GBM standard chemotherapeutic drug temozolomide. In a mouse model using highly resistant GBM stem cells, intratumoral injection of this OAV induced tumor lysis and prolonged survival of tumor bearing mice.

## SELECTED PUBLICATIONS

**Brennenstuhl H, Armento A, Braczynski AK, Mittelbronn M, Naumann U.** (2015) In glioma cells, IkbZ, an atypical member of the inhibitor of nuclear factor kappa B (NFkB) family, is induced by gamma irradiation, regulates cytokine secretion and is associated with bad prognosis. *Int. J. Oncol.* 47, 2015: 1971-1980.

Kumar P, **Naumann U**, Aigner L, Wischhusen J, Beier CP, Beier D. (2015) Impaired TGF-β induced growth inhibition contributes to the increased proliferation rate of neural stem cells harboring mutant p53. *Am. J. Cancer Res.* 5: 3436-3445.

**Naumann U**, Holm PS. (2015) Oncovirotherapy of glioblastoma – a kind of immunotherapy? *Brain Disorders and Therapy* 2015, <http://dx.doi.org/10.4172/2168-975X.S2-001>.

Mantwill K, **Naumann U, Seznec J**, Girbinger V, Lage H Surowiak P, Beier C, Mittelbronn M, Schlegel J, Holm PS. (2013) YB-1 dependent oncolytic adenovirus efficiently inhibits tumor growth of glioma cancer stem like cells. *Translational Med.* 11: 216.

**Höring E**, Harter PN, **Seznec J**, Schittenhelm J, Bühring HJ, **Bhattacharyya S**, von Hattingen E, Zachskorn C, Mittelbronn M, **Naumann U**. (2012) The “go or grow” potential of gliomas is linked to the neuro-peptide processing enzyme carboxypeptidase E and mediated by metabolic stress. *Acta Neuropathol* 124(1): 83-97.

Podlech O, Harter P. N, Mittelbronn M, Pöschel S, **Naumann U**. (2012) Fermented mistletoe extract as a multimodal antitumoral agent in gliomas. *Evid Based Complement Alternat Med* 501796. doi: 10.1155/2012/501796.

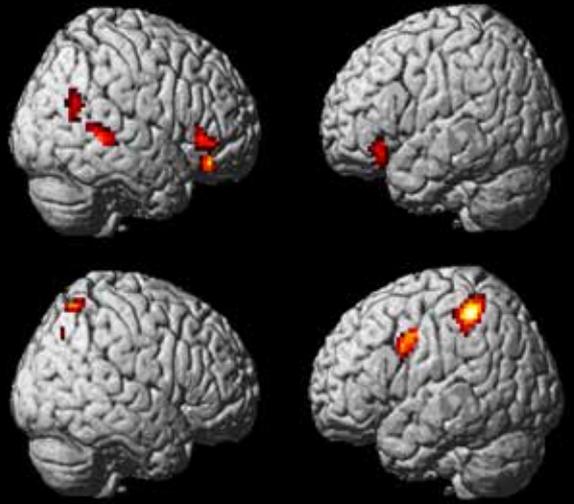
**Seznec J, Weit S, Naumann U.** (2010) Gene expression profile in a glioma cell line resistant to cell death induced by the chimeric tumor suppressor-1 (CTS-1), a dominant-positive variant of p53 – the role of NFkappaB. *Carcinogenesis* 31(3): 411-8.

# Neurophonetics

Head: Prof. Dr. Hermann Ackermann

Team: 4 members

Key words: speech production and perception /  
neurobiology of language / acoustic communication



**The Neurophonetics Group investigates the neural bases of speech communication – an unique capability of our species – based upon psycholinguistic methods and functional-imaging technology.**

*Die Arbeitsgruppe untersucht die neurobiologischen Grundlagen von Sprechmotorik und Sprachwahrnehmung insbesondere unter Verwendung funktionell-bildgebender Methoden.*

## **Blind subjects deploy visual cortex in order to better understand spoken language**

Blind individuals may learn to comprehend ultra-fast speech at a rate of up to about 22 syllables per second (syl/s), exceeding by far the maximum performance level of normal-sighted listeners (8-10 syl/s). Based upon previous functional Magnetic Resonance Imaging (fMRI) studies, a training experiment is currently under way. The data obtained so far support the notion that the recruitment of a “visual” strategy underlies the acquisition of this exceptional skill both in early as well as late-blind subjects. In addition to changes of hemodynamic activity in visual cortex, Diffusion Tensor Imaging (DTI) could demonstrate structural reorganization of white matter across a training period of several weeks (Dietrich et al., 2015b). Finally, evaluation of functional connectivity in terms of Dynamic Causal Modelling (DCM) revealed visual cortex (V1) to receive afferent auditory input via thalamic interfaces while the anterior supplementary motor area (SMA) represents its output target region. The strength of V1-SMA connectivity was found to correlate with behavioural performance of ultra-fast speech comprehension (Dietrich et al., 2015a).

## **The role of SMA and pre-SMA in speech perception**

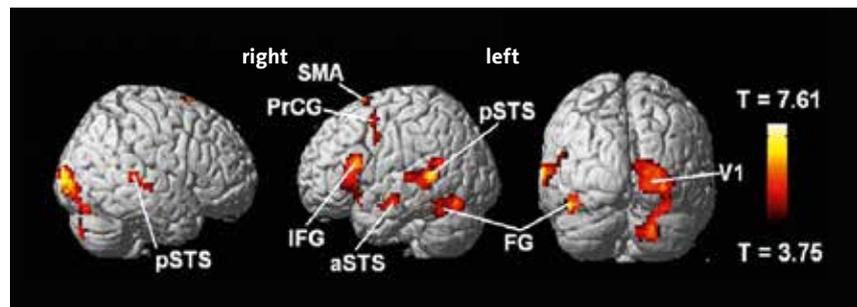
As an intriguing finding, the investigations of ultra-fast encoding of verbal utterances in blind subjects revealed activation of SMA proper and pre-SMA under these condition, indicating an involvement of mesiofrontal cortex in the control of top-down mechanisms of speech perception. Top-down prediction and reconstruction processes appear, by contrast, predominantly bound to the inferior-frontal speech generation system of the left hemisphere. Against this background, both a “phonological” and a “semantic” strategy may be deployed during ultra-fast speech perception, linked to different sub-networks of the language system. We propose that SMA proper is associated with a prearticulatory-phonological-auditory network comprising Brodmann area 44, connected to the so-called dorsal pathways of the perisylvian language network while pre-SMA, supporting semantic operations, is linked to more inferior and anterior frontal regions, connected to the ventral pathways of the language network. Transcranial Magnetic Stimulation (TMS) experiments (preliminary results in Dietrich et al., 2015c) are performed to further determine in how far SMA and pre-SMA are engaged in time-critical

aspects of speech processing (in cooperation with the Brain Networks & Plasticity Lab, see above).

## **Studies in neurophonetics and psycholinguistics**

As a final study of a research project on the German vowel system funded by the DFG (HE 1573/4-1), a psychoacoustic study was conducted which demonstrated that, indeed, vowel duration is used as the primary cue for the categorical phonological distinction between tense (long) and lax (short) vowels whereas subtle differences in vowel quality (centralization of short vowels) enhance the durational contrast by increasing the perceptual distance between short and long vowels (Tomaschek et al., 2015). Furthermore, our group was affiliated with Project B2 of the DFG-Sonderforschungsbereich 833, University of Tübingen, which addressed the semantic processing of so-called presuppositions. The behavioral, electroencephalographic (EEG), and magnetoencephalographic (MEG) studies conducted showed, among other things, that presuppositions - depending on linguistic context - may cause (i) increased reaction times (Tiemann et al., 2015), (ii) evoked EEG responses such as the N400 and P600, and (iii) altered auditory MEG responses to syllable onsets as well as widespread suppression of MEG alpha activity (Hertrich et al., 2015).

Whole-head fMRI analyses (14 blind, 12 sighted subjects) revealed activation clusters in right hemisphere primary-visual cortex (V1), left fusiform gyrus (FG), bilateral pulvinar (Pv) – not visible – and supplementary motor area (SMA), in addition to perisylvian “language zones”.



### Speech motor deficits in disorders of the cerebellum

In cooperation with L. Schöls and M. Synofzik, Center for Neurology, University of Tübingen, we try to clarify whether the syndrome of ataxic dysarthria separates into various subtypes, depending upon which component of the cerebellum is predominantly compromised. Patients with Friedreich ataxia or spinocerebellar ataxia (SCA3, SCA6) have been evaluated so far. Reduced speaking rate and voice irregularities were found specifically related to ataxia in other motor domains. In SCA-patients, by contrast, articulatory problems emerged as a predictor for ataxia severity (for a review see Ackermann & Brendel 2015).

### An evolutionary perspective on spoken language: vocal continuity between non-human and human primates

Any account of what is special about the human brain must specify the neural bases of our unique trait of articulate speech – and the evolution of these remarkable skills in the first place. Analyses of the disorders of acoustic communication following cerebral lesions/diseases as well as functional imaging studies in healthy subjects throw – together with paleo-anthropological data – some light on the phylogenetic emergence of spoken language, pointing at a two-stage model of the evolution of articulate speech:

- (i) monosynaptic refinement of the projections of motor cortex to the brainstem nuclei steering laryngeal muscles (brain size-associated phylogenetic trend), and a
- (ii) subsequent “vocal-laryngeal elaboration” of cortico-basal ganglia circuitries, driven by human-specific FOXP2 mutations.

A more extensive representation of laryngeal muscles within the basal ganglia should have allowed for the deployment of the vocal folds – beyond sound generation (“voice box”) – as an “articulatory organ” which can be pieced together with orofacial gestures into holistic “motor plans”, controlling syllable-sized movement sequences (for more details see Ackermann et al., 2014).

## SELECTED PUBLICATIONS

**Ackermann H, Brendel B.** The contribution of the cerebellum to speech and language. In: Hickok G, Small SL, editors. *The Neurobiology of Language*. Elsevier 2015:73-84.

**Ackermann H,** Hage SR, Ziegler W. Brain mechanisms of acoustic communication in humans and nonhuman primates: An evolutionary perspective. *Behavioral & Brain Sciences* 2014; 37: 529-546, 577-604.

**Dietrich S, Hertrich I, Ackermann H.** Network modelling for functional Magnetic Resonance Imaging (fMRI) signals during ultra-fast speech comprehension in late-blind listeners. *PLoS ONE* 2015a; 10: e0132196.

**Dietrich S, Hertrich I, Kumar V, Ackermann H.** Experience-related structural changes of degenerated occipital white matter in late-blind humans: A Diffusion Tensor Imaging (DTI) study. *PLoS ONE* 2015b; 10: e0122863.

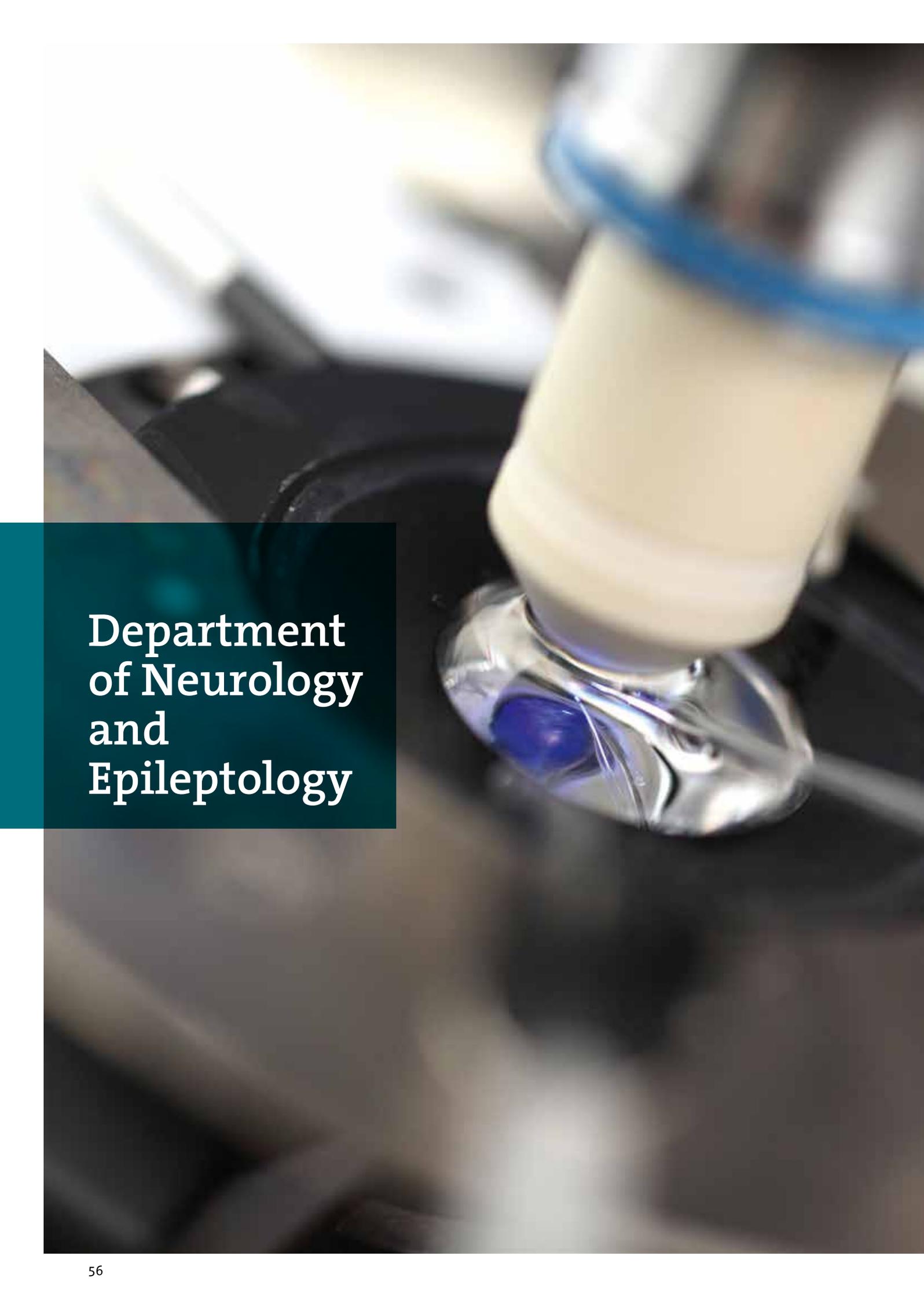
**Dietrich S,** Müller-Dahlhaus F, Ziemann U, **Ackermann H, Hertrich I.** The role of pre-SMA for time-critical speech perception: A transcranial magnetic stimulation (TMS) study. In: Wolters M et al., editors. Proceedings 18th International Congress of Phonetic Sciences. *Glasgow: University of Glasgow; 2015c:238.*

**Hertrich I,** Kirsten M, Tiemann S, Beck S, Wühle A, **Ackermann H,** Rolke, B. Context-dependent impact of presuppositions on early magnetic brain responses during speech perception. *Brain and Language* 2015a; 149:1-12.

**Hertrich I,** Mathiak K, **Ackermann H.** The role of the cerebellum in speech perception and language comprehension. In: Marien P, Manto M, editors. *The Linguistic Cerebellum*. Boston, MA: Academic Press, 2015b: 33-50.

Kirsten M, Tiemann S, Seibold V, **Hertrich I,** Beck S, Rolke B. When the polar bear encounters many polar bears: event-related potential context effects evoked by uniqueness failure. *Language, Cognition, and Neuroscience* 2014; 29: 1147-1162.

Marien P, **Ackermann H,** Adamaszek M et al. Consensus Paper: Language and the cerebellum – an ongoing enigma. *Cerebellum* 2014; 13: 386-410.



**Department  
of Neurology  
and  
Epileptology**



<b>DEPARTMENT OF NEUROLOGY AND EPILEPTOLOGY</b>	<b>58</b>
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## Departmental Structure



Prof. Dr. Holger Lerche heads the Department of Neurology and Epileptology

The Department of Neurology and Epileptology was founded with the generous support of the charitable Hertie Foundation and started its activities in November 2009. As part of the Center of Neurology and together with the other Neurological Departments, the Department of Epileptology is responsible for the clinical care of all neurological patients at the University Clinic Tübingen. A team of nurses, therapists and physicians trained for neurological disorders is available for the inpatient and outpatient clinics. The initial operations of the department have been focusing on establishing an effective structure to successfully support basic and clinical research in the field of epileptology and associated paroxysmal neurological disorders and provide excellence in patient care. Beside epileptology other foci are pain disorders, particularly headache and neuromuscular diseases. The clinic offers the whole spectrum of modern diagnostic and therapeutic procedures. The inpatient unit with 28 beds (Wards 45 and 42), running under the supervision of Prof. Dr. Y. Weber, PD Dr. N. Focke and PD Dr. T. Freilinger, includes acute care for epileptic seizures and status epilepticus, longterm complex treatment for difficult cases, and a Video-EEG-Monitoring Unit which is operated in cooperation with the Department of Neurosurgery. Within this unit, inpatients are continuously and simultaneously monitored with video and electroencephalography (EEG) for differential diagnostic and presurgical



evaluations. Epilepsy surgery, an effective treatment for patients resistant to anticonvulsive medication, deep brain stimulation of the thalamus and vagal nerve stimulation are provided in close cooperation with the Department of Neurosurgery (Dr. S. Rona, Prof. Dr. J. Honegger, Prof. Dr. A. Garabaghi). The epilepsy outpatient clinic (Prof. Dr. H. Lerche, Prof. Dr. Y. Weber and PD Dr. N. Focke) offers consulting and treatment in particular for difficult cases and specific questions including pregnancy under antiepileptic treatment and genetic aspects.

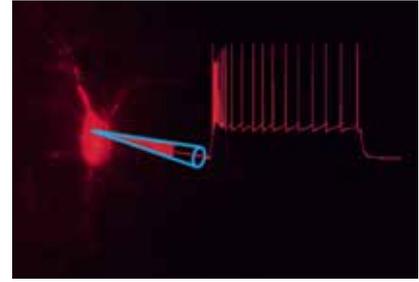
Other outpatient clinics are focused on headache and facial pain as well as other neurological pain syndromes (PD Dr. T. Freilinger), on neuromuscular diseases (Dr. C. Freilinger), and genetically determined paroxysmal neurological and ion channel disorders (Prof. Dr. H. Lerche and Prof. Dr. Y. Weber). Specific genetic diagnostic testing using parallel next generation sequencing of all known epilepsy genes in one step (also available for other neurological disorders) has been established together with PD Dr. S. Biskup who founded the company CeGaT in Tübingen. The department's study center has been involved in diverse medical trials to explore novel treatment options. The department supports the medical and neuroscientific education at the University of Tübingen by providing a comprehensive offer of lectures, seminars and courses.



The department stimulates synergies between physicians in the Neurological Clinic and basic research groups in the Hertie Institute with the aim to work on clinically driven basic research questions and rapidly transfer scientific progress into clinical practice.

Our main research topics are

- (i) the genetics and pathophysiology of hereditary epilepsy syndromes and related neurological disorders
- (ii) the closely related mechanisms of the excitability of nerve cells and neuronal networks
- (iii) the molecular function, pharmacology and localization of ion channels and transporters which are membrane proteins that regulate neuronal excitability
- (iv) the genetics and molecular pathophysiology of rare monogenic (e.g. hemiplegic migraine) as well as common types of migraine and other primary headache disorders
- (v) clinical characterization and genetics of neuromuscular diseases
- (vi) structural and functional brain imaging to detect epileptogenic lesions and foci, epileptogenic networks in the brain and to characterize cognitive consequences of epilepsy. This latter work is performed in close cooperation with the MEG Center and the Departments of Neuroradiology, Neuroimaging and Neurosurgery



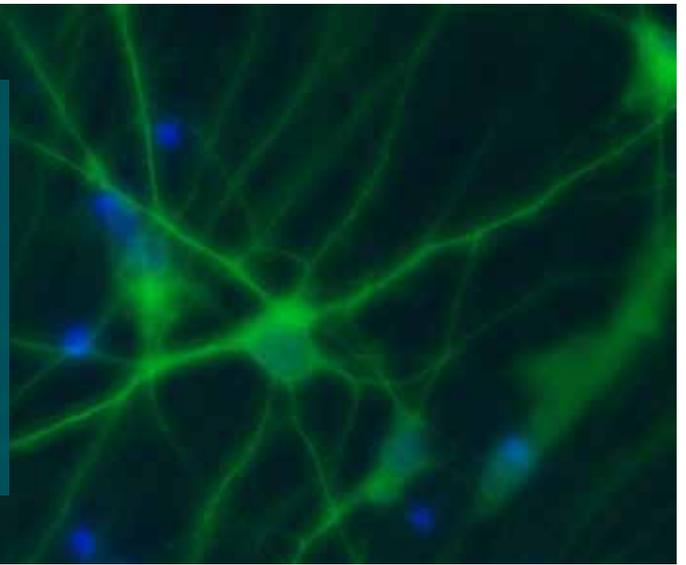
For electrophysiological recordings of neuronal activity in brain slices, a glass micropipette with a very fine tip (left) is brought in tight contact with a neuronal cell under microscopic control (middle). Recorded neurons are labelled with fluorescent dyes (red) to identify them after recordings; the picture shows a collage with a symbolized pipette and an original recording of a series of action potentials (right).

# Experimental Epileptology

Head: Prof. Dr. Holger Lerche

Team: 20 members

Key words: channelopathies / genetics / seizures / imaging / neuronal networks



Mouse primary hippocampal neurons expressing a GFP-tagged voltage gated potassium channel.

**The goal of our research is to link the molecular mechanisms of mainly genetic, neurological diseases caused by disturbed neuronal excitability to their clinical symptoms. We are recruiting well-defined cohorts of patients with epilepsies and related disorders (see group on Clinical Genetics of Paroxysmal Neurological Diseases), searching for disease-causing genetic defects with modern sequencing techniques, particularly in ion channels or transporters, and analyzing their functional consequences to understand the pathomechanisms and improve therapy. To study mechanisms of neuronal hyperexcitability on the molecular, cellular and network level, we use non-neuronal screening tools such as automated electrophysiology in oocytes and mammalian cells, neuronal expression systems including neurons derived from induced pluripotent stem cells, and gene-targeted mouse models.**

*Das Ziel unserer Forschung ist es, die molekularen Mechanismen vor allem genetischer, neurologischer Krankheiten mit einer gestörten neuronalen Erregbarkeit mit ihren klinischen Symptomen zu verknüpfen. Wir rekrutieren gut definierte Kohorten von Patienten mit Epilepsien und verwandten Krankheiten, suchen nach den genetischen Defekten mit modernen Sequenziermethoden, insbesondere in Ionenkanälen oder -transportern, und untersuchen deren funktionelle Auswirkungen, um die Pathomechanismen zu verstehen und die Therapie zu verbessern. Darüber hinaus untersuchen wir die Mechanismen neuronaler Übererregbarkeit auf molekularer, zellulärer und Netzwerkebene mit Screening-Methoden, wie automatisierter Elektrophysiologie in Oozyten oder Säugerzellen, in neuronalen Expressionssystemen einschließlich induzierter pluripotenter Stammzellen, und in genetisch veränderten Mausmodellen.*

Epilepsy affects up to 3 % of people during their life time, with a genetic component playing a major pathophysiological role in almost 50 % of cases. To analyze the genetic architecture of epilepsy we have been involved in national (National Genome Network, NGFNplus and German Network of Neurological and Ophthalmological Ion Channel Disorders, IonNeurONet), European (FP6: Epicure, ESF: EuroEPI-NOMICS, FP7: EpiPGX) and international (ILAE consortium on the genetics of complex epilepsies, collaboration with Epi4k, Epi25) research networks confined to the recruitment of large cohorts of

affected individuals and/or families and their genetic analyses. As examples, within the EuroEpinomics consortium and associated partners, we recently identified mutations in STX1B encoding the presynaptic protein syntaxin-1B in fever-associated epilepsy syndromes (Schubert et al. 2014), in genes encoding different GABA<sub>A</sub> receptor subunits (see also Functional Epilepsy Genetics group), in KCNA2 causing epileptic encephalopathies (Syrbe et al. 2015, see below), or in KCNC1 causing progressive myoclonus epilepsy (Muona et al. 2015). KCNC1 encodes an important K<sup>+</sup> channel

enabling high frequency firing of inhibitory neurons which is disturbed by the mutations. Many more rare and also common genetic factors were identified (see publications in the annex and the reports of the groups of Y. Weber and S. Maljevic), important to note is the first genome-wide significant GWAS analysis in epilepsy by the world-wide initiative of the ILAE consortium, which identified polymorphisms in the most important epilepsy gene (SCN1A encoding the main Na<sup>+</sup> channel in inhibitory neurons, see also below) as risk factors for epilepsy in general (ILAE consortium 2014).

Detected genetic variants are subjected to functional analysis in different heterologous systems. One screening method we use is the two-electrode voltage clamp system in *Xenopus laevis* oocytes. We could show that newly detected mutations in KCNA2, encoding the voltage-gated K<sup>+</sup> channel K<sub>v</sub>1.2, can lead to both gain and loss of channel function associated with different clinical entities (Syrbe et al. 2015). Strongly increased K<sup>+</sup> currents conducted by channels with gain-of-function mutations can be significantly reduced by application of 4-Aminopyridine, a licensed drug which is now tried as a precision therapy in patients to relieve disease symptoms, such as seizures, impaired cognition and ataxia (ongoing studies).

Functional implications of selected mutations are further examined in neuronal expression systems, such as transfected murine primary neurons and genetically-altered animal models carrying a human mutation (so-called “humanized mouse models”). The advantage of such mouse models is that altered channels can be studied in their natural environment and additionally, the consequences on intrinsic neuronal properties and network activity can be studied. Electrophysiological methods, including single cell patch clamp, extracellular recording or multielectrode array (MEA) techniques to analyze neuronal network activity are employed. With these techniques we characterized a knock-in mouse carrying a SCN1A mutation associated with generalized epilepsy with febrile seizures plus (GEFS+) and found a reduced excitability of inhibitory neurons in all examined brain regions. SCN1A is coding for the sodium Na<sub>v</sub>1.1 channel expressed in inhibitory neurons and our findings indicate a disinhibition to generate seizures in this model, with the key mechanism of interneuron dysfunction being a deficit of action potential initiation at the axon initial segment. This defect

leads to a widespread neuronal hyperexcitability which could be shown by network dysfunction in MEAs, in thalamocortical field recordings and by using Ca<sup>2+</sup> imaging of the hippocampus (Hedrich et al., 2014).

Taken together, our genetic and functional studies point to a pre- or postsynaptic GABAergic disinhibition as a major common pathway in several types of epilepsy. To take this further, we now use our mouse models to study network dysfunction in vivo with multi-electrode array recordings in cooperation with C. Schwarz (Systems Physiology group), and together with O. Garaschuk (Inst. Physiology II) we use in vivo 2-photon Ca<sup>2+</sup> imaging of the cortex for the same question.

Sudden unexplained death in epilepsy (SUDEP) is the main reason for a more than tenfold increased mortality of

epilepsy patients before the age of 50, and the SUDEP rate is particularly high in severe genetic epilepsies. In an ongoing project, we test the hypothesis if a dysfunction of the central regulation of breathing in the main underlying neuronal network (the PreBöttinger Complex) may be an important factor contributing to SUDEP. Therefore, we aim to unravel the consequences of known epilepsy-causing mutations in our genetic mouse models (SCN1A and SCN2A) on the function of the PreBötC.

Finally, we are reprogramming fibroblasts and keratinocytes obtained from patients carrying different epilepsy-causing mutations in ion channel genes to generate human induced pluripotent cells (hiPSC) (in cooperation with the Functional Epilepsy Genetics group).

## SELECTED PUBLICATIONS

Schubert J, Siekierska A, Langlois M, May P, Huneau C, Becker F, ... Biskup S, ... Crawford AD, Esguerra CV, Weber YG, Lerche H. Mutations in STX1B, encoding a presynaptic protein, cause fever-associated epilepsy syndromes. *Nature Genetics* 2014; 46: 1327-32.

Syrbe S\*, Hedrich UB\*, Riesch E\*, Djemie T\*, Muller S, ...Synofzik M, ... Loffler H, Detert K, ... Schols L, ... Weber YG, ... Biskup S, Wolff M, Maljevic S, Schule R, Sisodiya SM, Weckhuysen S, Lerche H\*\*, Lemke JR\*\*. De novo loss- or gain-of-function mutations in KCNA2 cause epileptic encephalopathy. *Nature Genetics* 2015; 47: 393-99.  
\*equally contributing authors; \*\* corresponding authors

Muona M, Berkovic SF, Dibbens LM, Oliver KL, Maljevic S, ... Lerche H, Palotie A, Lehesjoki AE. A recurrent de novo mutation in KCNC1 causes progressive myoclonus epilepsy. *Nature Genetics* 2015; 47: 39-46.

Hedrich UBS, Liautard C, Kirschenbaum D, Pofahl M, Lavigne J, Liu Y, Theiss S, Slotta J, Escayg A, Dihné M, Beck H, Mantegazza M, Lerche H. Impaired action potential initiation in GABAergic interneurons causes hyperexcitable networks in an epileptic mouse model carrying a human NaV1.1 mutation. *Journal of Neuroscience* 2014; 34: 14874-89.

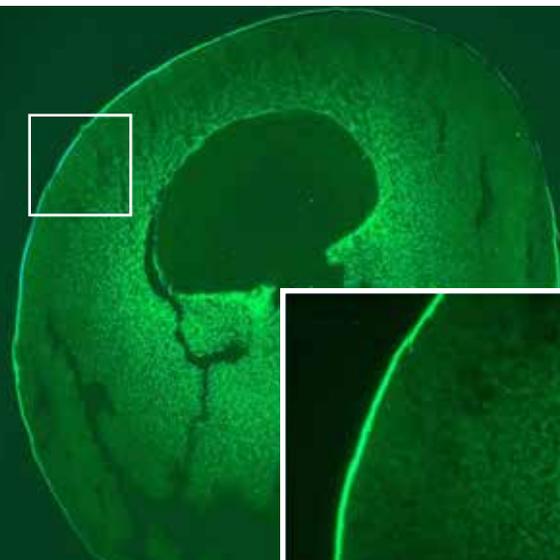
International League Against Epilepsy Consortium on Complex Epilepsies (incl. Weber Y and Lerche H). Genetic determinants of common epilepsies: a meta-analysis of genome-wide association studies. *Lancet Neurology* 2014; 13: 893-903.

# Clinical Genetics of Paroxysmal Neurological Diseases

Head: Prof. Dr. Yvonne Weber

Team: 9 members

Key words: paroxysmal neurological diseases / epilepsies / dyskinesia



Expression of the glucose transporter type 1 (Glut1) in *Xenopus laevis* oocytes.

**Paroxysmal neurological disorders include a broad spectrum of clinical entities. The research group is focused on the clinical genetics of epilepsies and paroxysmal dyskinesias, paroxysmal neurological disorders with overlapping clinical and pathophysiological features. In the last years, the main topics were the clinic-genetic evaluation of patients with PED (paroxysmal kinesigenic dyskinesia), EOAE (early-onset absence epilepsy), PKD (paroxysmal kinesigenic dyskinesia), the overlapping phenotype BFIS (benign familial infantile seizures) and different types of genetic epileptic encephalopathies leading to an intensive genetic and functional work on the genes SLC2A1 (Weber et al. 2008), PRRT2 (Schubert et al. 2012), CHD2 (Suls et al. 2013) and STX1B (Schubert et al. 2015). In 2015, mutations in the sodium channel gene SCN8A could be identified as a novel gene for PKD/BFIS in which also mutations in patients with epileptic encephalopathies were described before. This work emphasizes the joint pathophysiology of benign paroxysmal neurological disorders and severe epileptic phenotypes (Gardella et al. 2016).**

*Der Überbegriff der paroxysmalen neurologischen Erkrankungen beinhaltet ein breites Spektrum an klinischen Entitäten. Der wissenschaftliche Schwerpunkt der Arbeitsgruppe ist die klinisch genetische-genetische Untersuchung von Epilepsien und paroxysmalen Dyskinesien, die häufig pathophysiologische Überlappungen zeigen und ebenfalls zum Krankheitsspektrum der paroxysmalen neurologischen Erkrankungen zählen. In den letzten Jahren lag der Fokus auf den speziellen Formen PED (paroxysmale belastungsinduzierte Dyskinesie), EOAE (früh-beginnende Absence Epilepsie), der PKD (paroxysmale kinesiogene Dyskinesie), der überlappenden BFIS (benigne familiäre infantile Anfälle) und den verschiedenen Formen der genetischen epileptischen Enzephalopathien. Diese Arbeit resultierte in der intensiven genetischen und funktionellen Analyse der assoziierte Gene SLC2A1 (Weber et al. 2008), PRRT2 (Schubert et al. 2012), CHD2 (Suls et al. 2013) und STX1B (Schubert et al. 2014). In 2015 konnte erstmals mit der Detektion von Mutationen in der Natriumkanal-Untereinheit SCN8A ein weiteres Gen für PKD/BFIS beschrieben werden. Diese Arbeit unterstreicht erneut die enge pathophysiologische Verknüpfung benigner paroxysmaler neurologischer Erkrankungen mit den deutlich schweren Phänotypen der epileptischen Enzephalopathien (Gardella et al. 2016).*

Epilepsy is a very common neurological disease with a life time incidence of up to 3% in the general population. Epilepsies are divided in focal and generalized forms as well as in lesional (induced by e.g. scars, dysplasias or strokes) and genetic (idiopathic) forms looking from a pathophysiological point of view. Up to 30% of epilepsies are genetically determined.

Important groups of genetic epilepsies are:

- (i) idiopathic generalized epilepsies (IGE/GGE) like CAE, JAE, EGMA or JME
- (ii) the idiopathic focal epilepsies such as the Rolandic epilepsies
- (iii) the benign syndromes of early childhood such BFNS, BFIS and BFINS
- (iv) the epileptic encephalopathies such as the Dravet syndrome (SMEI)

Epilepsies are related to paroxysmal dyskinesias (PD) since both diseases can be found in the same family and can be based on the same genetic defect. Paroxysmal dyskinesias can be symptomatic (e.g. multiple sclerosis lesions found in the basal ganglia), but most of the described cases are of idiopathic/genetic origin. The genetic forms are divided in the following three subtypes:

- (i) non-kinesigenic dyskinesia (PNKD) induced by stress or alcohol
- (ii) kinesigenic dyskinesia (PKD), attacks induced by sudden voluntary movements
- (iii) exertion-induced dyskinesia (PED) induced by prolonged periods of exercise

#### Activities in 2015

We detected a novel gene for the PKD/BFIS complex. Previously only PRRT2 has been identified as the main gene for these phenotypes (see above). Now, we performed a detailed clinico-genetic and neurophysiological workup in three PRRT2-negative families. The exome analysis revealed a co-segregating heterozygous missense mutation in SCN8A encoding a voltage-gated sodium channel subtype ubiquitously expressed in the brain. A founder effect was excluded by linkage analysis. All individuals but one had normal cognitive and motor milestones, neuroimaging and interictal neurological status. Fifteen affected family members presented with afebrile focal or generalized tonic-clonic seizures during the first or second year of life. All patients stayed seizure-free, most of them without medication.

Five patients developed additional brief paroxysmal dyskinetic episodes in puberty, either dystonic/dyskinetic or “shivering” attacks, triggered by stretching, motor initiation or by emotional stimuli. Our studies establish SCN8A as a novel gene in which a recurrent mutation causes BFIS/PKD, expanding the clinical spectrum of combined epileptic and dyskinetic syndromes.

A second project focused on RBFOX1. Partial deletions of the gene encoding the neuronal splicing regulator have been reported in a range of neurodevelopmental diseases but also in GGE. In the recent study, deletions in RBFOX1 were also found in patients with several forms of focal epilepsy which underpins the hypothesis of several genetic pathophysiological factors also in those very common sporadic epilepsy forms.

## SELECTED PUBLICATIONS

**Weber YG et al.** GLUT1 mutations are a cause of paroxysmal exertion-induced dyskinesias and induce hemolytic anemia by a cation leak. *Journal of Clinical Investigation* 2008; 118: 2157-68.

**Schubert S, ..., Becker F, ..., Weber YG.** PRRT2 mutations are the major cause of benign familial infantile seizures (BFIS). *Hum Mutat* 2012; 33: 1439-43.

Suls A\*, Jaehn JA\*, Kecskés A\*, **Weber Y\***, et al. De novo loss-of-function mutations in CHD2 cause a fever-sensitive myoclonic epileptic encephalopathy sharing features with Dravet syndrome. *Am J Hum Genet* 2013; 93: 967-75. \* *equally contributing first authors.*

**Schubert S, ..., Becker F, ..., Weber YG\***, Lerche H\*. Mutations in STX1B encoding a presynaptic protein cause fever-associated epilepsy syndromes. *Nat Genet* 2014; 46: 1327-32. \* *contributed equally.*

Lal D, ..., **Becker F, ..., Weber YG.** Extending the phenotypic spectrum of RBFOX1 deletions: Sporadic focal epilepsy. *Epilepsia* 2015; 56: e129-33.

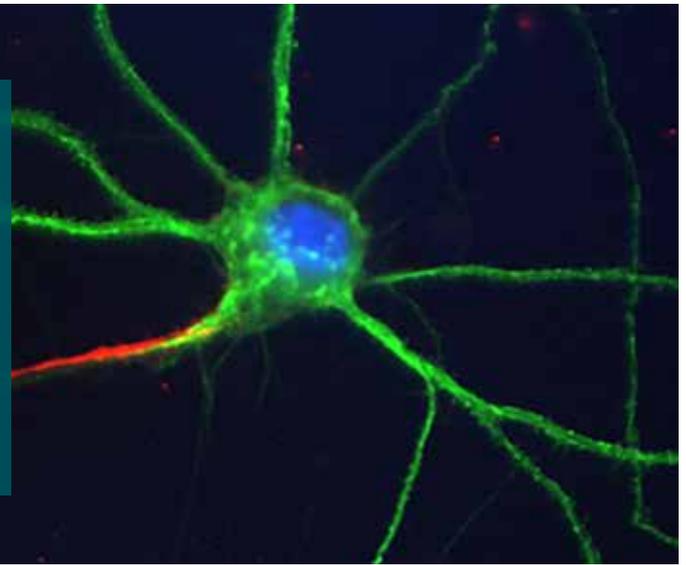
Gardella E, **Becker F, Møller RS, Schubert J, ..., Weber YG.** Benign infantile seizures and paroxysmal dyskinesia caused by an SCN8A mutation. *Ann Neurol* 2016; 79: 428-36.

# Functional Epilepsy Genetics

Head: Dr. Snezana Maljevic

Team: 6 members

Key words: GABA receptors / potassium channels / epilepsy / iPS cells

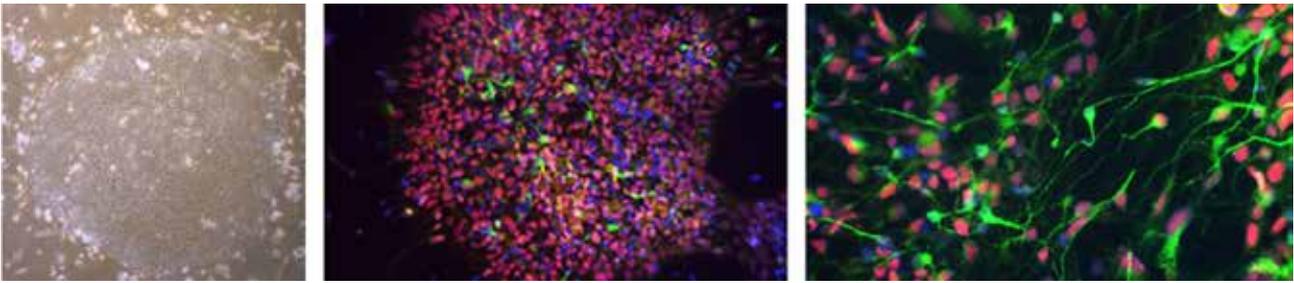


Mouse primary hippocampal neurons in culture, stained with a somatodendritic marker (MAP2, green) and a marker for the axon initial segment (AnkG, red).

**Our research focus is on two ion channel groups with a prominent role in the regulation of excitability, the neuronal Kv7 channels, and the GABA(A) receptors. Many genetic alterations in the corresponding KCNQ2/3 and GABRx genes have been linked to different epilepsy phenotypes, expanding from milder forms to severe epileptic encephalopathies. We use heterologous expression, murine primary neuronal cultures, genetic mouse models and human induced pluripotent stem (hiPS) cells to study the impact of the disease-causing mutations on the molecular, cellular and neuronal network level and understand better the biological processes these channels are involved in. Our main goal is to establish in vitro models that can be used in the precision medicine approaches and enable individualized treatment of the severely affected patients.**

*Unser Forschungsschwerpunkt liegt auf zwei Ionenkanalgruppen, die neuronalen Kv7 Kanälen und den GABA(A)-Rezeptoren, die eine prominente Rolle in der Regulierung der neuronalen Erregbarkeit spielen. Eine Reihe von genetischen Veränderungen in den entsprechenden KCNQ2/3 und GABRx Genen wurde mit unterschiedlichen epileptischen Phänotypen, von milderen Formen zu schweren epileptischen Enzephalopathien, in Verbindung gebracht. Wir verwenden heterologe Expression, murine primäre neuronale Kulturen, genetische Mausmodelle und menschliche induzierte pluripotente Stammzellen (hiPS) Zellen, um die Auswirkungen der krankheitsverursachenden Mutationen auf molekularer, zellulärer und neuronaler Netzwerkebene zu untersuchen. Unter anderem liegt unser Interesse daran, die gewonnenen Erkenntnisse zu nutzen um die biologische Prozesse an denen diese Kanäle beteiligt sind besser zu verstehen. Unser Hauptziel dabei ist es in vitro-Modelle zu schaffen, die in der personalisierten Medizin verwendet werden können und individualisierte Behandlung schwer betroffener Patienten ermöglichen.*

Detected genetic variants related to different epileptic syndromes are subjected to functional analysis in different heterologous systems. A useful screening strategy in our group is to use the *Xenopus laevis* oocyte expression system and examine effects of detected mutations using an automated two-voltage clamp system. In this way, we showed that newly detected variants affecting different subunits of the GABA(A) receptor diminish GABA-induced currents, which can explain the occurrence of seizures via reduced inhibition in the brain (Niturad et al., submitted, Johannesen et al., in revision, Moeller et al., in preparation). In addition, we analyzed a mutation affecting the KCNC1 gene encoding voltage-gated Kv3.1 channel, which occurred in 13 unrelated patients with progressive myoclonus epilepsy (PME). The mutation caused a loss of channel function with a dominant-negative effect since the mutant subunits impaired the function of the wild-type Kv3.1 (Muona et al., 2015). Because this channel is expressed in inhibitory neurons, our data suggested a disinhibition as a pathological mechanism of PME.



**Fig. 1** Induced pluripotent stem cells (iPSCs) derived from skin fibroblasts of an epilepsy patient (left) are used to generate neurospheres (middle) comprising cells expressing Sox2 (red), a neural progenitor marker, and TuJ1 (green) typical for young neurons. During the differentiation process (right), the culture is enriched with cells expressing neuronal marker MAP2 (green).

Mutations in the *KCNQ2* and *KCNQ3* genes encoding voltage-gated potassium channels Kv7.2 and Kv7.3 have been associated with a rare benign form of neonatal epilepsy (BFNS). However, recent studies in cohorts of severely affected children with refractory epilepsy and mental retardation linked de novo mutations in the *KCNQ2* gene to an epileptic encephalopathy (EE) phenotype. Functional analysis of mutations in these channels revealed a loss-of-function as a major pathomechanism, suggesting haploinsufficiency as a cause of neonatal seizures. Our previous study in *Xenopus laevis* oocytes indicated that a severe dominant-negative effect might present a pathomechanism underlining the epileptic encephalopathy (Orhan et al. 2014). To further explore the function of neuronal KCNQ channels and the pathomechanisms underlining related epileptic disorders, we have extended the initial characterization in *Xenopus laevis* oocytes with studies in the primary neuronal cultures. To this end, we use a *Kcnq2* knockout mouse to examine effects of a moderate and complete reduction of the Kv7.2 current on the activity of neuronal networks (Rosa et al., in preparation). Furthermore, an efficient lentivirus-based expression system has been applied to express several BFNS and EE mutations in

these neuronal cultures. Electrophysiological methods, including single cell patch clamp or multielectrode array (MEA) technique to analyze neuronal network activity, revealed increased excitability of single neurons and neuronal networks caused by *KCNQ2* mutations (Füll et al., in preparation).

In cooperation with the groups of Prof. Thomas Gasser and Prof. Stephan Liebau (Institute of Neuroanatomy, Tübingen), we have been generating induced pluripotent stem (iPS) cell lines from fibroblasts and

keratinocytes of patients with the *KCNQ2* EE and other epilepsy syndromes (see Experimental Epileptology). So far several differentiation protocols have been applied to obtain different types of neuronal cells from these lines to establish human disease models and examine mechanisms of epileptogenesis in a patient-derived system. The ongoing efforts aim at improving the maturity of obtained neuronal cells and networks and establishing appropriate electrophysiological, molecular and cellular assays to study the disease mechanisms.

## SELECTED PUBLICATIONS

Muona M, Berkovic SF, Dibbens LM, Oliver KL, **Maljevic S**,..., **Lerche H**, Palotie A, Lehesjoki AE. A recurrent de novo mutation in *KCNC1* causes progressive myoclonus epilepsy. *Nature Genetics* 2015; 47: 39-46.

**Orhan G**, **Bock M**, **Schepers D**, **Ilina EI**, **Reichel SN**, **Löffler H**, **Jezutkovic N**, **Weckhuysen S**, **Mandelstam S**, **Suls A**, **Danker T**, **Guenther E**, **Scheffer IE**, **De Jonghe P**, **Lerche H**, **Maljevic S**. Dominant-negative effects of *KCNQ2* mutations are associated with epileptic encephalopathy. *Annals of Neurology* 2014; 75: 382-94.

**Füll Y**, **Seeböhm G**, **Lerche H**, **Maljevic S**. A conserved threonine in the S1-S2 loop of Kv7.2 and Kv7.3 channels regulates voltage-dependent activation. *Pflugers Archive* 2013; 465: 797-804.

**Maljevic S**, **Naros G**, **Yalçin Ö**, **Blazevic D**, **Loeffler H**, **Cağlayan H**, **Steinlein OK**, **Lerche H**. Temperature and pharmacological rescue of a folding-defective, dominant-negative Kv7.2 mutation associated with neonatal seizures. *Human Mutation* 2011; 32: E2283-93.

**Maljevic S**, **Krampf K**, **Cobilanschi J**, **Tilgen N**, **Beyer S**, **Weber YG**, **Schlesinger F**, **Ursu D**, **Melzer W**, **Cossette P**, **Bufler J**, **Lerche H**, **Heils A**. A mutation in the GABA(A) receptor alpha(1)-subunit is associated with absence epilepsy. *Annals of Neurology* 2006; 59: 983-7.

# Migraine and Primary Headache Disorders

Head: PD Dr. Tobias Freilinger

Team: 8 members

Key words: migraine / headache / channelopathies / genetics / mouse models

Thalamocortical brain slice of a mouse strain expressing GFP in GAD67-positive inhibitory neurons

**Our group is interested in the genetic basis and molecular pathophysiology of migraine and other primary headaches as well as related paroxysmal neurological disorders and neurovascular phenotypes. We are covering the entire spectrum from rare monogenic entities to the common types of headache disorders, aiming at identifying clinically relevant biomarkers and establishing translational treatment approaches.**

*Unsere Gruppe interessiert sich für die genetische Basis und molekulare Pathophysiologie der Migräne, anderer primärer Kopfschmerzerkrankungen und verwandter paroxysmaler neurologischer / neurovaskulärer Phänotypen. Wir untersuchen dabei das gesamte Spektrum von sehr seltenen und schwer verlaufenden monogenen Entitäten bis hin zu den häufigen Formen der Erkrankung, mit dem Ziel, klinisch relevante Biomarker zu identifizieren und translationale Therapie-Ansätze zu etablieren.*

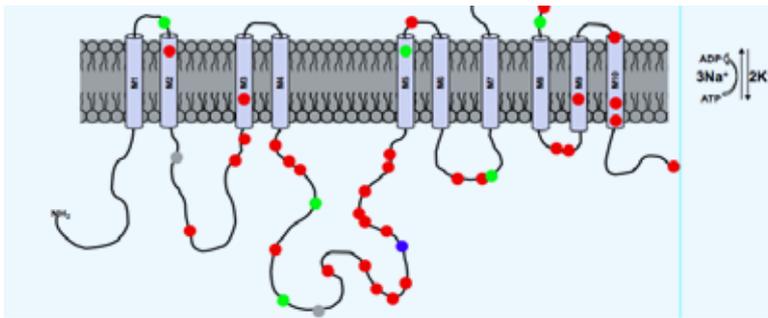
As a monogenic model disease, we are studying hemiplegic migraine (HM), a severe subtype of migraine with aura, characterized by some degree of hemiparesis in addition to other aura symptoms. In the past, we have made significant contributions to unraveling the genetics of HM, which is caused by mutations in three different genes (*CACNA1A*, *ATP1A2* and *SCN1A*) responsible for ion translocation in the central nervous system. In order to study mechanisms underlying cortical hyperexcitability in HM and better understand the differential pathophysiology of migraine vs. epilepsy, we are performing multimodal *in vitro* and *in vivo* analysis of a transgenic knock-in HM mouse model generated by our group, focusing specifically on cortical spreading depression (CSD), the likely correlate of migraine aura. Complementary to these functional studies we are conducting

translational pilot trials in affected patients aiming at both acute and prophylactic treatment. Finally, there are ongoing efforts aiming at further gene identification in HM.

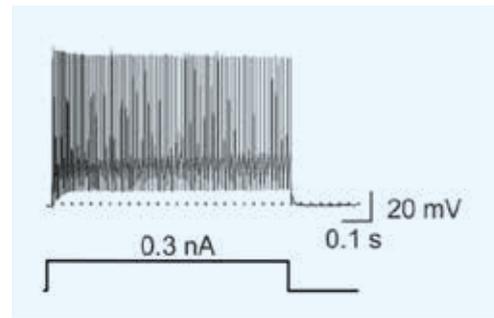
As a second major focus we are interested in the common genetically complex types of migraine. As part of the International Headache Genetics Consortium (IHGC) we are prominently involved in the identification of all currently established robust risk variants for sporadic migraine. Extending on these findings, we are currently interested in characterizing the genetic basis of common and clinically relevant comorbidities of migraine, with a special focus on the link between migraine and cerebrovascular disorders (in particular cervical artery dissection). Further, we are trying to correlate data from high-throughput genotyping studies

with clinically relevant parameters and extend genetic approaches also to other primary headache disorders, including in particular trigemino-autonomic cephalalgias (e.g. cluster headache or SUNCT).

Our research portfolio is complemented by epidemiological and clinical studies in the field of headache disorders including studies of reversible cerebral vasoconstriction syndrome (RCVS) and clinical studies evaluating the effect of placebo effects in neurological disorders, which are performed in collaboration with the Department of Psychosomatic Medicine and Psychotherapy. Finally, we are engaged in several multicenter clinical treatment trials in headache disorders, which are performed in the context of our outpatient headache unit.

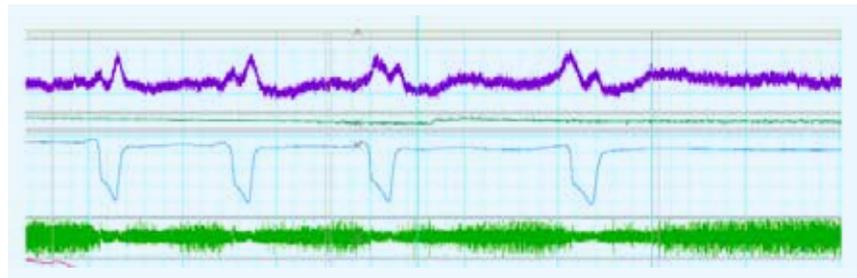


Membrane topology of the sodium/potassium transporter encoded by the HM gene ATP1A2, with localization of different causative mutations indicated by coloured circles.

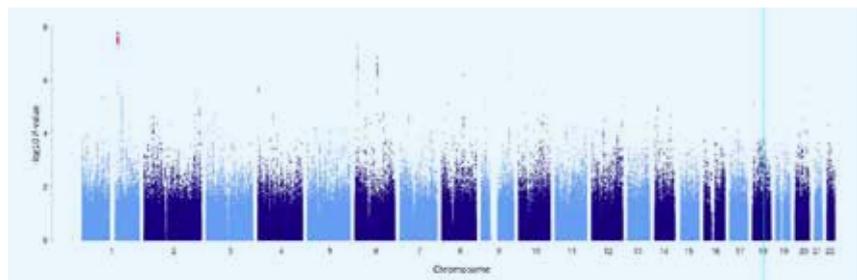


Firing activity of a hippocampal fast spiking interneuron as answer to a 0.3 nA current injection. The cell was clamped to -70 mV (dotted line).

Representative traces from multiparametric in vivo monitoring of transgenic HM animals



Graphical representation ('Manhattan plot') of several risk loci for the common types of migraine (adopted from Freilinger et al. 2012)



## SELECTED PUBLICATIONS

Malik R, Winsvold B, **Auffenberg E**, Dichgans M, **Freilinger T**. The migraine – vascular disease connection: a genetic perspective. *Cephalalgia* 2015. pii: 0333102415621055, *Epub ahead of print*.

Malik R, **Freilinger T**, [...]. Assessment of the shared genetic basis between ischemic stroke and migraine. *Neurology* 2015; 84(21):2132-45.

de Vries B, Anttila V, **Freilinger T**, Wessman M, Kaunisto MA, Kallela M, Artto V, Vijfhuizen LS, Göbel H, Dichgans M, Kubisch C, Ferrari MD, Palotie A, Terwindt GM, van den Maagdenberg AMJM, on behalf of the International Headache Genetics Consortium. Systematic re-evaluation of candidate migraine genes in a large genome-wide association data set. *Cephalalgia* 2015; pii: 0333102414566820, *Epub ahead of print*.

Winsvold B, Nelson CP, Malik R, Anttila V, [...], **Freilinger T**, [...], Palotie A. Assessing shared genetic risk between migraine and coronary artery disease using genome-wide association data. *Neurology Genetics* 2015; 1: DOI 10.1212/NXG.000000000000010, *Epub ahead of print*.

Roth C, **Freilinger T**, Kirovski G, Dunkel J, Shah Y, Wilken B, Rautenstraub B, Ferbert A. Clinical spectrum in three families with familial hemiplegic migraine type 2 including a novel mutation in the ATP1A2 gene. *Cephalalgia* 2014; 34(3): 183-90.

**Freilinger T**. Genetik primärer Kopfschmerzen. *Bundesgesundheitsblatt* 2014; 57(8): 919-27.

Anttila V, Winsvold B, ..., **Freilinger T**, Schoenen J, Frants R, et al. Genome-wide meta-analysis of 23 285 individuals with migraine. *Nature Genetics* 2013; 45(8): 912-7.

**Freilinger T**, Anttila V, de Vries B et al. Genome-wide association analysis identifies susceptibility loci for migraine without aura. *Nature Genetics* 2012; 44(7): 777-782.

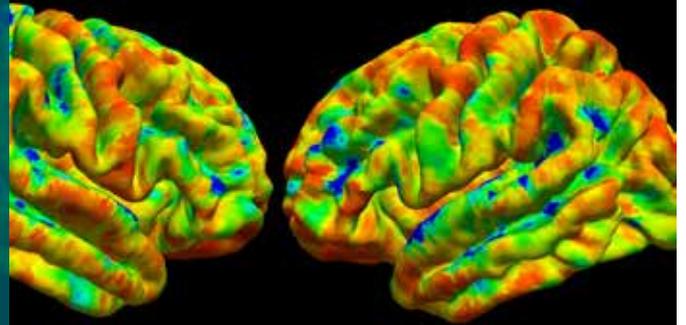
Dichgans M, **Freilinger T**, Eckstein G, Babini E, Lorenz-Depiereux B, Biskup S, Ferrari MD, Herzog J, van den Maagdenberg AM, Pusch M, Strom TM. Mutation in the neuronal voltage-gated sodium channel SCN1A in familial hemiplegic migraine. *Lancet* 2005; 366(9483): 371-7. \*equal contributions

# Translational Neuroimaging

Head: PD Dr. Niels Focke

Team: 9 members

Key words: multi-modal imaging / epilepsy / post-processing / classification methods



Cortical structural connectivity derived from whole brain fibre tracking

**The focus of our group is structural and functional imaging in neurological diseases with a particular focus on epilepsy. We are interested in better understanding the biology of pathological, neurological processes and translating these results to improved patient care and earlier diagnosis. We apply several computational, post-processing methods including voxel-based morphometry, machine learning and network analysis based on MRI, MEG, HD-EEG and PET.**

In epilepsy, we are interested in better defining the structural and functional abnormalities associated with seizure generation (“epileptogenic zone”) by means of imaging including high-field MRI (3T and 9.4T) and post-processing. Moreover, we apply diffusion-tensor imaging to analyze how epilepsy and seizures affect the structural networks of the brain. On the functional

side, we use functional MRI together with high-density EEG (256 channels) and MEG to assess functional networks characteristics and spread of ictal discharges i.e. epileptic activity. We also apply PET to study metabolic disease effects. This broad range of non-invasive methods provides us with comprehensive access to brain networks in humans and in-vivo.

## Imaging Modalities

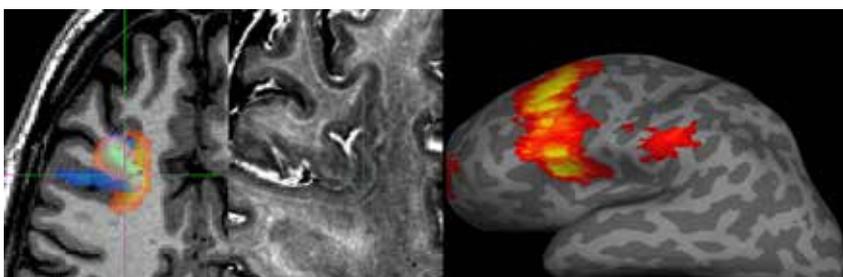
- MRI (structural and functional incl. simultaneous EEG-fMRI)
- HD-EEG (256 channels)
- MEG (275 channels, whole brain)
- PET-MRI (hybrid system, incl. simultaneous PET-MRI-EEG)

## Recent results

In patients with idiopathic/genetic generalized epilepsy (IGE/GGE) we could demonstrate microstructural network alterations based on diffusion tensor imaging although routine MR imaging

was completely normal (Focke et al., 2014). Moreover, based on functional imaging (MEG) we could show increased network connectivity in IGE/GGE in the resting state (Elshahabi et al., 2015). These findings will aid in better understanding the neurobiology of IGE/GGE with rapidly generalized seizures.

In focal epilepsy, we routinely apply our multi-modal imaging approach in patients being evaluated for epilepsy surgery. In a particularly interesting case with musicogenic epilepsy (seizures evoked by certain music) we could non-invasively predict the onset and propagation of epileptic activity using imaging (dynamic causal modelling). These predictions were later confirmed by invasive EEG and surgical resection (Klamer et al., 2015b). Also, we have worked on integrating and systematically comparing different imaging modalities (Klamer et al., 2015a).



Focal cortical dysplasia at 3T (including voxel-based morphometry) and at 9.4T, as well as regionally increased functional connectivity based on resting-state MEG (left to right).

Der Schwerpunkt unserer Forschungsgruppe ist die strukturelle und funktionelle Bildgebung neurologischer Erkrankungen mit besonderem Fokus auf die Epileptologie. Wir nutzen die technischen Methoden multi-modaler Bildgebung, um das Verständnis der Krankungsentstehung zu verbessern und in klinisch nutzbare Anwendungen zu überführen („Translation“). Ziele sind frühere Diagnosestellungen, automatisierte Läsion-Detektionen und Entwicklung bildgebungs-basierte Biomarker für die Klinik. Hierfür verwenden wir zahlreiche, Computer-basierte Techniken wie Voxel-basierte Morphometrie, Maschinen-Lernen und Netzwerk-Analysen basierend auf MRT, MEG, HD-EEG und PET.

In der Epileptologie sind wir daran interessiert, die strukturellen und funktionellen Veränderungen zu analysieren, die für die Entstehung von Anfällen verantwortlich sind (die s.g. „epileptogene Zone“). Eine wichtige Methode hierfür ist das Nachverarbeitung von strukturellen MRT Daten mit statistischen Techniken („Post-Processing“). Hiermit können sehr kleine, zuvor oftmals übersehene Läsionen entdeckt werden. Wir arbeiten hier mit Feldstärken von 3

bis 9.4 Tesla (Hochfeld-MRT). Weiterhin nutzen wir Diffusion-Bildgebung, um den Zusammenhang von Epilepsie und Anfällen auf die strukturellen Netzwerke zu untersuchen. Für die Analyse funktioneller Netzwerke verwenden wir ein sehr breites Spektrum von Methoden inkl. fMRT (BOLD), MEG, HD-EEG und PET. Die unterschiedliche räumliche und zeitliche Auflösung dieser Techniken ermöglicht einzigartige Einblicke in die sehr dynamischen neuronalen Prozesse beim Menschen in-vivo. So können wir mit EEG und MEG sowie parallelen EEG-fMRT die Quellen und Ausbreitung epileptischer Aktivität erfassen. Außerdem kann der „Ruhezustand“ (resting-state) des Gehirns untersucht werden.

#### Verwendete Bildgebungs-Modalitäten

- MRT (strukturell und funktionelle inkl. simultanes EEG-fMRT)
- HD-EEG (256 Kanäle)
- MEG (275 Kanäle, Ganzhirn)
- PET-MRT (Hybrid-System, inkl. simultanes PET-MRT-EEG)

#### Aktuelle Ergebnisse

Bei Patienten mit idiopathischer/genetischer generalisierter Epilepsie (IGE/GGE) konnten wir kürzlich deutliche

Veränderungen der Netzwerk-Mikrostruktur nachweisen, die in der Routine-MRT nicht sichtbar sind (Focke et al., 2014). Darüber hinaus konnten wir mit funktioneller Bildgebung (MEG) deutliche eine deutlich erhöhte Konnektivität, d.h. verstärkte Netzwerk-Verbindungen, bei IGE/GGE im Ruhezustand detektieren (Elshahabi et al., 2015). Diese Ergebnisse können uns helfen, die Neurobiologie der IGE/GGE mit schnell generalisierenden Anfällen besser zu verstehen. Weiter Studien beschäftigen sich mit Netzwerkanalysen bei definierten, s.g. mono-genetischen Epilepsien. Bei fokalen Epilepsien verwenden wir inzwischen routinemäßig unser multi-modales Bildgebungsprogramm in der prä-chirurgischen Diagnostik. So konnten wir z.B. bei einem Patienten mit musikogener Epilepsie (durch spezielle Musik ausgelöste Anfälle), die Entstehung und Ausbreitung der epileptischen Aktivität mit der multi-modalen Bildgebung korrekt vorherzusagen. Dies konnte später im invasiven EEG bestätigt werden (Klamer et al., 2015b). Weiterhin arbeiten wir an einer systematischen Integration und Vergleich der Modalitäten, z.B. fMRT, EEG und MEG (Klamer et al., 2015a) und auch tri-modal d.h. PET-MRT-EEG.

## SELECTED PUBLICATIONS

**Klamer S**, Rona S, **Elshahabi A**, **Lerche H**, Braun C, Honegger J, Erb M, **Focke NK**. Multimodal effective connectivity analysis reveals seizure focus and propagation in musicogenic epilepsy. *Neuroimage* 2015; 113:70-7.

**Elshahabi A**, **Klamer S**, **Sahib AK**, **Lerche H**, Braun C, **Focke NK**. Magnetoencephalography Reveals a Widespread Increase in Network Connectivity in Idiopathic/Genetic Generalized Epilepsy. *PLoS one* 2015; 10(9): e0138119.

**Focke NK**, Diederich C, Helms G, Nitsche MA, **Lerche H**, Paulus W. Idiopathic-generalized epilepsy shows profound white matter diffusion-tensor imaging alterations. *Human brain mapping* 2014; 35(7): 3332-42).

**Focke NK**, Yogarajah M, Symms MR, Gruber O, Paulus W, Duncan JS. Automated MR image classification in temporal lobe epilepsy. *Neuroimage* 2012; 59(1): 356-62.

**Focke NK**, Helms G, Scheewe S, Pantel PM, Bachmann CG, Dechent P, Ebentheuer J, Mohr A, Paulus W, Trenkwalder C. Individual voxel-based subtype prediction can differentiate progressive supranuclear palsy from idiopathic Parkinson syndrome and healthy controls. *Human brain mapping* 2011; 32(11): 1905-15.

Yogarajah M, **Focke NK**, Bonelli SB, Thompson P, Vollmar C, McEvoy AW, Alexander DC, Symms MR, Koeppe MJ, Duncan JS. The structural plasticity of white matter networks following anterior temporal lobe resection. *Brain: A journal of neurology* 2010; 133(Pt 8): 2348-64.

# Functional Neuronal Network And Neural Stem Cells

Head: PD Dr. Marcel Dihné  
*(since May 2013 head of department St. Lukas Klinik Solingen, but further associated to research at the HH)*

Team: 1 member

Key words: stem cells / functional neuronal networks

## **Development and pharmacological modulation of embryonic stem cell-derived neuronal network activity**

Neuronal network activity can be assessed by the microelectrode array (MEA) technology that allows simultaneous recording of the electrical activity exhibited by entire populations of neurons over several weeks or months in vitro. We demonstrated that ES cell-derived neural precursors cultured on MEAs for 5 to 6 weeks develop functional neuronal networks with oscillating and synchronous spike/burst patterns via distinct states of activity and towards late maturational processes. These processes were accompanied by an increasing density of presynaptic vesicles. Furthermore, we demonstrated that ES cell-derived network activity was sensitive to synaptically acting drugs indicating that pharmacologically susceptible neuronal networks were generated. Thus, the MEA technology represents a powerful tool to describe the temporal progression of stem cell-derived neural populations towards mature, functioning neuronal networks that can also be applied to investigate

pharmacologically active compounds. Actually, we are generating human functional neuronal networks from both native human embryonic and induced pluripotent stem cells.

## **Effects of inflammatory cytokines on neural stem cells**

Primary and secondary inflammatory processes are playing a role in nearly all brain pathologies. As endogenous neural stem cells supply the brain throughout life with new functional cells, it is important to verify the effect of inflammatory processes that include e. g. the up-regulation of cytokines on neural stem cells.

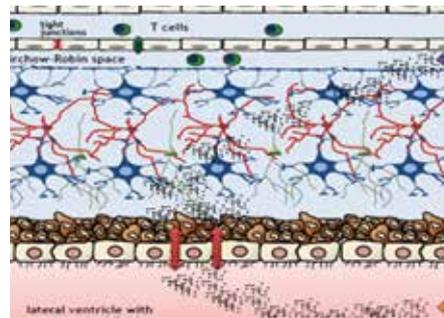
## **Epilepsy-associated alterations of invitro neuronal network activity**

The impact of epilepsy-associated mutation in genes encoding for ion channels on neuronal network activity is currently under investigation.

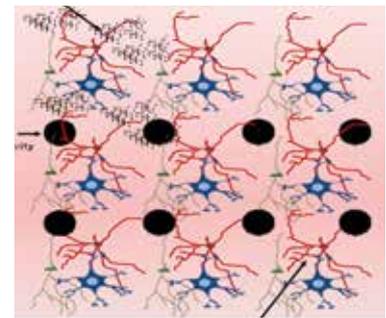
## **Volume transmission-mediated encephalopathies**

There is strong evidence that the composition of cerebrospinal fluid (CSF) influences brain development, neurogenesis and behavior.

The bi-directional exchange of CSF and interstitial fluid (ISF) across the ependymal and piagial membranes is required for these phenomena to occur. Because ISF surrounds the parenchymal compartment, neuroactive substances in the CSF and ISF can influence neuronal activity. Functionally important neuroactive substances are distributed to distant sites of the central nervous system by the convection and diffusion of CSF and ISF, a process known as volume transmission. It has recently been shown that pathologically altered CSF from patients with acute traumatic brain injury suppresses in vitro neuronal network activity (ivNN A) recorded by multielectrode arrays measuring synchronously bursting neural populations. Functionally relevant substances in pathologically altered CSF were biochemically identified, and ivNN A was partially recovered by pharmacological intervention. When considering the concept of volume transmission, it remains unclear whether the in vivo parenchymal compartment remains unaffected by pathologically altered CSF that significantly impairs ivNN A.



In vivo situation of neuropil with astrocytes (blue) and different kinds of neurons (red, green). The lateral ventricle (pale red) contains cerebrospinal fluid with neuroactive substances.



In vitro situation.

We hypothesize that the relevance of pathological CSF alterations goes far beyond the passive indication of brain diseases and that it includes the active and direct evocation of functional disturbances in global brain activity through the distribution of neuroactive substances, for instance, secondary to focal neurological disease. For this mechanism, we propose the new term “volume transmission-mediated encephalopathies” (VTE). Recording ivNN A in the presence of pure human CSF could help to identify, monitor and potentially suggest means for antagonizing functionally relevant CSF alterations that direct result in VTEs.

## SELECTED PUBLICATIONS

**Engelholm M**, Leo-Kottler B, Rempp H, Lindig T, Lerche H, Kleffner I, Henes M, **Dihné M**. Encephalopathic Susac's Syndrome associated with livedo racemosa in a young woman before the completion of family planning. *BMC Neurol.* 2013 Nov 25; 13: 185. doi: 10.1186/1471-2377-13-185.

Jantzen SU, Ferrea S, Wach C, Quasthoff K, Illes S, Scherfeld D, Hartung HP, Seitz RJ, **Dihné M**. In vitro neuronal network activity in NMDA receptor encephalitis. *BMC Neurosci.* 2013 Feb 5; 14: 17. doi: 10.1186/1471-2202-14-17.

Walter J, **Dihné M**. Species-dependent differences of embryonic stem cell-derived neural stem cells after Interferon gamma treatment. *Front Cell Neurosci.* 2012 Nov 8; 6: 52. doi: 10.3389/fncel.2012.00052. eCollection 2012.

Walter J, Hartung HP, **Dihné M**. Interferon gamma and sonic hedgehog signaling are required to dysregulate murine neural stem/precursor cells. *PLoS One.* 2012; 7(8): e43338. doi: 10.1371/journal.pone.0043338. Epub 2012 Aug 29.

**Wolking S**, **Lerche H**, **Dihné M**. Episodic itch in a case of spinal glioma. *BMC Neurol.* 2013 Sep 23; 13: 124. doi: 10.1186/1471-2377-13-124.



**Department  
of Neuro-  
degenerative  
Diseases**



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## Departmental Structure



Prof. Dr. Thomas Gasser is Chairman of the Department of Neurodegenerative Diseases.

The Department of Neurodegenerative Diseases was founded with the support of the Charitable Hertie Foundation and started operations on September 1, 2002. Since 2009, it is also a part of the Tübingen site of the German Center for Neurodegenerative Diseases (DZNE). The department pursues a comprehensive approach towards basic and clinical research in the field of neurodegenerative diseases and movement disorders, from their genetic basis and early diagnosis to innovative treatment and patient care. Through its clinical division, the department cares for patients with neurodegenerative diseases and movement disorders in one inpatient unit of 21 beds (Ward 43) and several specialized outpatient clinics. The clinical work is carried out by specially trained staff on all levels, including nurses, physiotherapists, occupational and speech therapists, as well as neurologists and neuropsychologists.

The department offers specialized and up-to-date diagnostic procedures for neurodegenerative diseases, including transcranial sonography of the brain parenchyma and genetic testing. Innovative treatment for patients with Parkinson's disease (PD) and other movement disorders include deep brain stimulation (in close collaboration with the Department of Neurosurgery), but also continuous apomorphine or levodopa infusion treatment in Parkinson's patients with severe fluctuations, or botulinum toxin treatment in patients with dystonias and spastic gait disorders. The close collaboration of the specialized

inpatient unit with the outpatient clinics for PD, dementia, dystonia, motor neuron diseases, ataxias, spastic paraplegias, and other rare neurogenetic disorders allows highly individualized patient management. The equally close interaction of clinicians with basic scientists within the Hertie Institute for Clinical Brain Research and the DZNE, on the other hand, allows truly translational research. This innovative concept includes active education and training of scientific and clinical junior staff. In 2015, the clinical department was named for the third time in a row as one of Germany's Top Ten hospital departments in Parkinson's Disease by the Magazine Focus.

Research is currently organized within 10 research groups. The group of Prof. T. Gasser investigates the genetic basis of Parkinson's disease and other movement disorders with high throughput array and next generation sequencing techniques. The group works closely with the team of Prof. D. Berg (Clinical Parkinson's Research) with its focus on clinical cohort studies, phenotyping and neuroimaging. The research section for Clinical Neurogenetics, headed by Prof. L. Schöls focuses on clinical and fundamental aspects of inherited ataxias, spastic paraplegias, motor neuron diseases and other rare neurogenetic conditions.

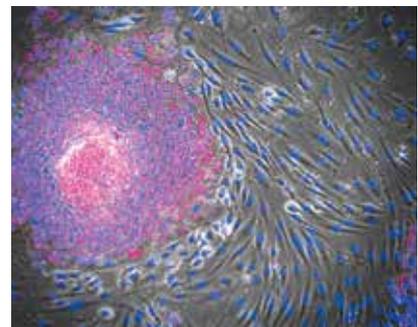
Dr. D. Weiss took over the lead of the deep brain stimulation (DBS) group from Prof. R. Krüger, who accepted a chair at the University of Luxembourg in June 2014 and develops novel DBS stimulation paradigms, while Prof. Krüger still maintains his own HIH

research group working on fundamental pathogenetic mechanisms of neurodegeneration in PD, with a particular focus on mitochondrial function and dysfunction. Prof. P. Kahle's group (section of Functional Neurogenetics) investigates also fundamental aspects of neurodegeneration. The group of Dr. M. Synofzik applies systems neurobiologic and genetic approaches to elucidate the basis and develop novel treatments of complex movement disorders including ataxias, but also dementias and motor neuron diseases, while Dr. R. Schüle rejoined the department, after a two-year Marie-Curie-funded stay at the University of Miami, as a group leader focusing on the genetic basis of spastic paraplegias. Dr. Dr. S. Biskup leads a research group on LRRK2-biology, but also a highly successful company that offers innovative methods of genetic diagnosis. Prof. W. Maetzler focuses on neurogeriatrics and gait disorders in Parkinson's disease and dementias. Finally, Dr. J. Simon-Sanchez, "Genetics and Epigenetics of Neurodegeneration" has recently established a group jointly supported by the Department and the German Center for Neurodegenerative Diseases (DZNE) with a primary interest in the genetics and genomics of neurodegenerative disorders.

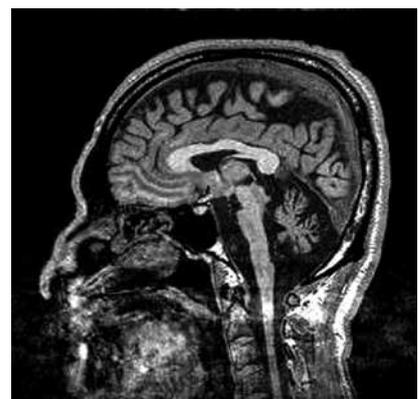
Thus, the wide spectrum of activities in the department covers all aspects from basic research to highly competent care of patients with Parkinson's disease and other neurodegenerative diseases.



Insertion of an electrode during deep brain stimulation for Parkinson's disease.



To study the effects of mutations related to Parkinson's disease, induced pluripotent stem cells (iPSC) with specific genetic alterations have been generated (red: iPSC, co-cultured with embryonic connective tissue (blue) from mice).



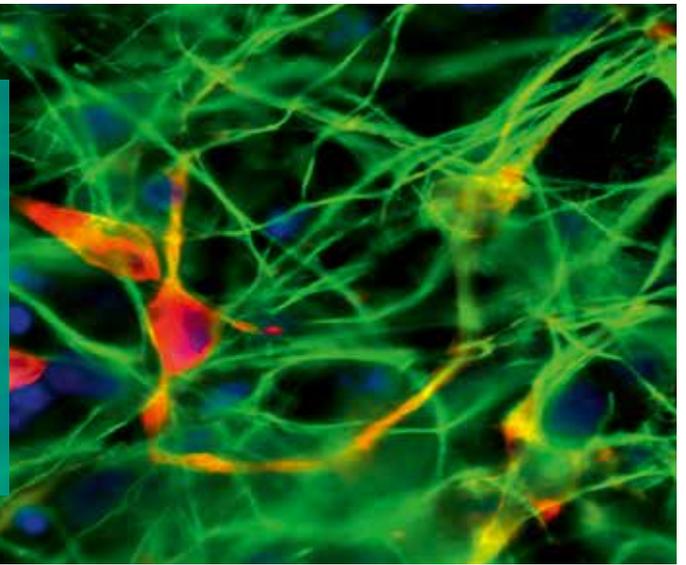
Both, fundamental mechanisms of neurodegeneration in Parkinson's disease and the effects of deep brain stimulation are investigated in Professor Krüger's group.

# Parkinson Genetics

Head: Prof. Dr. Thomas Gasser

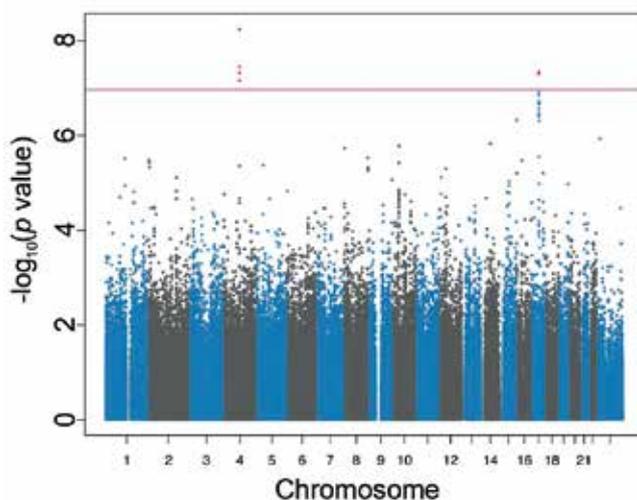
Team: 12 members

Key words: parkinson's disease / genetics / association studies / GWAS / mutation / induced pluripotent stem cells



**Although most patients with Parkinson's disease (PD) do not have affected parents or siblings, it is becoming increasingly clear that genetic factors greatly influence the risk to develop the disease and determine its course. As members of several international consortia, we are striving to identify these genetic variants by state-of-the-art high throughput techniques in conjunction with in depth clinical analyses.**

*Obwohl bei den meisten Parkinson-Patienten keine weiteren Familienmitglieder von dieser Erkrankung betroffenen sind, wird immer klarer, dass genetische Faktoren dennoch das Erkrankungsrisiko und den Verlauf wesentlich beeinflussen. Innerhalb großer internationaler Konsortien arbeiten wir mit modernen Hochdurchsatzmethoden verbunden mit genauen klinischen Analysen daran, diese genetische Varianten zu identifizieren.*

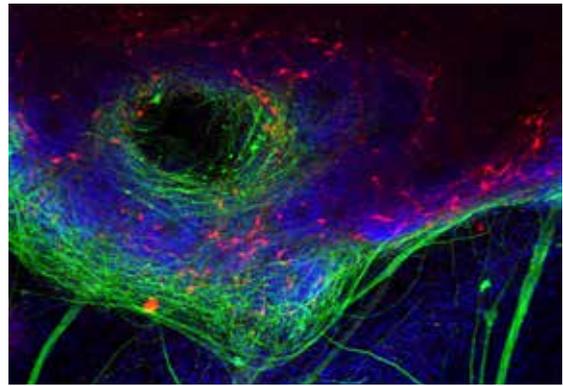


A large genome-wide study identified two genetic risk loci for sporadic PD. One is **MAPT**, containing the gene for the microtubule associated protein tau.

Specific mutations in some genes can cause rare inherited forms of Parkinson's disease (PD). Mutations in the **LR-RK2**-gene, causing the most prevalent autosomal-dominant form of PD, was discovered by us and collaborators in 2004 (Zimprich et al., Neuron 2004). A contribution of more common genetic sequence variants, often called single nucleotide polymorphisms (SNPs), in the etiology of the much more common sporadic (=non-familial) form is now equally well established.

In an attempt to identify these risk variants for the sporadic disease, we have conducted the first large genome-wide association study (GWAS), funded in part by the National Genome Research Network, **NGFN2**, in a collaboration with the laboratory for neurogenetics of the National Institutes of Health (NIH). (Simon-Sanchez et al., Nat Genet 2009). Since this initial study, we have worked with numerous collaborators who had performed similar studies to re-analyze the data, now based on a total sample size of almost 20,000 cases and 95,000 controls. This latest meta-analysis resulted in the confirmation of a total of 28 risk loci with genome wide significance (Nalls et al., Nat Genet 2014). These variants can also influence the course of the disease (Escott-Price et al., 2015).

Zu sehen ist ein Netzwerk aus Neuronen (also Nervenzellen) mit langen neuronalen Ausläufern (in grün). Sie sind aus reprogrammierten Fibroblasten (Hautzellen) eines Parkinson-Patienten entstanden. Nach einem speziellen Verfahren bei der Reifung von Stammzellen zu Neuronen entstehen dabei auch dopaminerge Neuronen (in rot). Also diejenigen Zellen, die beim Parkinson-Patienten am empfindlichsten sind und daher schneller absterben. Dies ermöglicht das Arbeiten an dopaminergen Neuronen von Parkinson-Patienten im „Reagenzglas“. Zellkerne sind blau dargestellt.



As genome-wide association studies only capture relatively common variants, a significant proportion of the total genetic risk remains to be discovered. This is sometimes called the “missing heritability”, and thought to be conferred mainly by rare genetic variants of moderate effect size. In order to identify the relevant variants, we are conducting whole-exome sequencing studies. Based in part on these studies, we have contributed to the development of a genotyping array, a novel tool to capture a large proportion of common and rare genetic variability contributing to neurodegenerative diseases (Nalls et al., 2015).

Knowing the genetic underpinnings of a complex neurodegenerative disorder such as PD is important, but it does not yet answer the question how these genetic abnormalities lead to disease. Until recently, studies on gene function have only been possible in animal and cellular models, which often fail to the specific features of human diseases. The revolutionary technology of “reprogramming cells” into so-called “induced pluripotent stem cells” (iPSC) has opened up a whole new research area: The iPSC can be differentiated into practically any cell type of the body, including nerve cells. We have successfully used this technology and have generated numerous iPSC-lines with specific

PD-related mutations. These cells allow us to study the consequences of PD causing mutations in their “natural” surrounding. We have thus recently been able to demonstrate that iPSC-derived neurons from patients with GBA-mutations exhibit specific alterations in calcium signaling (Schöndorf et al., Nat Commun 2014).

## SELECTED PUBLICATIONS

Brockmann, K., ... **T. Gasser** and D. Berg (2015). “GBA-associated Parkinson’s disease: reduced survival and more rapid progression in a prospective longitudinal study.” *Mov Disord* 30(3): 407-411.

Escott-Price, V., C., ... **T. Gasser**, et al. (2015). “Polygenic risk of Parkinson disease is correlated with disease age at onset.” *Ann Neurol* 77(4): 582-591.

Nalls, M. A., ... **T. Gasser**, et al. Parkinson’s Disease meta-analysis (2015). “NeuroX, a fast and efficient genotyping platform for investigation of neurodegenerative diseases.” *Neurobiol Aging* 36(3): 1605 e1607-1612.

Nalls, M. A., ..., **T. Gasser**, et al. (2014). “Large-scale meta-analysis of genome-wide association data identifies six new risk loci for Parkinson’s disease.” *Nat Genet* 46(9): 989-993.

Schöndorf, D. C., ... **T. Gasser** and M. Deleidi (2014). “iPSC-derived neurons from GBA1-associated Parkinson’s disease patients show autophagic defects and impaired calcium homeostasis.” *Nat Commun* 5: 4028.

Simon-Sanchez, J. and **T. Gasser** (2015). “Parkinson disease GWAS: the question of lumping or splitting is back again.” *Neurology* 84(10): 966-967.

Simon-Sanchez J, ..., **Gasser T**. Genome-wide association study reveals genetic risk underlying Parkinson’s disease. *Nat Genet*. 2009 Dec; 41(12): 1308-12.

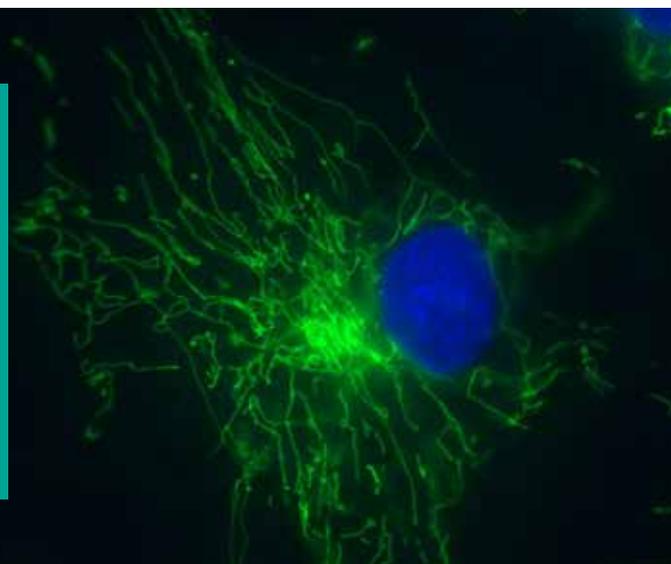
Zimprich A, Biskup S, ... **Gasser T**. Mutations in LRRK2 Cause Autosomal-Dominant Parkinsonism with Pleomorphic Pathology. *Neuron*. 2004 Nov 18; 44(4): 601-7.

# Functional Neurogenomics

Head: Prof. Dr. Rejko Krüger  
*(since 2014 also Professor for Clinical and Experimental Neuroscience, Luxembourg Centre for Systems Biomedicine, University of Luxembourg and affiliated)*

Team: 5 members in 2015

Key words: mitochondrial dysfunction / mitophagy / patient-based cellular models / unmet symptoms / motor networks / freezing of gait / learning



The Functional Neurogenomics Group is focused on the elucidation of molecular signaling pathways leading to neurodegeneration in Parkinson's disease (PD). We intensively study functional consequences of the identified mutations involved in pathogenesis of PD by investigating the underlying molecular signaling cascades. Here we have access to a unique collection of patient-based cellular models, including carriers of the A30P mutation in the alpha-synuclein gene (Krüger et al., 2001; Seidel et al., 2010) and the 'E64D' mutation in the DJ-1 gene (Hering et al., 2004; Obermaier et al., 2015; Abstract). Using patient fibroblasts to study mitochondrial function and dynamics the group pioneered in the field of mitochondrial pathologies in Parkinson's disease and defined first mitochondrial phenotypes related to mutations in the DJ-1 and the mortalin gene (Krebiehl et al., 2010;

Burbulla et al., 2014). Most interestingly characteristic mitochondrial alterations were already observed in cells from presymptomatic human mutation carriers indicating a potential role as a biomarker of the disease (Burbulla et al., 2010). Recent studies aim at the identification shared pathways of different PD-associated proteins linked to mitochondrial quality control. Here we found that PINK1/Parkin-mediated increase in mitophagy can rescue the loss of mortalin function phenotype characterized by intramitochondrial proteolytic stress (Burbulla, Fitzgerald et al., 2014). Moreover in collaboration with T. Rasse we confirmed a role of mortalin in neurodegeneration in flies in vivo (Zhu et al., 2013). Reduced levels of mortalin caused a Parkinsonian locomotor phenotype in flies that was related to a loss of synaptic mitochondria. In a mouse model of PD,

we are also investigating mitochondrial function and neuronal survival in vivo focusing on mutations in the mitochondrial serine protease Omi/HtrA2 (Casadei et al. 2016). We further extended our research on the characterization of neuron-specific phenotypes based on induced pluripotent stem cells (Reinhard et al., 2013) and are currently developing first individualized treatment strategies. After the identification of a novel mechanism for c.192G>C mutant DJ-1 that leads to complete protein loss due to defective splicing, we are currently applying targeted approaches for rescuing the correct splicing and restituting DJ-1 protein levels in neurons derived from stem cells of affected mutation carriers (Obermaier et al., 2015; Abstract).

With regard to the hitherto unmet therapeutic need on gait disturbances and falls we want to develop novel treatment strategies.



## SELECTED PUBLICATIONS

**Wüst R, Maurer B**, Hauser K, Woitalla D, Sharma M, **Krüger R**. Mutation analyses and association studies to assess the role of the presenilin-associated rhomboid-like gene in Parkinson's disease. *Neurobiol Aging*. Epub ahead of print.

Casadei N, **Sood P**, Ulrich T, Fallier-Becker P, Kieper N, Helling S, May C, Glaab E, Chen J, Nuber S, Marcus K, Rapaport D, Ott T, Riess O, **Krüger R**, **Fitzgerald JC**. Mitochondrial defects and neurodegeneration in mice overexpressing wild type or G399S mutant HtrA2. *Human Molecular Genetics*. Epub ahead of print.

**Fitzgerald JC, Burbulla L, Stegen K, Westermeier J**, Kato H, Mokranjac D, **Sauerwald J**, Martins LM, Woitalla D, Proikas-Cezanne T, Rapaport D, Riess O, Rasse T and **Krüger R**. (2014). Mitochondrial Proteolytic Stress Induced by Loss of Mortalin Function is Rescued by Parkin and PINK1. *Cell Death and Disease*. 5: e1180. DOI 10.1038/cddis2014.

Grau T, **Burbulla L**, Engl G, Oexle K, Leo-Kottler B, Roscioli T, **Krüger R**, Rapaport D, Wissinger B, Schimpf-Linzenbold S. (2013). A novel heterozygous OPA3 mutation located in the mitochondrial leader sequence results in an altered mitochondrial translocation and a fragmented mitochondrial network. *J Med Genet* 50: 848-858.

Reinhardt P, Schmid B, **Burbulla LF**, Schöndorf DC, Wagner L, Glatza M, Höing S, Hargus G, Heck SA, Dhingra A, Wu G, Müller S, Brockmann K, Kluba T Maisel M, **Krüger R**, Berg D, Tsytsyura Y, Thiel CS, Psathaki K, Drexler H, Klingauf J, Kuhlmann T, Klewin M, Müller H, Gasser T, Schöler H, Sternecker J. (2013). Gene-correction in iPSCs uncovers multiple pathogenic changes associated with LRRK2 G2019S. *Cell Stem Cell* 12: 354-67.

Zhu J, Sreekumar V, **Westermeier J**, Vereshchagina N, **Burbulla LF**, Daub K, Martins LM, Woitalla D, **Krüger R**, Rasse TM. (2013). Knockdown of Hsc70-5/mortalin induces loss of synaptic mitochondria in a Drosophila Parkinson's disease model. 2013; 8(12): e83714, *PLoS One* (doi: 10.1371/journal.pone.0083714).

Schüpbach WM, Rau J, Knudsen K, Haelbig TD, Hesekamp H, Navarro SM, Meier N, Falk D, Mehdorn M, Paschen S, Vorkmann J, Timmermann L, Maarouf M, Barbe MT, Fin GR, Kupsch A, Gruber D, Schneider GH, Krack P, Seigneuret E, Kistner A, Chaynes P, Ory-Magne F, Corubon CB, Vestper J, Schnitzler A, Wojtecki L, Houeto JL, Bataille B, Maltete D, Damier P, Raoul S, Sixel-Doering F, Hellwig D, Gharabaghi A, **Krüger R**, Pinsker MO, Amtage F, Regis JM, Witjas T, Thobois S, Mertens P, Kloss M, Hartmann A, Oertel WH, Post B, Speelman H, Agid Y, Schade-Brittinger C, Deuschl G. (2013). Neurostimulation for Parkinson's disease at an early disease stage? A randomized controlled trial (EARLYSTIM-Study). *N Engl J Med* 368: 610-622.

**Weiss D**, Walach M, Meisner C, Fritz M, Scholten M, Breit S, Plewnia C, Bender B, Gharabaghi A, **Wächter T, Krüger R**. (2013). Nigral stimulation for resistant axial motor impairment in Parkinson's disease? A randomized controlled trial. *Brain* 136: 2098-108, doi: 10.1093/brain/awt122. Epub 2013 Jun 11.

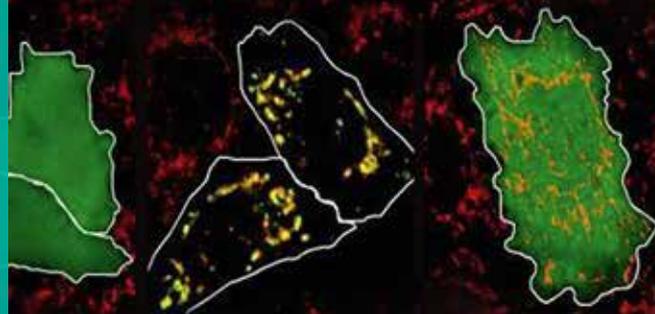
Plewnia C, Zwissler B, Längst I, **Maurer B**, Giel K, **Krüger R**. (2013). Effects of transcranial direct current stimulation (tDCS) on executive functions: influence of COMT Val/Met polymorphism. *Cortex* 49: 1801-1807.

# Functional Neurogenetics

Head: Prof. Dr. Philipp Kahle

Team: 8 members

Key words: parkinson's disease / amyotrophic lateral sclerosis / frontotemporal dementia / synuclein / ubiquitin / mitochondria / signal transduction / innate immunity



**We are elucidating the molecular mechanisms of neurodegeneration and physiological roles of genes linked to Parkinson's disease (PD) with emphasis on the major genetic and neuropathological hallmark  $\alpha$ -synuclein as well as the neuropathological disease entities characterized by the nucleic acid binding proteins TDP-43 and FUS, causing frontotemporal dementia (FTD) and amyotrophic lateral sclerosis (ALS). We are doing basic research using biochemical, molecular and cell biological methods as well as histological techniques, applying to cell culture, fly and mouse models, and patient-derived biomaterials.**

*Wir erforschen molekulare Mechanismen von neurodegenerativen Genprodukten, sowohl deren normale physiologische Funktionen als auch pathologische Aberrationen. Die untersuchten Gene verursachen Morbus Parkinson, hier vor allem das genetisch und neuropathologisch zentrale  $\alpha$ -Synuklein, sowie im Falle der RNA-bindenden Genprodukte TDP-43 und FUS frontotemporale Demenzen und amyotrophe Lateralsklerose. Unsere Grundlagenforscherguppe verwendet biochemische, molekularbiologische und histologische Techniken. Wir untersuchen Zellkultur- und Tiermodelle (Fliegen und Mäuse) sowie Patienten-Biomaterialien.*

We contribute to the ongoing efforts of the Department of Cellular Neurology (Prof. Jucker) to elucidate the seeding of proteinopathies, specifically PD  $\alpha$ -synucleinopathy. As a first result, it was published that extracts from phenotypic  $\alpha$ -synuclein transgenic mice maintained pathology-inducing potential after formaldehyde fixation (Schweighauser et al. Acta Neuropathologica). In astrocyte cell culture, we could demonstrate neuroinflammatory effects of exogenously added  $\alpha$ -synuclein, which mediated signal transduction events via the innate immunity receptor TLR4 (Rannikko et al. BMC Neuroscience). It is of note that the second most important PD gene LRRK2 also plays a role in innate immunity cells both in the brain (microglia) and the periphery (macrophages).

A major focus in the  $\alpha$ -synuclein research field worldwide is the identification of disease-mediating  $\alpha$ -synuclein species, oligomers being the most conspicuous ones (Kahle et al. *EMBO Journal*). In addition to extracellular and synaptic  $\alpha$ -synuclein, there might be a portion in the nucleus. The highly negative net charge of  $\alpha$ -synuclein would allow binding to histones. Thus our current research focus is on epigenetic effects of  $\alpha$ -synuclein (manuscript submitted), also as part of a new German-Canadian-French research consortium (decipherPD).

In the case of recessive PD genes, our main emphasis is on mitophagy. We continue to screen for novel modifiers, developing novel high-throughput systems, in collaboration with the DZNE Cellomics facility (Dr. Jain). Validations of already identified modulators are ongoing.

For the neurodegenerative proteinopathy causing FUS, we investigated nuclear import factors as disease modifiers in a newly generated *Drosophila* model (Jäckel et al. *Neurobiology of Disease*). We confirmed in vivo that transportin mediates nuclear import of FUS, and that this is modulated by arginine methyltransferase 1.

These events were directly correlated with motor neuron degenerative phenotypes, making it an attractive fly model for ALS-FUS. Knowing that arginine methylation also occurs in chromatin, disease-modifying effects could also involve epigenetic mechanisms (see above). Not only for FUS, but also particularly TDP-43 we continue our efforts to characterize post-translational modifications regulating function, cellular distribution and proteopathic aggregation. Mass spectrometric analyses are done in collaboration with the DZNE Proteomics facility (Dr. Glöckner).

## SELECTED PUBLICATIONS

Schweighauser M, Bacioglu M, Fritschi SK, Shimshek DR, **Kahle PJ**, Eisele YS, Jucker M. Formaldehyde-fixed brain tissue from spontaneously ill  $\alpha$ -synuclein transgenic mice induces fatal  $\alpha$ -synucleinopathy in transgenic hosts. *Acta Neuropathologica* 2015 Jan; 129(1): 157-9.

**Rannikko EH, Weber SS, Kahle PJ**. Exogenous  $\alpha$ -synuclein induces toll-like receptor 4 dependent inflammatory responses in astrocytes. *BMC Neuroscience* 2015 Sep 7; 16: 57.

**Kahle PJ, Sugeno N, Skodras A**.  $\alpha$ -Synuclein oligomers pump it up! *EMBO Journal* 2015 Oct 1; 34(19): 2385-7.

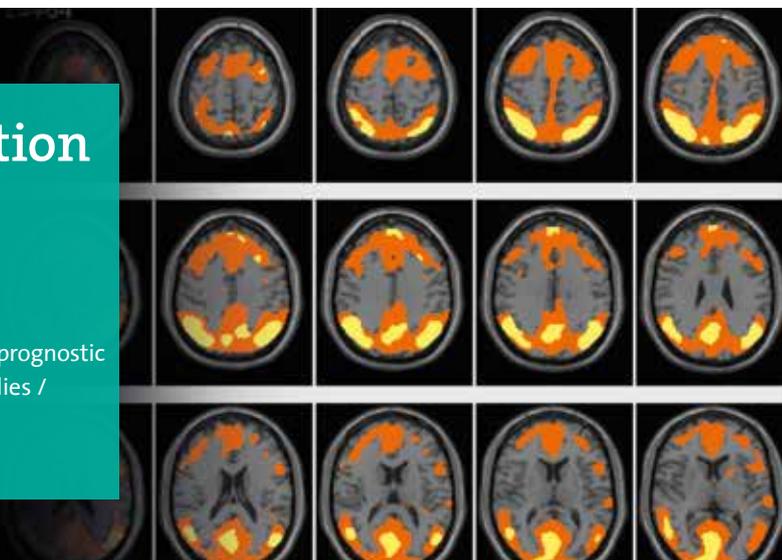
**Jäckel S, Summerer AK, Thömmes CM**, Pan X, Voigt A, Schulz JB, Rasse TM, Dormann D, Haass C, **Kahle PJ**. Nuclear import factor transportin and arginine methyltransferase 1 modify FUS neurotoxicity in *Drosophila*. *Neurobiology of Disease* 2015 Feb; 74: 76-88.

# Clinical Neurodegeneration

Head: Prof. Dr. Daniela Berg

Team: 24 members

Key words: parkinson's disease / alzheimer's disease / lewy-body disease / tremor / diagnostic and prognostic markers / imaging / prospective cohort studies / therapy



**With the aging society the prevalence of Parkinson's disease (PD) and neurodegenerative dementias increases steadily. Notably, neurodegenerative processes underlying these diseases start years before clinical diagnosis, and have progressed by large when therapy starts. Therefore, the group Clinical Neurodegeneration follows large cohorts of patients and yet healthy individuals with an increased risk for neurodegenerative diseases to identify markers for an earlier diagnosis and for an objective, individualized understanding and description of disease progression. Additionally novel medication and conservative therapeutic strategies are offered in numerous studies with a specific focus on individualized therapy.**

*In der immer älter werdenden Bevölkerung nimmt die Zahl an Patienten, die von Parkinson oder neurodegenerativen Demenzen betroffen sind, stetig zu. Gleichwohl sind noch viele Fragen bezüglich Ursachen, Entstehung und Verlauf dieser „Volkskrankheiten“ unklar. Von besonderer Bedeutung ist, dass der Nervenzelluntergang schon Jahre vor Auftreten der zur Diagnose führenden Symptome wie den typischen Bewegungsauffälligkeiten oder einer Demenz beginnt und zum Diagnosezeitpunkt schon fortgeschritten ist. Die AG Klinische Neurodegeneration untersucht deshalb in großen prospektiven Kohortenstudien charakteristische Veränderungen, die als diagnostische und prognostische Marker dienen können. Auffälligkeiten des Gehirns in bildgebenden Verfahren wie Ultraschall und MRT, klinische Veränderungen (z. B. bei bestimmten Bewegungsmustern oder beim Denken) sowie Marker im Nervenwasser wurden bereits als Diagnose- und Verlaufsmarker identifiziert. Zudem werden neue medikamentöse und konservative Therapiestrategien im Rahmen von Studien angeboten mit einem Schwerpunkt auf individualisierten Therapieansätzen.*

## Parkinson's disease

With a prevalence of about 2 % in the population older than 60 years, Parkinson's disease (PD) is one of the most common neurodegenerative disorders. As there is still a substantial lack of knowledge with regard to the correct and early diagnosis, as well as the course and etiology of PD, the group Clinical Neurodegeneration is conducting a number of large prospective longitudinal studies in national and international cooperations in patients and individuals at risk for the disease. Moreover, a special focus is being put on the identification and better understanding of subgroups of PD, i.e. monogenetic forms or forms in which

specific pathophysiological aspects play a major role – e.g. inflammation, mitochondrial dysfunction. Another focus is dementia in PD, especially the early diagnosis with the intention to intervene at a stage at which a greater benefit for patients and caregivers may be achieved. As a substantial impact on the activities of daily living function is mandatory for diagnosis of dementia varying scales and objective measurements are evaluated which might serve as diagnostic tools even in the pre-stage of dementia.

Selected examples of recent findings are

- (i) substantia nigra hyperechogenicity in healthy individuals older than 50 years determined by transcranial sonography indicates a more than 20 times increased risk to develop PD within five years,
- (ii) evaluation of progression markers in the pre-diagnostic phase of PD is feasible by assessing individuals with various combinations of risk and prodromal markers
- (iii) the neurodegenerative process in GBA-PD is associated with alterations of membrane phospholipid metabolism which might be also involved in abnormal  $\alpha$ -synuclein aggregation

(iv) Plasma ceramide and glucosylceramide metabolism is altered in sporadic Parkinson's disease and associated with cognitive impairment

A further focus of the group is standardization of assessments in collaboration with other experts. In cooperation with Prof. Walter Maetzler and colleagues simple to apply, unobtrusive accelerometer-based measurement systems as well as devices to test fine motor function are applied in many of the cohort studies to objectively assess subtle motor deficits.

In collaboration with the group of Prof. Thomas Gasser the group Clinical Neurodegeneration has been crucially involved in the development and maintenance of the Hertie-Biobank which is currently the basis for many national and international cooperations, promoting effective research in PD and other neurodegenerative disorders.

Moreover, based on the desire to improve therapy, the group is involved in a number of mono- and multicenter clinical phase II to IV studies, including also the allied health services, for all stages of PD with a focus on individualized interventions.

#### Atypical Parkinsonian syndromes

In the last years, huge effort of many groups has been put into a better characterization of the different endophenotypes of atypical Parkinsonian syndromes. The group Clinical Neurodegeneration is currently extending this effort by a comprehensive analysis of extensive clinical assessments and genetic data in individuals with atypical Parkinsonian syndromes.

#### Dementias with Lewy-bodies

With the demand for an early, individualized, and better treatment, one focus of the group is to identify patients with a potentially higher risk of dementia. In a cohort comprising 180 subjects with clinically defined idiopathic PD the

cognitive, neurobehavioral, motor and blood marker profile is being monitored longitudinally to discover factors which are associated with a more rapid cognitive decline. Further projects including first intervention strategies are followed in national and international cooperations.

#### Tremor

With a prevalence of 1 to 5 % essential tremor is the most frequent movement disorder. Understanding of the etiology is limited, which is at least in part due to a great phenotypic variance. Thus a large cohort of tremor patients is currently being characterized with thorough quantitative assessment batteries to better understand subtypes and facilitate differential diagnosis. In cooperation with national and international groups standardized protocols are being established and GWAS (genome-wide association studies) are being performed to disclose the secrets of this common movement disorder.

## SELECTED PUBLICATIONS

Liepert-Scarfone I, Pilotto A, ..., Gauss K, Wurster I, Streffer J, Berg D. Autonomic dysfunction in subjects at high risk for Parkinson's disease. *Journal of Neurology* 2015 Dec; 262(12): 2643-52.

Berg D, Postuma RB, ..., Liepert-Scarfone I, ..., Deuschl G. MDS research criteria for prodromal Parkinson's disease. *Movement Disorders* 2015 Oct; 30(12): 1600-11.

Postuma RB, Berg D, ..., Deuschl G. MDS clinical diagnostic criteria for Parkinson's disease. *Mov Disord*. 2015 Oct; 30(12): 1591-601.

Gaenslen A, Wurster I, Brockmann K, ..., Faust B, Lerche S, ..., Berg D. Prodromal features for Parkinson's disease – baseline data from the TREND study. *European Journal of Neurology* 2014 May; 21(5): 766-72.

Berg D, Postuma RB, ..., Deuschl G. Time to redefine PD? Introductory statement of the MDS Task Force on the definition of Parkinson's disease. *Movement Disorders* 2014 Apr; 29(4): 454-62.

Berg D, Lang AE, ..., Stern M. Changing the research criteria for the diagnosis of Parkinson's disease: obstacles and opportunities. *The Lancet Neurology* 2013 May; 12(5): 514-24.

Berg D, Marek K, Ross GW, Poewe W. Defining at-risk populations for Parkinson's disease: Lessons from ongoing studies. *Movement Disorders* 2012 Apr 15; 27(5): 656-65.

Berg D, ..., Liepert I, ..., Gaenslen A, ..., Poewe W. Enlarged Substantia Nigra Hyperechogenicity and Risk for Parkinson Disease. A 37-Month 3-Center Study of 1847 Older Persons. *Archives of Neurology*. 2011 Jul; 68(7): 932-7.

Brockmann K, ..., Schulte C, ..., Berg D, Hattingen E. GBA-associated PD: Neurodegeneration, altered membrane metabolism, and lack of energy failure. *Neurology* 2012 Jul 17; 79(3): 213-20.

Liepert-Scarfone I, Gauss K, ..., Berg D. Evaluation of Progression Markers in the Premotor Phase of Parkinson's Disease: The Progression Markers in the Premotor Phase Study. *Neuroepidemiology* 2013; 41(3-4): 174-82.

Mielke MM, ..., Deuschle C, ..., Gräber-Sultan S, Schleicher E, Berg D, Liepert-Scarfone I. Plasma ceramide and glucosylceramide metabolism is altered in sporadic Parkinson's disease and associated with cognitive impairment: a pilot study. *PLoS One* 2013 Sep 18; 8(9): e73094.

# Functional Neurogeriatrics

Head: Prof. Dr. Walter Maetzler

Team: 9 members

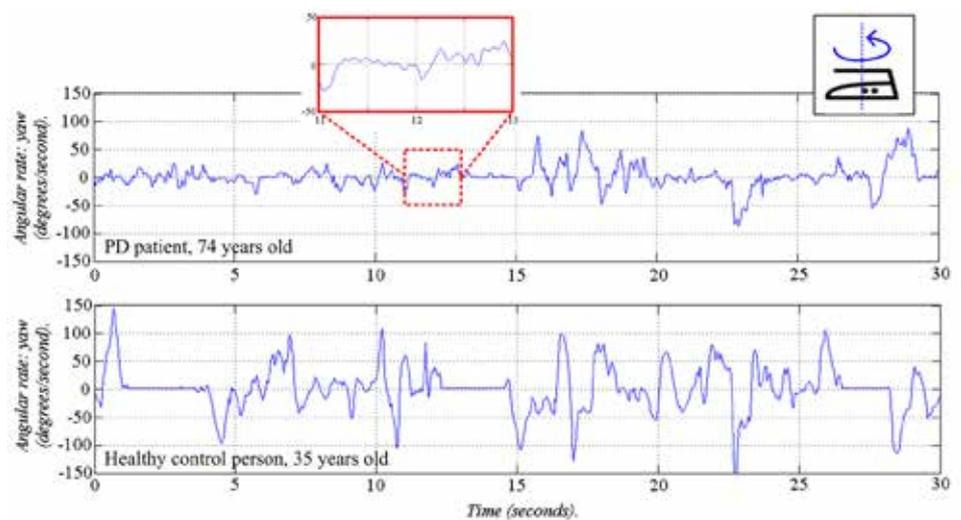
Key words: parkinson's disease / biomarkers / quantitative motor assessment / wearable sensors / quality of life / aging / self-empowerment

The functional neurogeriatrics group is dedicated to investigations of movement control of the elderly and individuals with prodromal and early stages of neurodegenerative disorders such as Parkinson's disease. Quantitative ambulatory assessment of axial and distal movements is performed with state-of-the-art measurement and investigation techniques. We are focusing on the association of movement deficits with quality of life in particular in chronic progressive diseases such as Parkinson's disease, and to give direct feedback to the user about movement deficits and resources to increase self-empowerment.

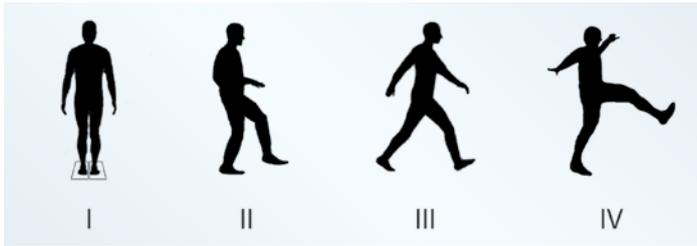
Empowering patients with chronic diseases to manage their own health and disease can result in improving health outcomes, encouraging patients to remain so, and in increased quality of life (Maetzler and Rochester, 2015). Moreover, it most probably leads to more cost-effective healthcare systems. The value of these activities is increasingly recognized not only by the patients and their doctors but also

by stakeholders and funding agencies. Our group is centrally involved in the development of a multimodal sensor information system for individuals with Parkinson's disease which exactly focuses on self-empowerment of the users. This system will be used in the home environment of patients with Parkinson's disease, and will be modular, extendible, adaptive and minimally obtrusive. Partners from England, Germany, Norway and Portugal contribute to this EU-funded ICT project (FP7, SENSE-PARK). First results have been published recently (Ferreira et al., 2015; Ramsperger et al., 2016).

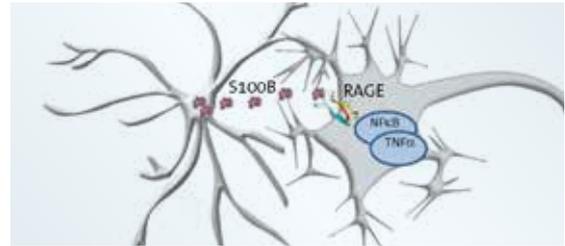
Another focus of our group is the investigation of quality of life aspects in Parkinson's disease. This disease has a major impact on the quality of life because it affects physical, mental and social life (van Uem et al., 2015). Since there are many factors contributing to a patient's quality of life, it is essential for clinicians and scientists to measure them as objectively as possible. In the EU-funded ITN project Moving beyond we aim at defining a conceptual framework that will quantitate quality



Example of movement detection in the home environment: Angular velocity of a gyroscope fixed on an iron while used by a patient with Parkinson's disease (top) and a healthy control (bottom), for ironing a shirt. Note also the 6 Hertz waves in the inset, representing action tremor.



The four postural control systems. I, Postural control during quiet stance; II, Postural control during step initiation; III, Postural control during walking; IV, Reactive postural adjustments (also including transitions and turning).



S100B is a calcium-binding protein secreted by astrocytes. We could show that high protein levels of S100B are associated with neurodegeneration in Parkinson's disease by involving the Receptor for Advanced Glycation Endproducts (RAGE) pathway (Sathe et al., 2012).

of life in patients with Parkinson's disease, using both cross-sectional and longitudinal objective measures generated in local study populations. Apart from this focus, the Moving beyond project spans the spectrum from basic understanding of mechanisms, over diagnostics to therapeutic applications of supraspinal motor control deficits (Maetzler et al., 2013).

#### **Markers for early detection, differential diagnosis and prediction of progression / subtypes in Parkinson's disease:**

Together with the groups of Prof. Daniela Berg and Prof. Thomas Gasser, our group is centrally involved in a structured and continuous development and maintenance of a local Neuro Biobank, and the collaboration with larger biobank efforts (Reijs et al., 2015). In this context, our group aims at investigating the association of biochemical markers with symptoms and signs associated with prediction / subtyping / differential diagnosis of Parkinson's disease (e.g. (Maetzler et al., 2014)).

#### **SELECTED PUBLICATIONS**

Ferreira JJ, Santos A, Godinho C, Domingos J, Abreu D, Larsen F, Serrano A, Weber P, Thoms A, Meckler S, Sollinger S, van Uem J, Hobert M, Maier K, Matthews HJD, Isaacs T, Duffen J, Graessner H, **Maetzler W**, 2015. Quantitative 12 weeks home-based assessment of Parkinson's symptoms: The SENSE-PARK feasibility and usability study. *BMC Neurol* 15, 89.

**Maetzler W**, Nieuwhof F, Hasmann SE, Bloem BR, 2013. Emerging therapies for gait disability and balance impairment: Promises and pitfalls. *Mov. Disord.* 28, 1576–1586. doi: 10.1002/mds.25682.

**Maetzler W**, Pilotto A, Apel A, Deuschle C, Kuebart G, Heinzel S, Liepelt-Scarfone I, Schulte C, Reusch D, Schleicher E, Rothfuss O, Schneider A, Dodel R, Gasser T, Berg D, 2014. In vivo markers of Parkinson's disease and dementia with Lewy bodies: current value of the 5G4  $\alpha$ -synuclein antibody. *Acta Neuropathol.* 128, 893–5. doi: 10.1007/s00401-014-1364-1.

**Maetzler W**, Rochester L, 2015. Body-worn sensors-the brave new world of clinical measurement? *Mov. Disord.* 30, 1203–5. doi: 10.1002/mds.26317.

Ramsperger R, Meckler S, Heger T, Uem J van, Hucker S, Braatz U, Graessner H, Berg D, Manoli Y, Serrano A, Ferreira JJ, Hobert MA, **Maetzler W**, Consortium SP, 2016. Continuous leg dyskinesia assessment in Parkinson's disease – clinical validity and ecological effect. *Parkinsonism Relat. Disord.* doi: 10.1016/j.parkreldis.2016.02.007.

Reijs BLR, Teunissen CE, Goncharenko N, Betsou F, Blennow K, Baldeiras I, Brosseron F, Cavedo E, Fladby T, Froelich L, Gabryelewicz T, Gurvit H, Kapaki E, Koson P, Kulic L, Lehmann S, Lewczuk P, Lleó A, **Maetzler W**, de Mendonça A, Miller A-M, Molinuevo JL, Mollenhauer B, Parnetti L, Rot U, Schneider A, Simonsen AH, Tagliavini F, Tsolaki M, Verbeek MM, Verhey FRJ, Zboch M, Winblad B, Scheltens P, Zetterberg H, Visser PJ, 2015. The Central Biobank and Virtual Biobank of BIOMARKAPD: A Resource for Studies on Neurodegenerative Diseases. *Front. Neurol.* 6, 216. doi: 10.3389/fneur.2015.00216.

# Dystonia

Head: Prof. Dr. Thomas Gasser, Prof. Dr. Ludger Schöls  
Dr. Ebba Lohmann

Team: 5 members

Key words: dystonia / torticollis / genetics / botulinum toxin



An artists depiction of a dystonic syndrom (above and below).

Dystonia is the third most common movement disorder, and mutations in a growing number of genes have been identified as causes for hereditary forms in many cases. The aim of the group, which brings together clinical experience in the diagnosis and treatment of the dystonias with expertise in molecular genetics, is to define the role of known genes in the etiology of dystonia, but especially to find new genes and therefore gain novel insight into the molecular pathogenesis of the disorder.

Patient recruitment is based on the departmental outpatient clinic for botulinum toxin treatment, on international collaborations but also on the work of Dr. E. Lohmann, who is presently working at the University of Istanbul in Turkey, supported by a Margarete von Wrangell-stipend. Turkey is a country with a consanguinity rate of up to 42 %, depending on the region, which greatly increases the prevalence of hereditary recessive diseases, thereby increasing the chances to find novel causative genetic variants. The project for which

Dr E. Lohmann received a funding from the Deutsche Forschungsgemeinschaft (DFG) builds on an existing cohort of patients with dystonia, mostly from consanguineous families in Turkey. Detailed phenotyping and a thorough work-up of the families will provide the basis for genetic analysis.



## SELECTED PUBLICATIONS

Clinical variability in ataxia-telangiectasia. **Lohmann E**, Krüger S, Hauser AK, Hanagasi H, Guven G, Erginel-Unaltuna N, Biskup S, **Gasser T**. *J Neurol*. 2015 Jul; 262(7): 1724-7. doi: 10.1007/s00415-015-7762-z. Epub 2015 May 10.

The CACNA1B R1389H variant is not associated with myoclonus-dystonia in a large European multicentric cohort. Mencacci NE, R'bibo L, Bandres-Ciga S, Carecchio M, Zorzi G, Nardocci N, Garavaglia B, Batla A, Bhatia KP, Pittman AM, Hardy J, Weissbach A, Klein C, **Gasser T**, **Lohmann E**, Wood NW. *Hum Mol Genet*. 2015 Sep 15; 24(18): 5326-9. doi: 10.1093/hmg/ddv255. Epub 2015 Jul 8.

A missense mutation in KCTD17 causes autosomal dominant myoclonus-dystonia. Mencacci NE, Rubio-Agusti I, Zdebik A, Asmus F, Ludtmann MH, Ryten M, Plagnol V, Hauser AK, Bandres-Ciga S, Bettencourt C, Forabosco P, Hughes D, Soutar MM, Peall K, Morris HR, Trabzuni D, Tekman M, Stanescu HC, Kleta R, Carecchio M, Zorzi G, Nardocci N, Garavaglia B, **Lohmann E**, Weissbach A, Klein C, Hardy J, Pittman AM, Foltynie T, Abramov AY, **Gasser T**, Bhatia KP, Wood NW. *Am J Hum Genet*. 2015 Jun 4; 96(6): 938-47. doi: 10.1016/j.ajhg.2015.04.008. Epub 2015 May 14.

A recurrent de novo mutation in KCNC1 causes progressive myoclonus epilepsy. Muona M, Berkovic SF, Dibbens LM, Oliver KL, Maljevic S, Bayly MA, Joensuu T, Canafoglia L, Franceschetti S, Michelucci R, Markkinen S, Heron SE, Hildebrand MS, Andermann E, Andermann F, Gambardella A, Tinuper P, Licchetta L, Scheffer IE, Criscuolo C, Filla A, Ferlazzo E, Ahmad J, Ahmad A, Baykan B, Said E, Topcu M, Riguzzi P, King MD, Ozkara C, Andrade DM, Engelsen BA, Crespel A, Lindenau M, **Lohmann E**, Saletti V, Massano J, Privitera M, Espay AJ, Kauffmann B, Duchowny M, Møller RS, Strausberg R, Afawi Z, Ben-Zeev B, Samocha KE, Daly MJ, Petrou S, Lerche H, Palotie A, Lehesjoki AE. *Nat Genet*. 2015 Jan; 47(1): 39-46. doi: 10.1038/ng.3144. Epub 2014 Nov 17.

Psychiatric disorders, myoclonus dystonia and SGCE: an international study. Peall KJ, Dijk JM, Saunders-Pullman R, Dreissen YE, van Loon I, Cath D, Kurian MA, Owen MJ, Foncke EM, Morris HR, **Gasser T**, Bressman S, Asmus F, Tijssen MA. *Ann Clin Transl Neurol*. 2015 Nov 20; 3(1): 4-11. doi: 10.1002/acn3.263. eCollection 2016 Jan.

Mutations in CIZ1 are not a major cause for dystonia in Germany. Dufke C, Hauser AK, Sturm M, Fluhr S, Wächter T, Leube B, Auburger G, Ott T, Bauer P, **Gasser T**, Grundmann K. *Mov Disord*. 2015 Apr 15; 30(5): 740-3. doi: 10.1002/mds.26198. Epub 2015 Mar 17. No abstract available.

# Clinical Neurogenetics

Head: Prof. Dr. Ludger Schöls

Team: 12 members

Key words: ataxias / spastic paraplegias / are neurogenetic diseases / axonal transport / translational medicine / clinical trials



Immunocytochemical staining of iPSC-derived cortical neurons (in green: neuronal marker  $\beta$ -III-tubulin; in red: cortical marker CTIP2; in blue: nucleus)

**The Section of Clinical Neurogenetics is dedicated to rare neurodegenerative disorders like ataxias, spastic paraplegias, amyotrophic lateral sclerosis, fronto-temporal dementia, mitochondriopathies and leukodystrophies. Focusing on the genetic basis of these diseases and defining the disease causing mutations helps us to decipher the underlying pathogenesis of neurodegeneration from its very beginning. The close interplay of clinical work at the Department of Neurology and basic research at the HIH enables us to address essential clinical questions to the lab and in return to bring back cutting edge results from the bench to the patient and run early clinical trials.**

*Die Sektion Klinische Neurogenetik widmet sich seltenen neurodegenerativen Erkrankungen wie Ataxien, spastischen Spinalparalysen, der Amyotrophen Lateralsklerose, Mitochondriopathien sowie Leukodystrophien. Durch die Erforschung des genetischen Hintergrunds dieser Erkrankungen und der krankheitsverursachenden Mutationen versuchen wir die zugrundeliegenden Krankheitsmechanismen aufzudecken und die neurodegenerativen Prozesse von ihrem Ursprung her zu verstehen. Die enge Vernetzung von Patientenbetreuung in der Klinik für Neurologie einerseits mit der Grundlagenforschung am Hertie-Institut für Hirnforschung andererseits ermöglicht es uns, essentielle klinische Fragen in der experimentellen Laborforschung zu berücksichtigen. Umgekehrt können neueste Ergebnisse der Grundlagenforschung schnell vom Labor in die Anwendung gelangen und in klinische Studien einbezogen werden.*

## Ataxia

In preparation for interventional trials in spinocerebellar ataxias (SCA) we participated in the EUROSCA consortium supported by the European Union ([www.euroasca.org](http://www.euroasca.org)) and set up a European registry with more than 3,000 patients suffering from this rare disease. We extended the SCA cohort to individuals at risk to develop SCA, i.e. first degree relatives of a patients who carry a 50 % risk to inherit the mutation. In this RISCA cohort we could show early changes in

eye movements and in MR imaging before onset of ataxia. This allows to apply future therapies in a presymptomatic stage and raises the chance to modify disease progression (Jacobi et al *Lancet Neurol* 2015).

Early onset ataxias are a major challenge to physicians as they divide into numerous genetic subtypes, almost all of them being extremely rare. To gather a representative cohort of such patients we established a

national network for early onset ataxias and set-up standards for diagnostic work-up and clinical characterization. All patients are registered in a newly created web based databank. This database is also open for European partners including paediatricians. The complex genetics of early onset ataxias leave most patients without a molecular diagnosis. To overcome this problem we developed an ataxia gene panel tool using next generation sequencing techniques to analyse all known ataxia genes and several neurometabolic ataxia-mimics in one single approach by massive parallel sequencing. With this new tool we identified several patients with very rare ataxia subtypes including ARSCAS, Niemann Pick Type C and LBSL (leucocapopathy with brainstem and spinal cord involvement and increased lactate) (Synofzik et al. *Orphanet J* 2015, Schicks et al. *Neurology* 2015a, Schicks et al. *Neurology* 2015b).

Families negative for all known ataxia genes underwent whole exome sequencing to search for new genes. By this approach we identified two new genes for autosomal recessive ataxia. WWOX is responsible for recessive ataxia with generalized tonic-clonic epilepsy and mental retardation (Synofzik et al. *Brain* 2015 epub). PNPLA6 causes ataxia with hypogonadism, a syndrome known as Gordon Holmes syndrome

or as Boucher-Neuhauser syndrome if ataxia and hypogonadism are accompanied by chorioretinal dystrophy (Synofzik et al. *Brain* 2015, epub).

In terms of therapy we have shown that active coordinative training is effective to reduce ataxia especially if performed on regular basis. Motivation and frustration frequently hinders every day training sessions especially in young patients. To address this problem we aimed to combine coordinative training with a fun factor and used whole-body controlled video game technology for highly interactive and motivational coordinative training for children with ataxia. Despite progressive cerebellar degeneration, children were able to improve motor performance by intensive coordination training. We could show that whole-body controlled video games present a highly motivational, cost-efficient, and home-based rehabilitation strategy to train dynamic balance and interaction with dynamic environments in kids (Ilg et al. *Neurology* 2012).

### Hereditary spastic paraplegia (HSP)

HSP is characterized by mostly selective degeneration of the corticospinal tract. Thereby the longest axons to the legs are much more severely affected than the relatively shorter axons to the arms. In two HSP mouse models we revealed molecular mechanisms for such a length dependent axonopathy. SPG10 is caused by mutations in the kinesin KIF5a, the motor of anterograde axonal transport. In a knockout model we found axonal transport to be affected but not only in anterograde but also in retrograde direction suggesting an essential interplay between both (Karle et al., *Neurogenetics* 2015). In a mouse model of SPG15 generated by the group of Hübner in Jena we could show that lack of SPASTIZIN leads to endolysosomal abnormalities and impaired axonal outgrowth (Khundadze et al. *PLoS Genet* 2015).

Rapid progress in genetic technologies allows for time- and cost-effective analyses of whole exomes providing

sequencing data of all coding regions of a genome within weeks. This turns out to become a highly efficient tool in the analysis of so far undefined genetic diseases. Using whole exome sequencing we found seven new genes causing HSP within 2 years: Reticulon 2 (RTN2) causing SPG12 (Montenegro et al. *JCI* 2012), B4GALNT1 causing SPG26 (*AJHG* 2015), DDHD1 causing SPG28 (*AJHG* 2012), GBA2 causing SPG46 (*AJHG* 2015), adaptor protein complex 4 (AP4B1) causing SPG47 (Bauer et al. *Neurogenetics* 2012), CYP2U1 causing SPG49 (*AJHG* 2012) and DDHD2 causing SPG54 (Schüle et al. *Eur J Hum Genet* 2015). This success in gene discovery became possible because of longstanding set-up of a large HSP cohort in national and European networks and close cooperation with the patient support groups.

Further effort is made to coin improved understanding of the molecular pathogenesis of HSP into therapeutic progress. Here we focus on SPG5, a subtype of HSP caused by mutations in CYP7B1. Lack of CYP7B1 leads to the accumulation of oxysterols (especially

27-OH sterol) in serum and even more pronounced in CSF (Schüle et al, *J Lipid Res* 2010). In cell cultures we could show that 27-OH sterol levels similar to concentrations in CSF of patients impair motorneuron-like cells. First results from a pilot trial with the cholesterol-lowering drug atorvastatin indicated lowering of 27-OH sterol in patients with SPG5.

### Trilateral project in Arab societies

In 2011 we started a new trilateral DFG project involving Israeli, Palestinian and German groups in the discovery of new genetic diseases in consanguineous families of the Arab population. After successful set-up of the consortium more than 50 families have been identified in Israel and the West Jordan land. Microarray based homozygosity mapping and high-throughput sequencing approaches allow for the identification of the molecular cause of the disease in an increasing number of families including the identification of new genes (Bauer et al. *Neurogenetics* 2012, Mallaret et al. *Brain* 2015, epub).

## SELECTED PUBLICATIONS

Jacobi H, Reetz K, du Montcel ST, Bauer P, Mariotti C, Nanetti L, Rakowicz M, Sulek A, Durr A, Charles P, Filla A, Antenora A, Schöls L, Schicks J, Infante J, Kang JS, Timmann D, Di Fabio R, Masciullo M, Baliko L, Melegh B, Boesch S, Burk K, Peltz A, Schulz JB, Dufaure-Gare I, Klockgether T (2015). "Biological and clinical characteristics of individuals at risk for spinocerebellar ataxia types 1, 2, 3, and 6 in the longitudinal RISCA study: analysis of baseline data." *Lancet Neurol* 12: 650-658.

Karle, K. N., S. Biskup, R. Schule, K. J. Schweitzer, R. Kruger, P. Bauer, B. Bender, T. Nagele and L. Schols (2015). "De novo mutations in hereditary diffuse leukoencephalopathy with axonal spheroids (HDLS)." *Neurology* 81: 2039-2044.

Mallaret M, Synofzik M, Lee J, Sagum CA, Mahajnah M, Sharkia R, Drouot N, Renaud M, Klein FAC, Anheim M, Tranchant C, Mignot C, Mandel JL, Bedford M, Bauer P, Salih MA, Schüle R, Schöls L, Aldaz CM and Koenig M (2015). The tumour suppressor gene WWOX is mutated in autosomal recessive cerebellar ataxia with epilepsy and mental retardation. *Brain*, epub Dec 24.

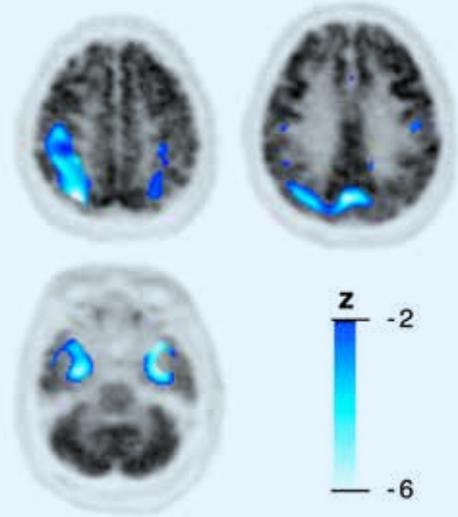
Martin E, Schüle R, Smets K, Rastetter A, Boukhris A, Loureiro JL, Gonzalez MA, Mundwiller E, Deconinck T, Wessner M, Jornea L, Oteyza AC, Durr A, Martin JJ, Schöls L, Mhiri C, Lamari F, Zuchner S, De Jonghe P, Kabashi E, Brice A, Stevanin G (2015). "Loss of function of glucocerebrosidase GBA2 is responsible for motor neuron defects in hereditary spastic paraplegia." *Am J Hum Genet* 92: 238-244.

# Systems Neurodegeneration

Head: PD Dr. Matthias Synofzik

Team: 7 members

Key words: rare neurogenetic diseases / ataxias / frontotemporal dementias / early-onset dementias / Amyotrophic Lateral Sclerosis / next-generation sequencing / neurorehabilitation / neurogeriatrics



FDG-PET bei einem Patienten mit frontotemporaler Demenz bei C9orf72-Repeatexpansion.

**Our research focuses on the investigation of the genetic basis, systems neuroscience and paradigmatic therapy approaches in**

- **movement disorders (e.g. degenerative ataxias, in particular early-onset ataxias, neurometabolic diseases, and rare complex movement disorders)**
- **frontotemporal dementias and other complex dementias (e.g. FTD spectrum diseases, early-onset dementias, rare variants and complex presentations of Alzheimer's Disease, genetic dementias)**
- **motor neuron diseases (Amyotrophic Lateral Sclerosis, in particular genetic variants; ALS-FTD spectrum diseases, lysosomal motor neuron diseases)**

**We use a broad spectrum of very different methods, reaching from recent molecular genetics techniques (e.g. whole exome and target sequencing panel analyses) and protein biomarker profiling to deep clinical phenotyping, neuropsychology and pioneering neuro-rehabilitation and neurogeriatrics approaches.**

## Early-onset ataxias and other rare movement disorders

Elaborating on the prospective longitudinal international multicenter Early-Onset Ataxia registry (EOA) established by us in 2012, we were able to establish a large national and international network on early-onset ataxias. Our registry and our work in next-generation genetics was the basis for a successful EU Erare JTC grant application „PREPARE“ in 2015. This novel EU consortium, which will be coordinated by Dr. Synofzik, aims at preparing targeted treatment trials for rare autosomal-recessive ataxias.

A substantial part of our early-onset ataxia research in 2015 focussed on investigating the clinical, genetic and biochemical properties of novel or still underdiagnosed recessive ataxia genes. Jointly with the HIH groups of Prof. Ludger Schöls and Dr. Rebecca Schüle, we helped to delineate the phenotypic spectrum of PNPLA6 [8], to characterize the enzymatic properties of GBA2/SPG46 [4], to identify novel diagnostic approaches for Niemann Pick Type C (NPC) [5], to establish novel MRI imaging techniques for ARSACS [2], and to explore the neuropathology of SPG7 [9]. Our large web-based cohort of ataxia exome data-sets,

*Unsere Forschungsgruppe ist spezialisiert auf die Erforschung genetischer Grundlagen, system-neurologischer Charakteristika und paradigmatischer Therapieansätze bei*

- *Bewegungsstörungen (v.a. degenerative Ataxien, insbesondere frühbeginnende Ataxien; neurometabolische Erkrankungen; komplexe seltene Bewegungsstörungen)*
- *frontotemporale Demenzen und andere komplexe Demenzen (u.a. frühbeginnende Demenzen, seltene Varianten der Alzheimer-Demenz, genetische Demenz-Formen)*
- *Motorneuronenerkrankungen (Amyotrophe Lateralsklerose, v.a. hereditäre Formen; ALS-FTD-Spektrum-Erkrankungen; lysosomale Motorneuronenerkrankungen).*

*Wir verwenden dabei ein breites Spektrum unterschiedlicher methodischer Ansätze. Diese reichen von neuen molekulargenetischen Techniken (z.B. whole exome oder Panel-Analysen) und Protein-Biomarker Profilen bis zu tiefer klinischer Phänotypisierung, Neuropsychologie und Pionier-Ansätzen in den Bereichen der Neurorehabilitation und Neurogeriatrie. 'Trial Readiness' für seltene Erkrankungen zu fördern.*

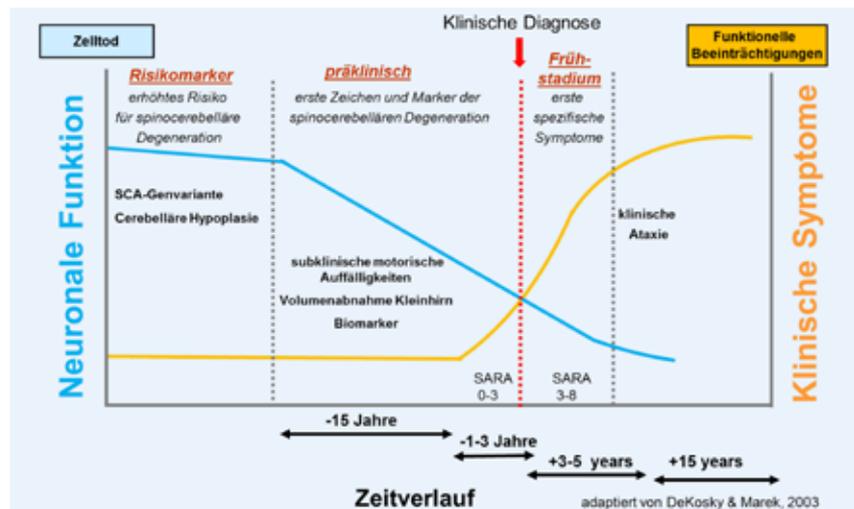
which has been continuously increased throughout 2015, allowed us to identify one novel early onset ataxia and epilepsy gene (KCNA2) in cooperation with the HIH group by Prof. Holger Lerche [7].

## Frontotemporal dementias and other complex dementias

We established all the infrastructure needed to become an important center for Frontotemporal Dementias (FTD) in Germany and internationally. We joined two large international prospective multi-center networks: (1) the Germany-based „FTLD network“, which systematically aggregates

### Hypothetisches Modell zum Erkrankungsverlauf spinocerebellärer Ataxien vor dem klinischen Beginn.

Auf dem Weg zur Früherkennung und für frühstmögliche Interventionsoptionen untersucht unsere Forschungsgruppe die Veränderungen in motorischen Maßen und Gehirnstrukturen bei spinocerebellären Ataxien noch vor ihrem klinischen Beginn.



patients, clinical and imaging data, and biomaterials from all FTD-spectrum diseases; and (2) the London-based „GENFI consortium“ which focuses more specifically on longitudinal clinical, imaging and biomaterial data collection from presymptomatic and symptomatic subjects from families with known genetic FTD types. In parallel, in cooperation with Prof. Peter Heutink (DZNE Tübingen) we established a large cohort of exome data-sets from subjects with FTD or with other early-onset dementias. First analyses of these exomes revealed that mutations in the Alzheimer

Disease genes PSEN1 and PSEN2 are much more common in Germany than previously thought and include presentations of complex or atypical Alzheimer Disease [1].

### Neurogeriatric approaches towards neurodegenerative disease

Together with Prof. Walter Maetzler from the HIH Tübingen, we were able to develop a novel perspective that might help to rethink one's individual process of getting older and how this process of aging might be seen in neurology [6]. The key questions here should not only be: how can we treat

age-related disorders? But also: how can we prevent age-related disorders in the first place or at least substantially delay their onset? [6]

Studies investigating the complex systems neuroscience of ageing –like our study on the interaction between vestibulo-ocular reflex suppression, gait, and fall risk in ageing [3]- might help to better understand the systems that become increasingly deficient during ageing and to prevent their deterioration by targeted interventions and daily life activities still during the healthy and younger period of life.

## SELECTED PUBLICATIONS

Blauwendraat C, Wilke C, Jansen IE, Schulte C, Simon-Sanchez J, Metzger FG, Bender B, Gasser T, Maetzler W, Rizzu P, Heutink P, Synofzik M (2015) Pilot whole-exome sequencing of a German early-onset Alzheimer's disease cohort reveals a substantial frequency of PSEN2 variants. *Neurobiology of Aging; Epub ahead of print.*

Holler M, Ehrlicke HH, Synofzik M, Klose U, Groeschel S (2015) Clinical Application of Fiber Visualization with LIC Maps Using Multidirectional Anisotropic Glyph Samples (A-Glyph LIC). *Clinical Neuroradiology. Epub ahead of print.*

Srulijes K, Mack DJ, Klenk J, Schwickert L, ..., Synofzik M, Schneider E, Ilg U, Berg D, Maetzler W, Becker C (2015) Association between vestibulo-ocular reflex suppression, balance, gait, and fall risk in ageing and neurodegenerative disease: protocol of a one-year prospective follow-up study. *BMC Neurology 15: 192.*

Sultana S, Reichbauer J, Schule R, Mochel F, Synofzik M, van der Spoel AC (2015) Lack of enzyme activity in GBA2 mutants associated with hereditary spastic paraplegia/cerebellar ataxia (SPG46). *Biochemical and Biophysical Research Communications 465:35-40.*

Synofzik M, Harmuth F, Stampfer M, Muller Vom Hagen J, Schols L, Bauer P (2015) NPC1 is enriched in unexplained early onset ataxia: a targeted high-throughput screening. *Journal of Neurology 262: 2557-2563.*

Synofzik M, Maetzler W (2015) Successful aging: what can neurology and geriatrics contribute? *Nervenarzt 86:475-480.*

Syrbe S, Hedrich UB, ..., Synofzik M, ..., Lerche H, Lemke JR (2015) De novo loss- or gain-of-function mutations in KCNA2 cause epileptic encephalopathy. *Nature Genetics 47:393-399.*

Tarnutzer AA, Gerth-Kahlert C, Timmann D, Chang DI, Harmuth F, Bauer P, Straumann D, Synofzik M (2015) Boucher-Neuhauser syndrome: cerebellar degeneration, chorioretinal dystrophy and hypogonadotropic hypogonadism: two novel cases and a review of 40 cases from the literature. *Journal of Neurology 262:194-202.*

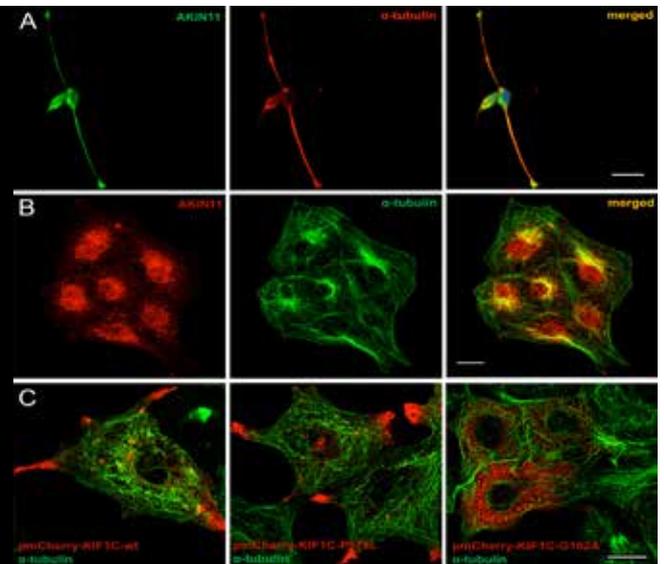
Thal DR, Zuchner S, Gier S, Schulte C, Schols L, Schule R, Synofzik M (2015) Abnormal Paraplegin Expression in Swollen Neurites, tau- and alpha-Synuclein Pathology in a Case of Hereditary Spastic Paraplegia SPG7 with an Ala510Val Mutation. *International Journal of Molecular Sciences 16: 25050-25066.*

# Genomics of Rare Movement Disorders

Head: Dr. R. Schüle

Team: 7 members

Key words: whole exome sequencing /  
whole genome sequencing / rare diseases /  
spastic paraplegia / ataxia / translational medicine /  
clinical trials



**Rare disease by definition affect not more than 5 in 10.000 people and yet, about 6–8 % of the population are affected by on of ~6.000 rare disease recognized by the European Union. Our team systematically adresses challenges specific to rare diseases, including definition of standard of care, validation of trial outcome parameters, performance of natural history studies, identification of novel disease genes and new therapeutic targets, disease modeling in cell culture and iPS derived human systems, preclinical and clinical trials. Supported by the European Union, the National Institutes of Health (NIH), the Spastic Paraplegia Foundation Inc. and others we team up with collaborators all over the world to promote trial readiness in rare diseases.**

*Seltene Erkrankungen betreffen definitionsgemäß nicht mehr als 5 in 10.000 Personen und doch sind ca. 6–8 % der Bevölkerung in der Europäischen Union von einer der rund 6.000 seltenen Erkrankungen betroffen. Unsere Forschungsgruppe stellt sich systematisch den Herausforderungen, die einer Therapieentwicklung für seltene Erkrankungen im Wege stehen: Definition von Therapiestandards, Validierung von Zielparametern für klinische Studien, Studien des natürlichen Verlaufes, Identifizierung neuer Erkrankungsgene und Ansatzpunkte für Therapien, Erkrankungsmodellierung in Zellkultur und humanen Stammzellmodellen, sowie die Durchführung präklinischer und klinischer Studien. Gefördert durch die Europäische Union, die amerikanischen 'National Institutes of Health' (NIH), die Spastic Paraplegia Foundation Inc. und andere kooperieren wir hierbei mit Forschungsgruppen aus der ganzen Welt, um 'Trial Readiness' für seltene Erkrankungen zu fördern.*

Hereditary spastic paraplegias (HSP) and ataxias are rare neurodegenerative disorders primarily affecting the corticospinal tract motoneurons and/or cerebellar Purkinje cells. Initially defined as independent disease groups the clinical and genetic overlap between HSPs and ataxias is increasingly recognized. With over 150 genes causing the conditions known, they are one of the genetically most heterogeneous groups of Mendelian diseases.

Mutations in known genes still explain only about half of the cases. To identify novel disease genes and ultimately novel therapeutic targets we have performed whole exome and whole genome sequencing in > 400 families with HSP, spastic ataxia and ataxia and led and participated in the identification of > 10 novel genes for these conditions.

HSP type SPG5 is caused by mutations in the 7 $\alpha$ -hydroxylase CYP7B1, an enzyme involved in degradation of cholesterol to primary bile acids. Lowering of cholesterol levels might lower the levels of pathologically elevated oxysterol levels we have identified in SPG5 patients. In 2015 we have thus started a clinical trial in SPG5, the first ever causal treatment trial in HSP.

### Subcellular localization of endogenous and overexpressed KIF1C.

- A. Endogenous KIF1C: In the mouse motor-neuron like spinal chord cell line NSC-34, endogenous KIF1C is found throughout the cell body with an accumulation in the pericentrosome, along the neurites, and strong accumulation at the neurite tips. In fibroblast-like COS-7 cells, endogenous KIF1C is sparsely distributed throughout the cell and accumulates perinuclear in a reticular pattern. In COS-7 cells displaying cellular processes, accumulation at the tips of these processes can be seen (not shown).
- B. Overexpressed, mCherry-tagged KIF1C accumulates at the tips of cellular processes in the COS-7 monkey fibroblast cell line (left). The same localization pattern can be observed for mCherry-tagged KIF1CPro176Leu (middle). In contrast, mCherry-tagged KIF1CGly102Ala (right) fails to reach cellular processes and instead is observed in a reticular pattern around the nucleus.
- 200um scale bar

To promote trial readiness in HSP we have initiated and coordinate a global network of major national HSP initiatives, the Alliance for Treatment in HSP and PLS. The network that is funded by the Spastic Paraplegia Foundation Inc. includes national HSP networks from Canada, the US, France, Belgium and other countries. The Alliance will institute a global HSP registry and perform systematic studies to identify

new biomarkers and other potential trial outcome parameters in HSP.

The Clinical Research in ALS and Related Disorders for Therapeutic Development (CREATe) Consortium is an NIH funded network. The goals of CREATe are to promote therapeutic development for neurodegenerative disorders through study of genotype-phenotype correlation and discovery and

development of biomarkers. Diseases in the focus of CREATe include amyotrophic lateral sclerosis, frontotemporal dementia, primary lateral sclerosis, hereditary spastic paraplegia and progressive muscular atrophy. With the PI Dr. R. Schüle the University of Tübingen is the only European partner in this otherwise U.S. American consortium.

## SELECTED PUBLICATIONS

Syrbe S, Hedrich UB, Riesch E, Djemie T, Muller S, Moller RS, Maher B, Hernandez-Hernandez L, Synofzik M, Caglayan HS, Arslan M, Serratos JM, Nothnagel M, May P, Krause R, Loffler H, Detert K, Dorn T, Vogt H, Kramer G, Schols L, Mullis PE, Linnankivi T, Lehesjoki AE, Sterbova K, Craiu DC, Hoffman-Zacharska D, Korff CM, Weber YG, Steinlin M, Gallati S, Bertsche A, Bernhard MK, Merkschlager A, Kiess W, Euro ER, Gonzalez M, Zuchner S, Palotie A, Suls A, De Jonghe P, Helbig I, Biskup S, Wolff M, Maljevic S, **Schule R**, Sisodiya SM, Weckhuysen S, Lerche H, Lemke JR. De novo loss- or gain-of-function mutations in KCNA2 cause epileptic encephalopathy. *Nature Genetics*, 2015; 47(4): p. 393-9.

Schmidt WM, Rutledge SL\*, **Schule R\***, Mayerhofer B, Zuchner S, Boltshauser E, Bittner RE. Disruptive SCYL1 Mutations Underlie a Syndrome Characterized by Recurrent Episodes of Liver Failure, Peripheral Neuropathy, Cerebellar Atrophy, and Ataxia. *American Journal of Human Genetics*, 2015; 97(6): p. 855-61.

Rossor AM, Oates EC, Salter HK, Liu Y, Murphy SM, **Schule R**, Gonzalez MA, Scoto M, Phadke R, Sewry CA, Houlden H, Jordanova A, Tournev I, Chamova T, Litvinenko I, Zuchner S, Herrmann DN, Blake J, Sowden JE, Acsadi G, Rodriguez ML, Menezes MP, Clarke NF, Auer Grumbach M, Bullock SL, Muntoni F, Reilly MM, North KN. Phenotypic and molecular insights into spinal muscular atrophy due to mutations in BICD2. *Brain*, 2015; 138(Pt 2): p. 293-310.

Sultana S, **Reichbauer J**, **Schule R**, Mochel F, Synofzik M, van der Spoel AC. Lack of enzyme activity in GBA2 mutants associated with hereditary spastic paraplegia/cerebellar ataxia (SPG46). *Biochemical and Biophysical Research Communications*, 2015; 465(1): p. 35-40.

Rieber N, Singh A, Oz H, Carevic M, Bouzani M, Amich J, Ost M, Ye Z, Ballbach M, Schafer I, Mezger M, Klimosch SN, Weber AN, Handgretinger R, Krappmann S, Liese J, Engeholm M, **Schule R**, Salih HR, Marodi L, Speckmann C, Grimbacher B, Ruland J, Brown GD, Beilhack A, Loeffler J, Hartl D. Pathogenic fungi regulate immunity by inducing neutrophilic myeloid-derived suppressor cells. *Cell Host & Microbe*, 2015; 17(4): p. 507-14.

Gonzalez M, Falk MJ, Gai X, Postrel R, **Schule R\***, Zuchner S\*. Innovative Genomic Collaboration Using the GENESIS (GEMapp). *Platform Hum Mutat*, 2015; 36(10): p. 950-6.

\* equal contribution



# Genetics and Epigenetics of Neurodegeneration

Head: Javier Simón-Sánchez, PhD

Team: 3 members

Key words: Parkinson's disease / whole genome sequencing / whole exome sequencing / genetics / genomics

The group of “Genetics and Epigenetics of Neurodegeneration” has been jointly established at the Department of Neurodegenerative Diseases within the Hertie Institute for Clinical Brain Research (HIH) and the German Center for Neurodegenerative Diseases (DZNE). We are a young research team with primary interest in the genetics and genomics of neurodegenerative disorders, especially Parkinson's disease (PD).

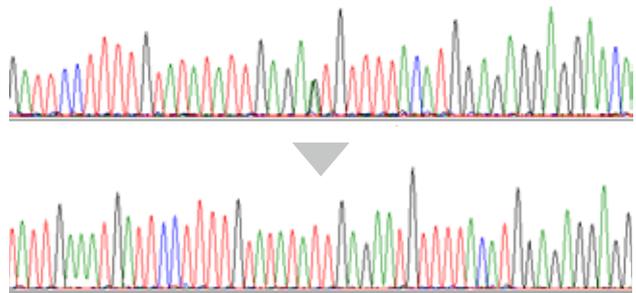
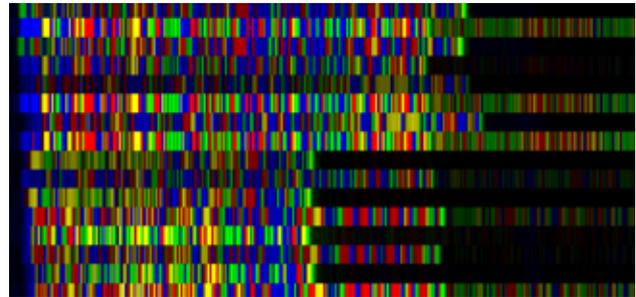
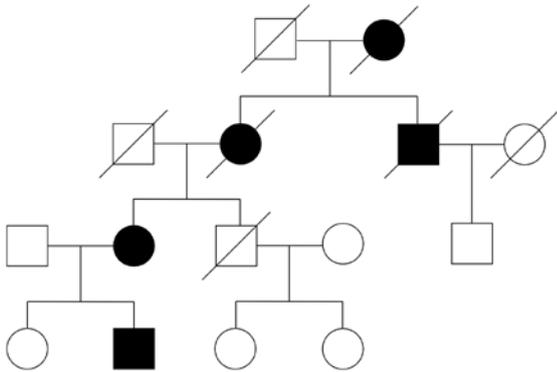
During 2015 we have been active partners of a European collaborative project (Courage-PD, JPND) aiming to further understand the genetic architecture of PD. As part of this project, we have collected 180 individuals from 117 families with autosomal dominant or autosomal recessive PD, from our partners in the Netherlands, Turkey, Spain, Tunisia, Italy, Portugal, and Germany. All these

samples were subjected to whole-genome sequencing (WGS) in Macrogen ([www.macrogen.com](http://www.macrogen.com)), and analyzed with standard bioinformatics tools. Variants segregating with PD in these families have been sent to our collaborators in Illumina so that they are included in a custom array that will be used to genotype a large cohort of PD cases and controls from different populations. Data derived from these genotyping experiments will help us understanding the genetic etiology of PD across different ethnic groups.

The mentioned WGS experiments have also helped us to identify the genetic cause of PD in two of the Turkish families included (1)(2). For two other families we have identified segregating mutations in genes not previously associated with PD. At this moment, in collaboration with our partners in Luxembourg, we are developing a

series of functional assays to confirm the role of these gene in PD. If confirmed, these results will help us understanding the genetic etiology of this devastating disorder.

During 2015, we have also used whole-exome sequencing data available through our collaboration with the International Parkinson's disease Consortium (IPDGC) to rule out PARK10 as a risk locus for sporadic PD (3, 4). These data has also been used to further understand the role of rare genetic variation in genes involved in mitochondrial pathways in the onset of PD. Results derived from this analyses will be followed up in a series of clinical assays as part of another European collaboration (MitoPD).



## SELECTED PUBLICATIONS

Hasmet A. Hanagasi, **Anamika Giri**, Gamze Guven, Başar Bilgiç, Ann-Kathrin Hauser, Murat Emre, Peter Heutink, Nazl Basak, Thomas Gasser, **Javier Simón-Sánchez**, and Ebba Lohmann. Movement Disorders. *In press*.

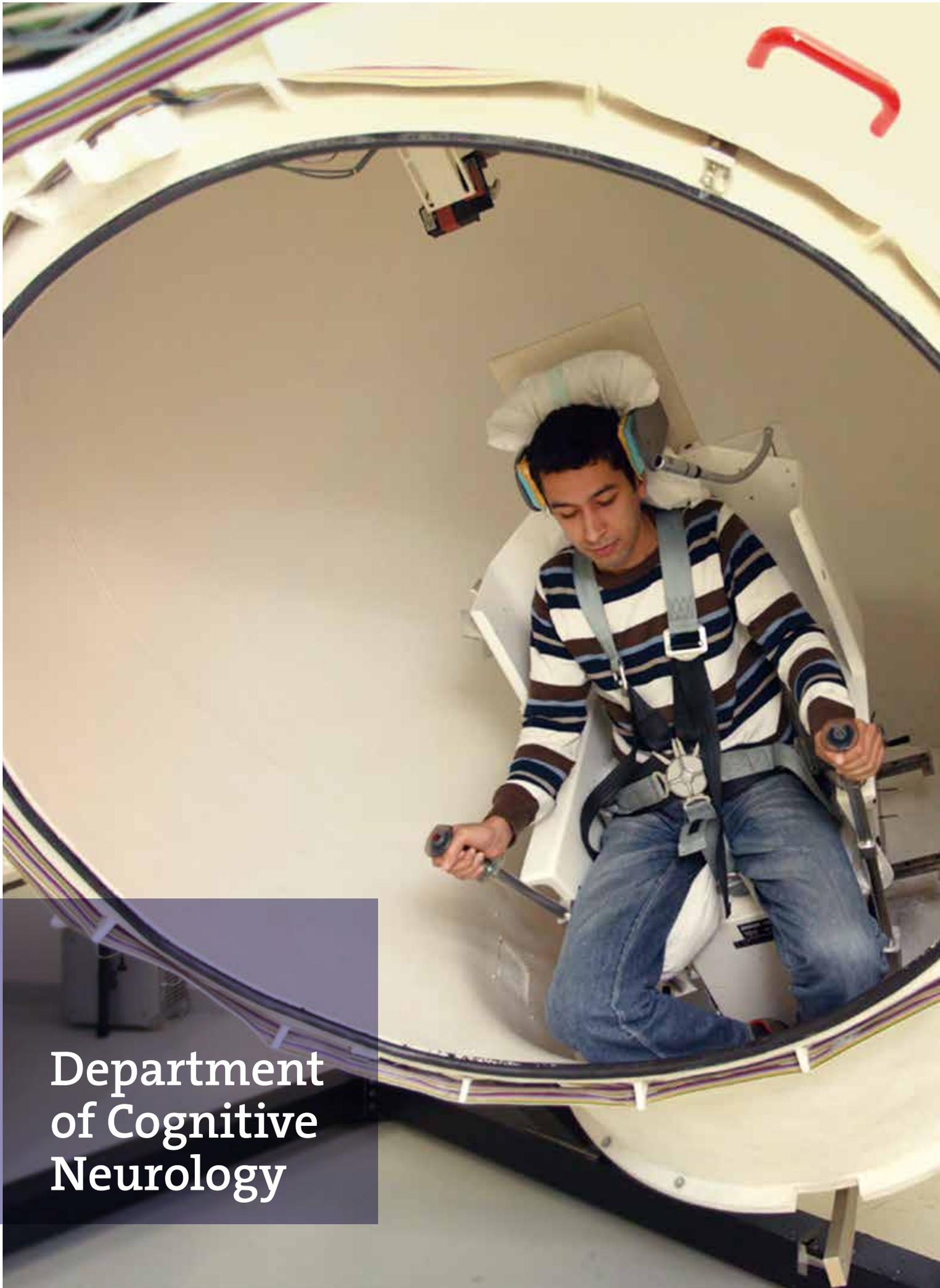
**Anamika Giri**, Gamze Guven, Hasmet Hanagasi, Ann-Kathrin Hauser, Nihan Erginul-Unaltuna, Basar Bilgic, Hakan Gurvit, Peter Heutink, Thomas Gasser, Ebba Lohmann, and **Javier Simón-Sánchez**. PLA2G6 mutations related to distinct phenotypes: a new case with early onset Parkinsonism. Tremor and Other Hypokinetic Movements. *In press*.

**Simón-Sánchez J**, Heutink P, Gasser T; International Parkinson's Disease Genomics Consortium (IPDGC). Variation in PARK10 is not associated with risk and age at onset of Parkinson's disease in large clinical cohorts. *Neurobiology of Aging*. 2015.

**Simón-Sánchez J**, Gasser T. Parkinson disease GWAS: the question of lumping or splitting is back again. *Neurology*. 2015.

Nalls MA, Bras J, Hernandez DG, Keller MF, Majounie E, Renton AE, Saad M, Jansen I, Guerreiro R, Lubbe S, Plagnol V, Gibbs JR, Schulte C, Pankratz N, Sutherland M, Bertram L, Lill CM, DeStefano AL, Faroud T, Eriksson N, Tung JY, Edsall C, Nichols N, Brooks J, Arepalli S, Pliner H, Letson C, Heutink P, Martinez M, Gasser T, Traynor BJ, Wood N, Hardy J, Singleton AB; **International Parkinson's Disease Genomics Consortium (IPDGC)**; Parkinson's Disease meta-analysis consortium. NeuroX, a fast and efficient genotyping platform for investigation of neurodegenerative diseases. *Neurobiol Aging*. 2015.

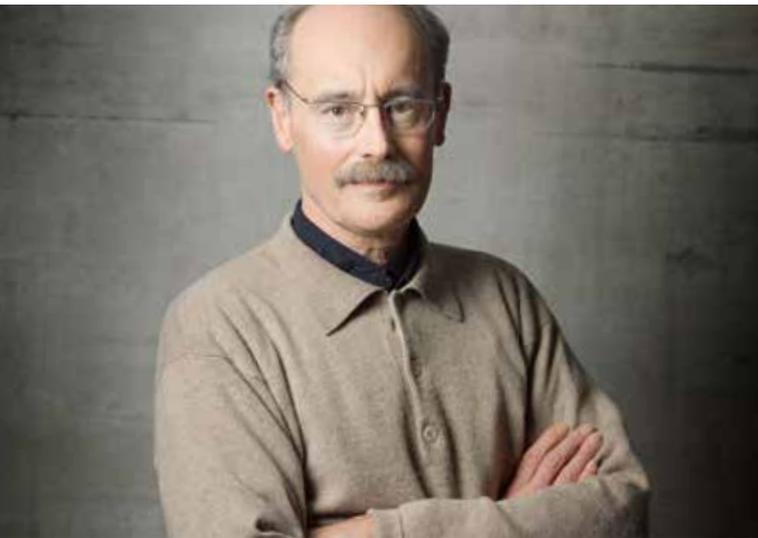
Blauwendraat C, Wilke C, Jansen IE, Schulte C, **Simón-Sánchez J**, Metzger FG, Bender B, Gasser T, Maetzler W, Rizzu P, Heutink P, Synofzik M. Pilot whole-exome sequencing of a German early-onset Alzheimer's disease cohort reveals a substantial frequency of PSEN2 variants. *Neurobiol Aging*. *Epub ahead of print*.



Department  
of Cognitive  
Neurology

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Prof. Dr. Peter Thier heads the Department of Cognitive Neurology.

## Departmental Structure

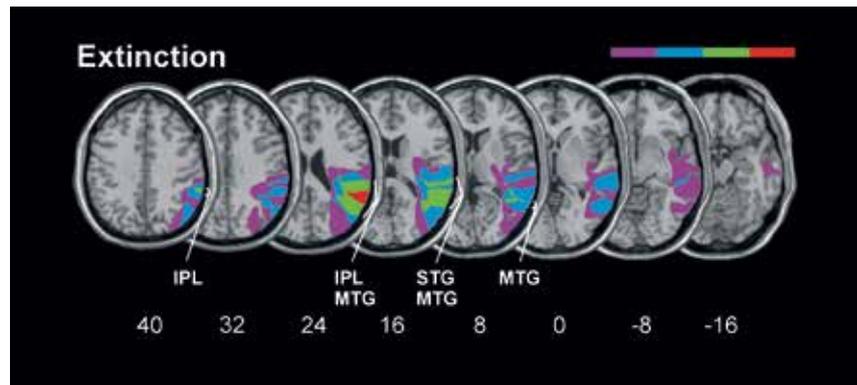
The Department of Cognitive Neurology was founded in the year 2000 with support from the program “C4-Department of Neuroscience at Neurology Clinics” of the Hermann and Lilly-Schilling Foundation. In the year 2002, in which the Neurology Clinic was reorganized, the Department of Cognitive Neurology became a constitutional part of the newly founded twin institutions, namely the Center of Neurology and the Hertie Institute for Clinical Brain Research. In the beginning of 2004, it was reinforced by the formation of a Section of Neuropsychology associated with a professorship for neuropsychology both taken over by Hans-Otto Karnath. In summer 2008 the Section of Computational Sensomotorics, headed by the newly appointed professor Martin Giese and funded co-jointly by the Hertie Foundation and the German Research Council within the framework of the Excellence Cluster “Centre for Integrative Neuroscience” (CIN), was installed at the department. In 2009 Cornelius Schwarz was appointed professor and head of the research group on Systems Neurophysiology within the CIN.

This group was integrated into the Department of Cognitive Neurology. The department currently comprises seven independent labs, namely, the Sections of Neuropsychology (H.-O. Karnath) and Computational Sensomotorics (M. Giese) respectively, the Systems Neurophysiology Lab (C. Schwarz), the Functional Neuroanatomy Lab (Fahad Sultan), the Neuropsychology of Action Control Lab (Marc Himmelbach), originally set up with funds from a 2007 ERC starting grant, the Sensorimotor Lab, headed by Peter Thier, who also serves as the chairman of the department, and the Oculomotor Lab of Uwe Ilg. The latter is also head of the Neuroscience Lab for Pupils at the Werner Reichardt Centre for Integrative Neuroscience (CIN). Two independent young investigator groups, namely the Neurobiology of Decision Making Lab headed by Axel Lindner and the Neuropsychology of Attention group headed by Bianca de Haan are also part of the department. The Lindner group operates under the roof of the Sensorimotor Lab, while the de Haan group is part of the Section of Neuropsychology.

The Department of Cognitive Neurology is devoted to research on the underpinnings of higher brain functions and their disturbances due to diseases of the nervous system. The spectrum of research topics is wide – which is a consequence of the existence of quite a few independent research groups with individual interests. The topics addressed comprise among others the basis and disturbances of spatial processing and orientation including the mechanisms of perceptual stability with respect to ego-motion, of attention, of motor learning and motor rehabilitation, as well as of social interactions. To this end, the Department of Cognitive Neurology adopts multifarious approaches: the consequences of circumscribed brain lesions are analyzed using classical neuropsychological techniques in conjunction with state-of-the-art psychophysical, behavioral and brain imaging methods, ‘motion capturing’ and virtual reality. Transcranial magnetic stimulation is used to simulate virtual lesions in the healthy brain. In order to explore the neuronal underpinnings of higher human brain functions in more detail, non-human primate as well as rodent models are used, allowing recordings of single- and multi-neuron signals and the correlation of these signals with well-defined behaviors or perceptual states as well as the targeted manipulation of neurons and neuronal circuits and their consequences for function. Recently, with the help of the CIN, 2P-imaging of cortical circuits has been added. Experiments using genetically modified non-human primates as a model system for autism are currently being established. In-vitro techniques such as whole cell patch clamp recordings from isolated brain slices are being applied in an attempt to characterize the membrane and synaptic properties of identified neurons participating in neuronal circuits underlying higher brain functions, such as perception and learning. The tools for theoretical approaches and modeling offered by the Giese group are used to integrate the obtained data and to generate

experimentally testable predictions. The variety of methods responds to the need to examine complex brain functions and their disturbances due to disease at various levels and from various perspectives. Starting point is always a clinical problem as for example a better understanding of the pathophysiology of cerebellar ataxia, an indispensable prerequisite for any attempt to alleviate or mitigate this condition. These questions can be answered only if the normal operation of the structure, compromised by brain disease is understood. We believe that any promising attempt to understand complex cognitive or motor disturbances like neglect, ataxia or autism will require a better understanding of the normal functional architecture of the underlying healthy systems.

In past and present, the Department of Cognitive Neurology has played a central role in the development and coordination of various research networks. For instance, the DFG-funded Collaborative Research Center (SFB) 550, that ended in 2009, as well as a preceding research unit were coordinated by P. Thier. Many members of the department are also part of the excellence cluster 'Werner Reichardt Centre for Integrative Neuroscience (CIN)', currently involving more than 80 principle investigators associated with three faculties of the University of Tübingen and several non-university research institutions in Tübingen and its vicinity, which is now in its second funding period and likewise coordinated by P.T. The DFG-funded transregional research unit FOR 1847 'Primate Systems Neuroscience', which brings together research groups from Göttingen, Marburg, Frankfurt and Tübingen working with non-human primates, is conjointly coordinated by P. T. and Stefan Treue, German Primate Center Göttingen, the latter actually a former member of the department. The research unit took up work in March 2014. Currently, three members of the Department of Cognitive Neurology are part of an initiative to implement a new international



Extinction patients can detect a single stimulus at any spatial location. However, when two stimuli are presented simultaneously, subjects are impaired at perceiving the contralesional item. In the Department of Cognitive Neurology both neurologically healthy subjects and neurological patients are studied with the aid of methods like TMS, fMRI, lesion mapping and behavioral studies to resolve questions concerning the anatomy and the underlying mechanisms of extinction.



The displayed system allows the application of external mechanical perturbations to the body in order to study the motor control during complex walking.

graduate school together with colleagues at the NIPS, conjointly funded by the CIN and the NIPS and H.-O. Karnath and P. Thier are members of an initiative to set up a DFG-funded interdisciplinary graduate school on 'Person, Self, Agent: Towards a Unified Picture of Ourselves', coordinated by Profs. Sattig and Döring from the Tübingen School of Philosophy, which was assessed positively recently and will start its work in summer 2016. C. Schwarz is a member of the transregional research unit 'Barrel Cortical Functions', coordinated by H. Luhmann, Mainz, and M. Giese is a member of the EC-supported Human Brain Project. Finally, M. Giese, A. Lindner, C. Schwarz, P. Thier are members of the Tübingen Bernstein Centre for Computational Neuroscience that was launched with funding from the BMBF.

All members of the department contribute significantly to research-oriented teaching at the Graduate Training Centre of Neuroscience, which currently involves the International Graduate School for Neural and Behavioural Sciences, the Graduate School of Cellular and Molecular Neuroscience and the Graduate School for Neural Information Processing. Martin Giese has been instrumental in helping to set up the latter, which started in October 2011. Uwe Ilg heads the 'Schülerlabor Neurowissenschaften' (Pupils' Lab Neurosciences) funded by the CIN, that aims at making senior pupils familiar with neuroscientific topics. Further teaching is deployed at the Faculties of Biology (Uwe Ilg) and Informatics (Martin Giese and Winfried Ilg) and, of course, at Tübingen Medical School.

# Sensorimotor Laboratory

Head: Prof. Dr. Peter Thier

Team: 16 members

Key words: mirror neurons / attention / autism /  
social cognition / motor learning / fatigue / ataxia



Mirror neurons, a class of neurons in premotor cortex of monkeys, are driven not only by the observation of naturalistic actions but also by filmed actions. In both cases, the same neurons show similar responses.

**The lab works on the underpinnings of social interactions and the mechanisms underlying motor learning and their disturbances due to disease.**

*Das Labor befasst sich mit den neuronalen Grundlagen sozialer Interaktionen und denen motorischen Lernens sowie deren krankheitsbedingter Störungen.*

One of the key interests of the sensorimotor laboratory concerns the underpinnings of social interactions and their disturbances due to disease such as in schizophrenia or autism: how is it possible to understand the intentions, the desires and beliefs of others, in other words to develop a theory of (the other one's) mind (TOM)?

Establishing a TOM requires the identification of the focus of attention of another person as well as an understanding of the purpose of her/his actions. Attention allows us to select particular aspects of information impinging on our sensory systems, to bring them to consciousness and to choose appropriate behavioral responses. Social signals such as eye, head or body orientation are a particularly powerful class of sensory cues attracting attention to objects of interest to the other one. The sensorimotor laboratory tries to unravel the neuronal mechanisms affording joint attention. The working hypothesis,

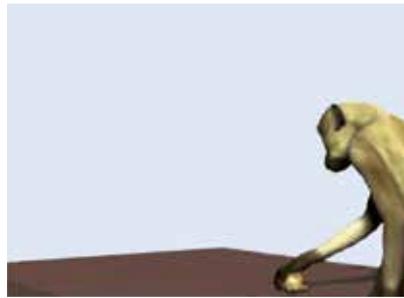
well-supported by previous and ongoing work of the lab, is that joint attention is based on specific parts of cerebral cortex (areas in the superior temporal sulcus [STS]), extracting the relevant visual features, allowing the characterization of eye and head gaze direction and converting them into spatial coordinates, taking the prevailing geometrical relationships into account. The lab hypothesizes that malfunction of these areas may actually underlie the inability of patients with autism to efficiently exploit gaze cues when interacting with others.

Complementary work on the underpinnings of social cognition addresses the role of the mirror neuron system in premotor cortex in action understanding. Mirror neurons are a class of neurons in premotor cortex of monkeys that are activated by specific types of goal-directed motor acts such as grasping a piece of apple in order to eat it. Unlike typical motor neurons, mirror neurons are also activated,

if the animal observes somebody unfolding similar behavior. This basic finding has suggested that we may understand the actions of others by mapping observed actions onto our motor repertoire, an idea that is varyingly referred to as simulation or resonance theory.

Although these ideas have received wide attention way beyond the confines of the neurosciences, the major tenet of the mirror neuron concept has never been rigorously tested. In an attempt to better understand the complex features of mirror neurons, to put the simulation theory to a critical test and to assess alternative concepts such as a role of the mirror neuron system in response selection, the lab is carrying out experiments on premotor cortical area F5. In a nutshell, our past work has shown that this particular area has access to streams of information which are obviously very important for the evaluation of the actions of others such as information

Mirror neurons are activated by the execution of specific types of goal-directed motor acts such as grasping a piece of apple in order to eat it as well as by the observation of such motor acts carried out by others.



on the operational distance between actor and observer or the subjective value, the observed action has for the observer or that observation-related responses of mirror neurons are to some extent viewpoint-invariant. This is important as the perspective under which we see the actions of others is of course not fixed.

A second major interest of the sensorimotor laboratory pertains to the role of the cerebellum in motor control. Using short-term saccadic adaptation as a model of motor learning, the sensorimotor lab has been able to develop a detailed model of the neuronal underpinnings of cerebellum-based learning. Its central idea is that a climbing fiber signal, representing information on the adequacy of the behavior prunes a simple spike population signal, which in turn, controls the behavior. A distinctive feature of cerebellum-based learning worked out by the group, is its extreme speed, accommodating behavioral adjustments within seconds, allowing the cerebellum to compensate imperfections of movements due to fatigue. The notion that the biological purpose of cerebellum-based learning is the compensation of motor or cognitive fatigue has also allowed the group to suggest a new perspective on cerebellar ataxia, the key deficit of patients suffering from cerebellar disease. Ataxia, characterized by the lack of precision, reduced velocity and increased variance is an inevitable consequence of the motor system's inability to compensate fatigue. Based on studies of perceptual disturbances from cerebellar disease, the group has been able to provide evidence for the

notion that such disturbances are a consequence of a loss of the ability to generate fast, optimized predictions of the consequences of movements, deploying the same neuronal principles that enable short-term motor learning and fatigue compensation.

Maladapted sensory predictions may lead to dizziness but also to disturbances of agency attribution, the latter arguably a key disturbance in schizophrenia. These pathophysiological concepts are pursued in patient studies.

## SELECTED PUBLICATIONS

Chen C-Y, Ignashchenkova A, **Thier P**, Hafed Z. Neuronal response gain enhancement prior to microsaccades. *Current Biology* 2015; 25(16): 2065-74.

**Marciniak K, Dicke PW, Thier P**. Monkeys' head gaze following is fast, precise and not fully suppressible. *Proceedings of the Royal Society B* 2015; 282: 20151020.

Daddaoua N, **Dicke PW, Thier P**. Eye position information is used to compensate the consequences of ocular torsion on V1 receptive fields. *Nature Communications* 2014; 5: 3047. doi: 10.1038/ncomms4047.

Dash S, **Thier P**. Cerebellum-dependent motor learning: lessons from adaptation of eye movements in primates. *Progress in Brain Research*. 2014; 210: 121-55.

**Marciniak K, Atabaki A, Dicke PW, Thier P**. Disparate substrates for head gaze following and face perception in the monkey superior temporal sulcus. *eLife*. 2014 Jul 14; e03222. doi: 10.7554/eLife.03222.

Caggiano V, **Pomper J, Fleischer F, Fogassi L, Giese MA, Thier P**. Mirror neurons in monkey area F5 do not adapt to the observation of repeated actions. *Nature Communications* 2013; 4: 1433.

**Pomper JK, Gebert L, Fischer M, Bunjes F, Thier P**. Does chronic idiopathic dizziness reflect an impairment of sensory predictions of self-motion? *Frontiers in Neurology* 2013; 4: 181.

Caggiano V, Fogassi L, Rizzolatti G, Casile A, **Giese MA, Thier P**. Mirror neurons encode the subjective value of an observed action. *Proceedings of the National Academy of Sciences USA* 2012; 109(29): 11848-53.

Prsa M, **Dicke PW, Thier P**. The absence of eye muscle fatigue indicates that the nervous system compensates for non-motor disturbances of oculomotor function. *Journal of Neuroscience* 2010; 30: 15834-42.

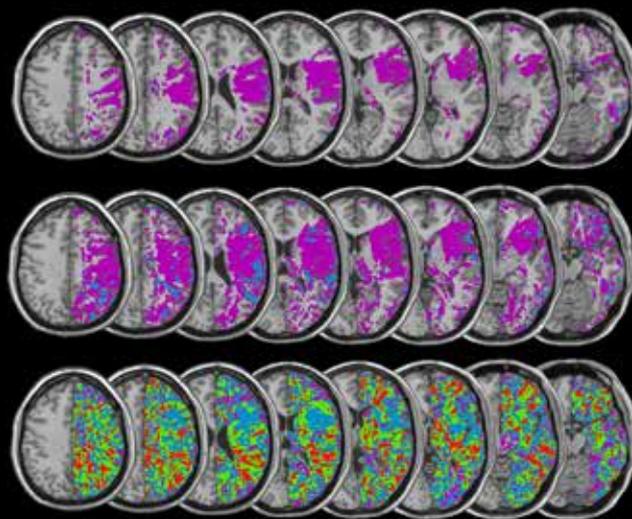
Caggiano V, Fogassi L, Rizzolatti G, **Thier P**, Casile A. Mirror neurons differentially encode the peri- and extrapersonal space of monkeys. *Science* 2009; 324: 403-6.

# Neuropsychology

Head: Prof. Dr. Dr. Hans-Otto Karnath

Team: 19 members

Key words: cognitive neuroscience / neuropsychology



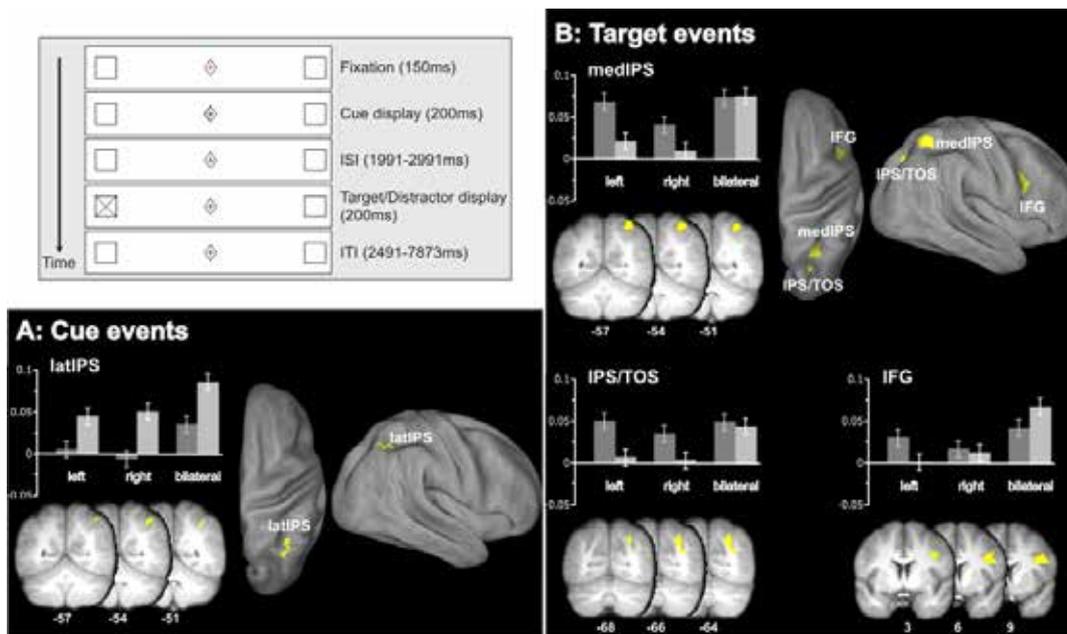
Evaluation of methods for detecting perfusion abnormalities after stroke in dysfunctional brain regions.

**The Section Neuropsychology focuses on the investigation of spatial cognition and object recognition in humans. The current issues of our work comprise the action control and sensorimotor coordination, object identification, perception of body orientation, spatial attention and exploration, grasping and pointing movements, and auditory localization in space.**

*Die Sektion Neuropsychologie arbeitet im Themengebiet Kognitive Neurowissenschaften. Arbeitsschwerpunkte der Sektion sind die Untersuchung der Raumorientierung und des Objekterkennens des Menschen, der Wahrnehmung der eigenen Körperorientierung, Prozesse der Aufmerksamkeit und Raumexploration, die visuomotorischen Koordinationsprozesse beim Zeigen und Greifen sowie die akustische Raumorientierung.*

The Section of Neuropsychology's main areas of research are the study of spatial orientation and object perception in humans. By using techniques such as functional magnetic resonance imaging (fMRI), Transcranial Magnetic Stimulation (TMS), eye tracking, and the motion capture of hand and arm movements in both patients with brain-damage and healthy subjects, the mechanisms and processes of human perception of objects, attention, the exploration of space, as well as visuomotor coordination during pointing, grasping and object interaction/manipulation are examined.

However, the greater question driving the Section of Neuropsychology's research is "how do organisms perform sensorimotor coordination processes?" For example, in order to generate successful motor actions (e.g. pointing or grasping movements) we must actively deal with the problem of spatial exploration and orientation. In order to do this it is necessary to process a multitude of sensorimotor information that is derived from constantly changing coordination systems. How the human brain accomplishes this task is a main focus of the cognitive neurosciences. The findings to our research questions not only allow us to have a better basic scientific understanding of these processes but will also aid us to develop new strategies for the treatment of patients with brain damage who show deficits in these areas.



Attending to multiple targets presented at multiple spatial locations simultaneously is crucial in everyday dynamic multi-target scenes (for example traffic scenes), yet little is known about the neural substrates of this ability. We performed a functional magnetic resonance imaging study to determine the neural anatomy associated with attending and responding to simultaneously presented targets (de Haan et al., *Cerebral Cortex* 2015; 25: 2321-31).

Our unique combination of a cued target detection task with a high proportion of catch trials allowed our study to be the first to separately assess both the neural activation specifically associated with the cue-driven top-down direction of attention to multiple potential target locations and the neural activation specifically associated with the bottom-up detection of multiple targets simultaneously. The main novel outcome of our study was that while the intraparietal sulcus was sensitive to both cue-related and target-related signals during multi-target situations, different areas of the right intraparietal sulcus appear to provide different contributions to our ability to attend and respond in multi-target environments.

## SELECTED PUBLICATIONS

Smith DV, Clithero JA, Rorden C, **Karnath H-O**. Decoding the anatomical network of spatial attention. *Proceedings of the National Academy of Sciences of the USA* 2013; 110: 1518-23.

**Borchers S, Himmelbach M**, Logothetis N, **Karnath H-O**. Direct electrical stimulation of human cortex – the gold standard for mapping brain functions? *Nature Reviews Neuroscience* 2012; 13: 63-70.

**Karnath H-O, Borchers S, Himmelbach M**. Comment on “Movement intention after parietal cortex stimulation in humans”. *Science* 2010; 327: 1200.

**Karnath H-O**, Rüter J, Mandler A, **Himmelbach M**. The anatomy of object recognition – visual form agnosia caused by medial occipitotemporal stroke. *Journal of Neuroscience* 2009; 29: 5854-62.

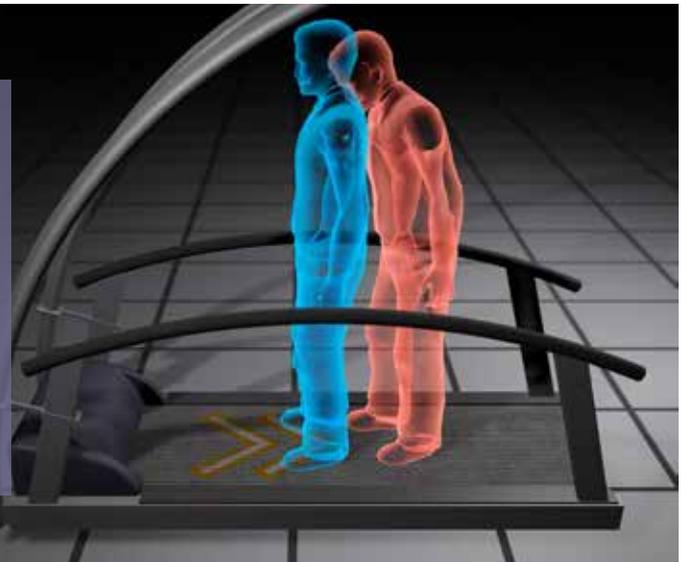
**Karnath H-O**, Baier B, Nägele T. Awareness of the functioning of one’s own limbs mediated by the insular cortex? *Journal of Neuroscience* 2005; 25: 7134-8.

# Computational Sensomotorics

Head: Prof. Dr. Martin Giese

Team: 15 members

Key words: sensorimotor control / neural modeling / movement quantification / motor learning and rehabilitation / biologically-motivated technical applications



**The Section Computational Sensomotorics investigates theoretical principles in the perception and control of motor actions. Research is organized around three main topics:**

- 1) **Clinical movement control and rehabilitation**
- 2) **Neural and computational principles of action processing**
- 3) **Biomedical and biologically-motivated technical applications**

**Research is highly interdisciplinary, including psychophysical and clinical experimentation, the development of mathematical and computational models, and the development of technical systems that exploit brain-inspired principles or support accurate diagnosis and rehabilitation training in neurological diseases.**

## **Clinical movement control and rehabilitation**

Many neurological disorders, such as cerebellar ataxia or Parkinson's disease, are associated with characteristic movement deficits. Their detailed quantitative characterization can help to understand underlying neural mechanisms and to improve the differential diagnosis of such disorders. In addition, it supports the pre-clinical diagnosis of such diseases as well as the quantification of therapeutic benefits, which is extremely difficult for complex body movements with relevance for every-day life.

We exploit advanced methods for movement analysis, motion capture, biomechanics and machine learning in order to quantify and study changes in complex whole-body movements. In cooperation with clinical partners we develop motor neurorehabilitation strategies based on scientific principles of motor learning, e.g. for cerebellar disorders.

We were able to show that physiotherapeutic training induces beneficial long-term effects in cerebellar ataxia patients, contrasting with the common opinion that cerebellar

*Die Sektion Theoretische Sensomotorik erforscht die theoretischen Prinzipien der Erkennung und Steuerung motorischer Handlungen. Die Forschungsarbeiten der Arbeitsgruppe gruppieren sich um drei Hauptthemen:*

- 1) *Klinische Forschung zur Bewegungskontrolle und Rehabilitation;*
- 2) *neuronale Mechanismen der Bewegungserkennung und -ausführung;*
- 3) *biologisch inspirierte und medizintechnische Anwendungen.*

*Die Forschungsarbeiten sind hochgradig interdisziplinär und umfassen psychophysische und klinische Experimente, die Entwicklung mathematischer und neuronaler Modelle, und die Entwicklung technischer Systeme, die auf Informationsverarbeitungsprinzipien des Gehirns beruhen, oder dabei helfen, die Diagnose sowie die Rehabilitation bei neurologischen Erkrankungen zu unterstützen.*

patients do not profit from motor training. Using commercial and own computer games (e.g. based on the Microsoft Kinect sensor) we investigate (in collaboration with M. Synofzik and L. Schöls, Dept. Neurodegeneration) novel training paradigms for patients suffering from cerebellar ataxia. Such training has been shown to be effective in ataxic children, and it might be also interesting at pre-clinical stages of these diseases in order to delay the progression of disease symptoms. We also study the relevance of cerebellar structures for perception-action coupling and different

forms of motor learning, collaborating with D. Timmann (University Clinic Essen), and exploiting patients with focal cerebellar lesions. In addition, we are interested in the neural mechanism of non-invasive brain stimulation (TMS) and its interaction with motor learning, which we study in collaboration with U. Ziemann (Dept. Vascular Neurology).

### Neural and computational principles of action processing

A vast amount of evidence shows that neural representations for the perception and planning of actions are overlapping. However, the precise mechanisms of this interaction are largely unclear. Within a close collaboration that links theoretical modeling in our group and electrophysiological experiments with nonhuman primates in the group of P. Thier, we investigate underlying neural circuits in premotor cortex (area F5). These studies are fundamental to clarify the exact neural mechanisms of action processing, which cannot be derived from behavioral or functional imaging studies. For example this research showed (contradicting common belief) that mirror neurons in premotor cortex represent visual viewing parameters and fail to show adaptation for repeated stimulus presentation. Our present research investigates the joint neural codes for perceived and planned actions and facial expressions, where we exploit high-end computer animation technology for the generation of stimuli and for the online animation of monkey avatars. Combining these experiments with quantitative neural modeling we hope to clarify the neural and computational mechanism of action encoding and its interaction with social perception.

The visual processing of social communication signals, such as bodily and facial movements, is impaired in certain psychiatric diseases. Exploiting techniques from machine learning and Virtual Reality, we study the processing of dynamic emotional body and facial expressions in the closed-loop

interaction with virtual agents and investigate critical underlying features. In collaboration with the Clinic for Psychiatry and Psychotherapy (A. C. Ehlis and A. Fallgatter) we investigate neural correlates of the processing of emotion stimuli using Near Infrared Spectroscopy (NIRS), and with international partners (Vanderbilt University, Nashville, USA; McMaster University, Hamilton, Canada), we investigate characteristic deficits in perception of emotional body expressions in schizophrenia patients and aging people.

### Biomedical and biologically-motivated technical applications

Many technical systems require highly flexible, accurate and versatile representations of complex human body movements. Inspired by the principles of human motor representations, and exploiting appropriate methods from machine learning and dynamical systems theory, we develop technical systems for the representation of complex adaptive movements with applications in computer graphics and for the control of humanoid robots. Models of this type are also used to study the perception of emotional signals in interaction between real participants and automatically reacting virtual agents. In collaboration with H.-O. Karnath (Section of



Using movement-controlled video games the group devised (in collaboration with L. Schöls, Dept. Neurodegeneration) novel training paradigms for children suffering from cerebellar ataxia at different levels of severity.

Neuropsychology), exploiting machine learning algorithms, we also investigate how semantic representations of actions are altered in patients with apraxia. In the domain of biomedical engineering, we have developed a low-cost (< 5000 EUR) gait analysis system (based on Microsoft Kinect sensors) which enables multi-center studies of rare neurological diseases, and which has been successfully applied in field studies on SCA2 patients in Cuba.

## SELECTED PUBLICATIONS

**Giese MA**, Rizzolatti G. Neural and Computational Mechanisms of Action Processing: Interaction between Visual and Motor Representations. *Neuron* 2015; 88(1): 167-80.

Goldberg H, **Christensen A**, Flash, T, **Giese MA**, Malach R. Brain activity correlates with emotional perception induced by dynamic avatars. *NeuroImage* 2015; 122: 306-17.

**Christensen A**, **Giese MA**, **Sultan F**, Mueller OM, Goericke SL, **Ilg W** et al. An intact action-perception coupling depends on the integrity of the cerebellum. *Journal of Neuroscience* 2014; 34(19): 6707-16.

**Giese MA**. Mirror representations innate versus determined by experience: A viewpoint from learning theory. *Behavioural and Brain Sciences* 2014; 37(2): 201-2.

**Ilg W**, Bastian A, Boesch S, Burciu R, Celnik P, Claassen J et al. Consensus Paper: Management of Degenerative Cerebellar Disorders. *Cerebellum* 2014; 13(2): 248-68.

# Systems Neurophysiology Laboratory

Head: Prof. Dr. Cornelius Schwarz

Team: 10 members

Key words: neocortex / tactile coding and perception / active scanning / motor coding and movements / associative learning



Rodents deploy their whiskers to explore their environment.

**We study the operating principles of the neocortex using modern multi-neuron electrophysiology and optical methods. We have established methods to observe tactile sensorimotor behavior and learning in rodents that let us study neocortical function during highly defined and precisely monitored behavior. The similarity of neocortex in animals and humans suggests that the results can be transferred easily to research on human disease (Alzheimer's, Parkinson's, schizophrenia, and depression).**

*Wir erforschen die Funktion des Großhirns (Neokortex) mit Hilfe moderner Multineuronen-Elektrophysiologie und bildgebender Verfahren auf zellulärer Ebene. Dazu haben wir neuartige Methoden entwickelt, mit denen wir beobachten können, wie Nagetiere ihren Tastsinn einsetzen und taktile Assoziationen lernen. Damit sind wir in der Lage, funktionelle Aspekte der Großhirnfunktion für genau definiertes und präzise vermessenes Verhalten zu untersuchen. Die Ähnlichkeit des Neokortex bei Tieren und Menschen legt nahe, dass unsere Resultate sehr einfach auf die Erforschung von Dysfunktion bei menschlichen Großhirnerkrankungen übertragbar sein werden (Alzheimer, Parkinson, Schizophrenie und Depression).*

## Cortex function, tactile learning, active perception

The generality of cortical neuronal architecture related to vastly diverse functions renders it likely that, beyond specific signal processing, there must be a generic function common to all cortical areas. We hypothesize that the neocortex is a giant associative storage device, which handles flexible combinations of sensory, motor and cognitive functions that the individual has learned in his/her life.

Research into cortex function requires the combination of a macroscopic and microscopic view – i.e. the study of representation of memories on the cellular level locally and their linkage between cortical areas globally. We employ modern methods of multiple neuron electrophysiology and optical

imaging/stimulation and combine it with behavioral observation at highest precision. Our model for studying these questions is the sensorimotor vibrissal system (vibrissae = whiskers) of rodents. These animals use an 'active' strategy of sampling tactile information about their immediate environment by actively moving their vibrissae across objects in their vicinity. We examine the establishment of memories (learning) and their execution (active perception) in tactile representations.

## Achievements learning

We use a Pavlovian learning paradigm, the so-called 'trace eye blink conditioning'. In this paradigm the conditioned tactile stimulus (CS, a whisker twitch) is related to the unconditioned stimulus (US, an aversive corneal air

puff) only across a stimulus-free time interval, through which the subject has to keep a memory 'trace' (hence the name), to be able to associate the two stimuli. It is known that this variant of the task has a decidedly 'cognitive' flavor, requiring firstly 'awareness' of the task and secondly an intact primary sensory cortex receiving the CS (in our case area S1). Using temporarily a precise optogenetic blockade we have found that S1 is required during presentation of the CS but not during the trace period. Using two-photon imaging of dendritic spines during task acquisition, we have found that S1 undergoes strong structural plasticity in a strictly delineated area representing the stimulated whisker.

Based on our findings, we predict that for 'trace learning', a cortical network



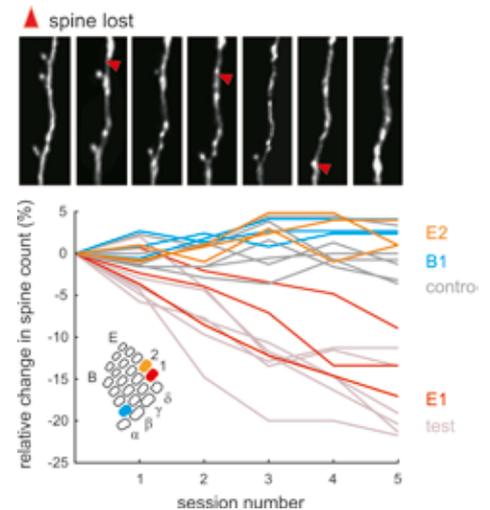
Active tactile exploration of the environment using vibriss.

including prefrontal cortex and S1 is required that establishes a ‘cognitive’ association of CS and US, in parallel to the classic, ‘sensorimotor’ association known to be established in the cerebellum.

#### Active perception

We use psychophysical methods combined with electrophysiological recording or two-photon imaging to assess how the sensorimotor neuronal system represents signals that are relevant for a subject’s decision. Along the ascending tactile pathway to S1 we found that neurons only contain information about short snippets of the vibrotactile signal. Also, behaviorally, they do not tend to integrate the vibrotactile signal across time, which is an utmost surprising finding after decades of tactile research based on Mountcastle’s seminal work about frequency and intensity discrimination (to find out about these variables, one obviously needs to integrate the vibrotactile signal). We interpret our findings that rodents use instantaneous analysis of frictional movements, short-lived stick-slip motions of the vibrissae, to discriminate textures, rather than using temporal integration of a detailed vibrotactile signal. We found that active sensor movement can systematically influence the occurrence of frictional movements, and we study how these movements affect tactile processing. These results have opened new avenues of research to find out if, why and how the animals adapt sensor movements to optimize active perception.

Our findings also inspire ideas about possible exciting parallels of function of whiskers and human fingertips. Presumed frictional movements of the papillar ridges of the fingerprint may help to put our knowledge about tactile perception on a conceptual base embracing active touch using vibriss and fingertips.



Top: Structural plasticity (spine loss) during trace conditioning.

Bottom: Structural plasticity is specific to whisker representation E1 (the stimulated whisker). No spine loss is observed in representations of not-stimulated whiskers (E2, B1), or in control animals that did not learn (Inset shows the investigated whisker representations)

## SELECTED PUBLICATIONS

**Joachimsthaler B**, Brugger D, Skodras A, **Schwarz C**. Spine loss in primary somatosensory cortex during trace eyeblink conditioning. *Journal of Neuroscience* 2015; 35: 3772-81.

**Schwarz C**, **Chakrabarti S**. Whisking control by motor cortex. *Scholarpedia* 2015; 10(3): 7466.

**Waiblinger C**, Brugger D, **Schwarz C**. Vibrotactile discrimination in the rat whisker system is based on neuronal coding of instantaneous kinematic cues. *Cerebral Cortex* 2015; 25: 1093-106.

**Waiblinger C**, Brugger D, Whitmire CJ, Stanley GB, **Schwarz C**. Support for the slip hypothesis from whisker-related tactile perception of rats in a noisy environment. *Frontiers in Integrative Neuroscience* 2015; 9: 53.

**Chagas AM**, Theis L, Sengupta B, Stüttgen MC, Bethge M, **Schwarz C**. Functional analysis of ultra high information rates conveyed by rat vibrissal primary afferents. *Frontiers in Neural Circuits* 2013; 7: 190.

Gerdjikov TV, Bergner CG, Stüttgen MC, **Waiblinger C**, **Schwarz C**. Discrimination of vibrotactile stimuli in the rat whisker system – behavior and neurometrics. *Neuron* 2015; 65: 530-40.

Stüttgen MC, **Schwarz C**. Psychophysical and neurometric detection performance under stimulus uncertainty. *Nature Neuroscience* 2008; 11: 1091-99.

# Oculomotor Laboratory

Head: Prof. Dr. Uwe Ilg

Team: 5 members

Key words: eye movements / saccades / video game play / attention



**Video-game play is a very widely distributed leisure activity in our society. Especially younger individuals do play video games every day. Actually, there is a vivid debate about possible consequences, either positive or negative, of these activities. We decided to examine the differences in oculomotor control and perceptual performance in video-game players (VGP) and non-players (NVGP). Based on the results of several studies, we find shorter latencies and higher eye velocities in VGPs compared to NVGPs. However, there is no difference in the precision of eye movements between VGPs and NVGPs. In addition, VGPs outperform NVGPs in various perceptual tasks.**

*Das Spielen von Videospiele ist eine sehr weit verbreitete Freizeitaktivität in unserer Gesellschaft. Vor allem jüngere Menschen vergnügen sich täglich mit Videospiele. Es findet derzeit eine sehr lebendige Diskussion über die möglichen Konsequenzen dieser Aktivitäten statt. Wir haben uns entschlossen, die Unterschiede in den Blickbewegungen und den Wahrnehmungsleistungen von Computerspielern und Nicht-Spielern zu untersuchen. Im Rahmen von verschiedenen Untersuchungen konnten wir kürzere Latenzen und höhere Augengeschwindigkeiten von Computerspielern im Vergleich zu Nicht-Spielern zeigen. Wir konnten jedoch keinen Unterschied in der Präzision der Augenbewegungen nachweisen. Außerdem konnten wir belegen, dass Computerspieler in verschiedenen Wahrnehmungsaufgaben besser abschneiden als Nicht-Spieler.*

Nowadays, video games are an omnipresent medium. In Germany, a recent study showed that over 46 % of the teenagers between 12 and 19 are consuming video games on a daily basis. Despite this strong prevalence, the effects of video-game consumption are still under debate. We decided to examine possible effects of video-game play in a wide battery of tests addressing eye movements and the allocation of attention.

## **Please do not look at the target! – Anti-Saccades**

We applied a very simple oculomotor task in our first study. We asked our subject to perform a saccade to the mirror position of a visual target. In some cases, the subject is unable to suppress the gaze shift towards the target, triggered by a reflexive

shift of attention towards the target. These saccades are directional errors quite similar to the visually-guided saccades called pro-saccades. There is compelling evidence that the fast visual orienting responses (directional errors) are generated by the superior colliculus in the midbrain. In contrast, the cognitively driven anti-saccades are mediated by the frontal eye field (area 8) in the frontal cortex. So the frequency of directional errors can be used as a direct measure for the strength of the executive control function of the frontal cortex upon the midbrain circuit. We tested a total of 55 subjects aged 15 to 31 years in our experiment. All subjects were either classified as VGPs or as non-players depending on their daily gaming time: VGPs (n=35) played at least one hour per day video games.

Firstly, we analyzed the saccadic reaction times of our subjects. A general finding was that the directional errors had about 100 ms shorter reaction times than the correctly executed anti-saccades. More strikingly, the reaction times of both types of saccades were decreased for approximately 10 ms in VGPs compared to non-players. The eye speed during gaze shifts reaches very high values. In our data, the maximal velocity of the eye during a 10-degree saccade is between 350 and 400 degrees/second. In other words, if the eyes could rotate without limitations, a complete rotation of the eyeball would occur within one second. As reported by others, direction errors reach higher peak velocities than anti-saccades. Surprisingly, both types of saccades of VGPs reach higher peak velocities gaze shifts executed by non-VGPs.

In order to reveal the shifts of attention, we measure precisely the gaze movements of our subjects. A high-speed camera is connected to the laptop, whose software is able to determine the position of the pupil 220 times each second. Another computer generates the visual stimuli presented on the screen in front of our subject.

To address the cognitive control function, which might be reduced in VGPs as supposed by others, we examined the frequency of direction errors in the anti-saccade task. VGPs as well as non-players showed an error rate of approximately 40%, there was no significant difference between players and non-players. In general, there is a speed-accuracy trade-off during the execution of anti-saccades. Subjects with short reaction times tend to show higher error rates than subjects with longer latencies. Despite this general relationship, we failed to find an increased amount of errors in VGPs compared to non-players. Since the frequency of directional errors is a direct measure for the amount of executive control function, our results show that this function is not impaired in VGPs compared to non-VGPs. Therefore, we conclude that playing video games does not produce negative effects on the control function of the frontal lobe. We used a modification of this paradigm to disentangle visual processing from motor preparation in the human superior colliculus. Here, the subjects had to reach out to the mirror position of a visual target. Brain activity revealed by functional MRI could be associated to either processing of visual information to one side and preparation of reaching movement to the opposite side.

Finally, we also compared the latency of the pupil light reflex in VGPs and non-VGPs. To our surprise, even this reflex was faster in VGPs compared to non-VGPs.

### Speed of shifting the spotlight of attention?

The above described decrease of reaction times in VGPs compared to non-VGPs may be attributed to the ability of VGPs to shift their spotlight

of attention faster. To test this hypothesis, we designed an experiment in which subjects had to report the identity of a specific visual target presented at a cued location. By varying the cue leading times between 0 and 600 ms, we were able to measure the benefit of changing the focus of attention towards the cued location. In this study, we examined 116 subjects, 63 identified as VGPs and 53 as non-VGPs, respectively.

We observed a better overall performance of VGPs in our experiments. We were especially interested in the speed of shifting the spotlight of attention. Therefore, we determined the cue leading time that resulted in peak performance of a given subject. Although peak performance was higher in VGPs compared to non-VGPs, we found no difference in the optimal cue leading time between VGPs and non-players. Therefore, our data do not support the hypothesis that VGPs are able to shift their spotlight of attention faster compared to

non-players. Alternatively, VGPs might have a larger spotlight of attention or the ability to process visual information more efficiently.

### Number competence

We asked our subject to identify the higher number in a parallel presentation of two stimuli containing different or identical numbers of dots. We selected stimuli ranging between 5 and 10 dots. We fitted logistic functions to the responses of our subjects. We used a Chi<sup>2</sup> goodness of fit test to ensure appropriate fits. From the logistic functions, we determined the just noticeable difference (JND) as well as the slope at the point of subjective equality (PSE). Both values were higher in VGPs compared to non-VGPs for all tested numerosities.

### Future directive

We are currently designing a training study to document a causal relationship between video-game play and superior attentional performance of VGPs.

## SELECTED PUBLICATIONS

Mack DJ, Ilg UJ. The effects of video game play on the characteristics of saccadic eye movements. *Vision Research* 2014; 102C: 26-32.

Himmelbach M, Linzenbold W, Ilg UJ. Dissociation of reach-related and visual signals in the human superior colliculus. *Neuroimage* 2013; 82C: 61-7.

Ilg UJ. The role of areas MT and MST in coding of visual motion underlying the execution of smooth pursuit. *Vision Research* 2008; 48: 2062-9.

Ilg UJ, Schumann S. Primate area MST-I is involved in the generation of goal-directed eye and hand movements. *Journal of Neurophysiology* 2007; 97: 761-71.

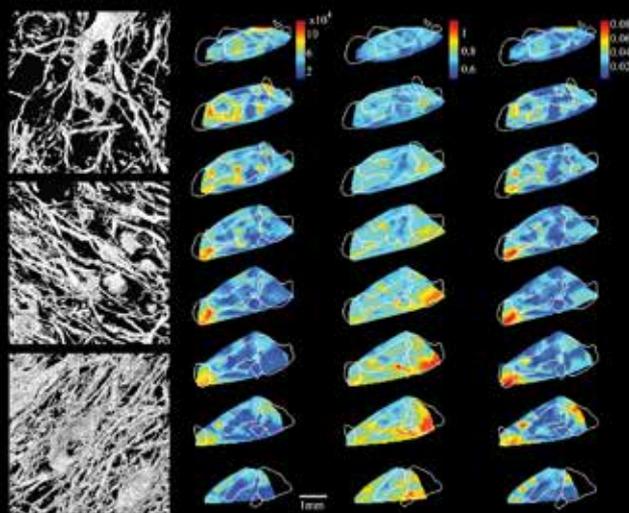
Ilg UJ, Schumann S, Thier P. Posterior parietal cortex neurons encode object motion in world-centered coordinates. *Neuron* 2004; 43: 145-51.

# Functional Neuroanatomy Laboratory

Head: PD Dr. Fahad Sultan

Team: 2 members

Key words: neuroimaging / functional magnetic resonance / cerebellum / motor control / brain evolution / electrical stimulation / quantitative neuroanatomy



**Capturing the complexity of the human brain requires gathering diverse information at multiple scales. Imaging the brain is evidently the most proximate approach to reveal these details. Functional neuroimaging includes all imaging techniques that help us to understand the functioning of the nervous system, either via detecting signals (e.g. fMRI) related to neuronal activity in defined brain regions or by linking anatomy to function via comparative, quantitative or computational approaches. In this lab unit, we use different imaging methods that allow us to visualize the complex molecular and cellular architecture of the brain at the microscopic level and also widely-distributed brain networks (electrical stimulation with fMRI) at the meso-macroscopic level. We image the neuropil of subcortical regions (with laser confocal microscopy) in the cerebellum in rodents and primates and relate these to specializations in network architecture of the human brain. We also use imaging techniques in order to address functional aspects of subcortical networks in targeting and influencing cortical network activity.**

*Die Komplexität des menschlichen Gehirns zu erfassen, erfordert Informationen aus multiplen Ebenen und Bereichen. Bildgebende Verfahren sind augenscheinlich die nächstliegende Methode zur Entschlüsselung dieser Details. Funktionelle Bildgebungsverfahren beinhalten zahlreiche Verfahren, die uns helfen die Funktion des Nervensystems zu verstehen. Diese Verfahren funktionieren entweder dadurch, dass sie aktivitätsabhängige Signale (z. B. BOLD in fMRT) erfassen, oder sie benutzen verschiedene Ansätze, die eine Verbindung zwischen der Anatomie und der Funktion herstellen. Diese Verbindungen können erreicht werden z.B. mittels vergleichender oder quantitativer Methoden oder über rechnerbasierte Modellierungen. In diesem Labor verwenden wir Bildgebungsverfahren, die uns die Visualisierung der komplexen molekularen und zellulären Architektur des Gehirns auf mikroskopischer Ebene und auch weitverteilter Hirnnetzwerke (elektrische Stimulation mit fMRT) auf der meso- bis makroskopischen Ebene erlauben. In unseren Untersuchungen können wir das Neuropil in subkortikalen Netzwerken des Kleinhirns in Nagetieren und Primaten visualisieren und quantifizieren und damit zur speziellen Netzwerkarchitektur des menschlichen Gehirns in Verbindung setzen. Unsere Ergebnisse zeigen erstmalig, dass diese tiefen Hirnstrukturen einen bedeutenden Einfluss auf weitreichende Großhirnnetzwerke haben.*

## **1. Decrease in dentate thickness as a remarkable ape-typical trait**

Compared to most mammals the cerebellar hemispheres are expanded in size in primates and even more so in apes and humans. In this study we re-examined the human dentate morphology using detailed 3D surface models. Our reconstructions showed that the major part of the nucleus is similar to the phylogenetically older microgyric dorsal motor part of the dentate. Therefore, the characteristic of the human dentate is its folding and surface increase and not the

emergence of a ventral macrogyric region. We studied the thickness of cortical sheets in different species and show systematic changes that are related to the cortex's (cerebral or cerebellar cortex or cerebellar nuclei) neuronal architecture. For instance, one major difference between the cerebral and cerebellar cortex is the larger increase in the thickness of the former. This is presumably caused by an increase in the wiring required by an associative network (Palm, 1987; Braitenberg and Schüz, 1991; Sultan, 2002). In addition, it is well known that

the cetacea have a thinner cerebral cortex than expected for their body/ brain size, which is due to the lack of layer IV in these animals. The data of the cerebral and cerebellar thickness are taken from previously published studies, while the dentate thickness is derived from the current study and based on the 3D models. In monkeys (and in other mammals), the thickness of the dentate gray matter increases with brain size. However, this cannot be said of the apes, in which dentate thickness remains close to 0.15mm regardless of brain size. Interestingly,

### Imaging the neuropil of the rodents' deep cerebellar nuclei.

We have analyzed the major components of the neuropil in the four deep cerebellar nuclei of the rat's brain using a quantitative 3D immunohistochemical method. We segmented and traced the neuropil stained with antibodies.

0.15mm is the thinnest sheet-like neuronal tissue that has ever been recorded (the thinnest retinae are around 0.143mm and the thinnest cerebellar cortices are probably also below 0.25mm), suggesting that we have reached the lower limit of neuronal tissue thickness. These results are in line with the hypothesis that we suggested, that the marked differences of the ape dentate is its remarkably reduced thickness.

### 2. Cellular and molecular architecture of the mammalian cerebellar nuclei: searching for the human-typical traits in the dentate nucleus

A common view of the architecture of different brain regions is that despite their heterogeneity they optimized their wiring schemes to make maximal use of space. Based on experimental findings, computational models have delineated how about 2/3 of the neuropil is filled out with dendrites and axons optimizing cable costs and conduction time whilst keeping the connectivity at the highest level. However, whether this assumption can be generalized to all brain regions has not yet been tested. In this project, we have used semi-automated 3D reconstructions of immune-stained rat brains to quantify and chart the components of the neuropil in the four deep cerebellar nuclei (DCN). Our approach allowed us to be sufficiently fast to systematically sample all DCN regions and reconstruct the neuropil with detail. We observe differences in dendritic and axonal fiber length density, average fiber diameters and volume fraction within the four different nuclei that comprise the DCN. We observe a relative increase in the length density of dendrites and Purkinje cell axons in two of the DCN, namely the posterior interposed nucleus and the lateral nucleus (also called dentate in primates). Furthermore, the DCN have a surprisingly low volume fraction of their dendritic

length density, which we propose is related to their special circuitry.

### 3. Correlating synaptic and ion channel markers with electrical excitability of brain regions

Electrical stimulation, combined with functional magnetic resonance imaging (es-fMRI), revealed a striking difference within the different deep cerebellar nuclei (DCN) of the monkey. Stimulating the phylogenetically older DCN we observed stimulation-induced BOLD activity in classical cerebellar receiving regions such as primary motor cortex, as well as in a number of additional areas in insular, parietal and occipital cortex, including all major sensory cortical representations. Independent of the specific cerebral area activated, responses were strongest for very high stimulation frequencies ( $\Rightarrow$  400Hz), suggesting a projection system optimized to mediate fast and temporarily precise information.

However, stimulation of the dentate nucleus failed to show activation in cerebral cortical areas. We hypothesize, that the dentate does not respond optimally to high frequency stimulation. Therefore, in our current attempt we wanted to elucidate differences in the synaptic and ion channel composition of the DCN that could explain these different responses to electrical stimulation. We have now established a multiple immunofluorescence protocol that allows to study different vesicular glutamate transporters (1 and 2) as markers of the excitatory presynapses and also potassium channels that are known to underly high-frequency firing (Kv3.3). Together with the structural markers MAP2a,b and PCP2, which mark either dendritic or Purkinje cell axons, we can study the colocalization patterns and look for systematic differences between the DCN that could explain the differences in neuronal behavior.

## SELECTED PUBLICATIONS

**Hamodeh S**, Sugihara I, Baizer J, **Sultan F**. Systematic analysis of neuronal wiring of the rodent deep cerebellar nuclei reveals differences reflecting adaptations at the neuronal circuit and internuclear level. *Journal of Comparative Neurology* 2014; 522: 2481-97.

**Sultan F**. From cerebellar texture to movement optimization. *Biological Cybernetics* 2014; 108: 677-88.

**Sultan F**, Augath M, **Hamodeh S**, Murayama Y, Oeltermann A, Rauch A, **Thier P**. Unravelling cerebellar pathways with high temporal precision targeting motor and extensive sensory and parietal networks. *Nature Communications* 2012; 3: 924.

Glickstein M, **Sultan F**, Voogd J. Functional localization in the cerebellum. *Cortex* 2011; 47: 59-80.

**Sultan F**, Augath M, Murayama Y, Tolia AS, Logothetis NK. esfMRI of the upper STS: further evidence for the lack of electrically induced polysynaptic propagation of activity in neocortex. *Magnetic Resonance Imaging* 2011; 29: 1374-81.

Logothetis NK, Augath M, Murayama Y, Rauch A, **Sultan F**, Goense J, Oeltermann A, Merkle H. The effects of electrical microstimulation on cortical signal propagation. *Nature Neuroscience* 2010; 13: 1283-91.

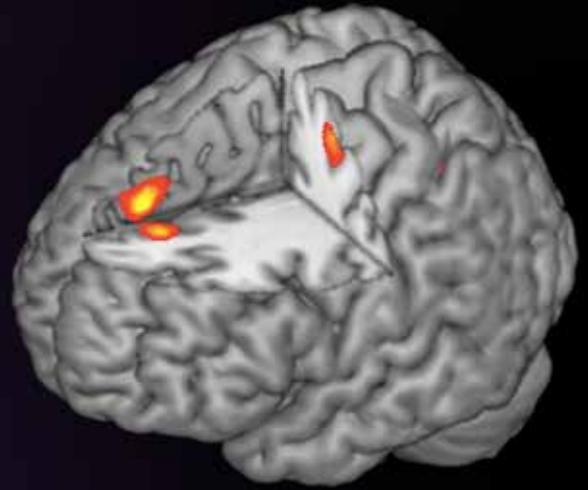
**Sultan F**, **Hamodeh S**, Baizer JS. The human dentate nucleus: a complex shape untangled. *Neuroscience* 2010; 167: 965-8.

# Neuropsychology of Action

Head: Dr. Marc Himmelbach

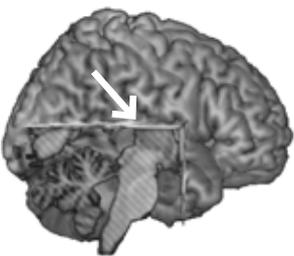
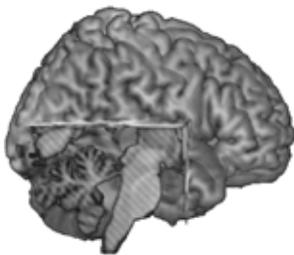
Team: 7 members

Key words: reaching / grasping / optic ataxia / apraxia / visual agnosia



**The Research Group “Neuropsychology of Action” is dedicated to investigations of human action control. Our work combines neuropsychological examinations of brain-damaged patients with state-of-the-art techniques for behavioral and brain activity measurements (functional neuroimaging; transcranial magnetic stimulation; motion and eye tracking systems).**

*Die Forschungsgruppe „Neuropsychologie der Handlungskontrolle“ widmet sich der Erforschung motorischer Kontrollprozesse beim Menschen. Unsere Arbeit kombiniert neuropsychologische Untersuchungen hirngeschädigter Patienten mit modernsten experimentellen Methoden der Verhaltens- und Hirnaktivitätsmessung.*



The superior colliculi are part of the tectum which additionally comprises the inferior colliculi right below. Traditionally the superior colliculi have been associated with visual and oculomotor functions.

Our work addresses higher order motor control deficits. With ‘higher order’ we want to express that these deficits are not simply caused by a loss of muscular strength. Our individual research projects investigate the neural and functional foundations and conditions that are associated with such disorders.

## **Evaluation of object functionality and mechanical reasoning in humans**

Human action control is characterized by its impressive complexity and flexible adjustment in tool use and object manipulation. We aim to investigate the cognitive control mechanisms involved in the evaluation of action affordances associated with an object and their neuronal correlates. How do we recognize a usable tool for a particular technical problem? How do memory and acquired knowledge about tools on the one hand and visual analysis and deductive reasoning on the other hand contribute

to our respective decision? A small group of brain damaged patients are especially impaired in using novel, unfamiliar tools while they are less impaired in using familiar tools. The examination of such patients and further behavioral and neuroimaging studies based on observations in these patients can help us to understand the way different cognitive sources are combined to come up with a motor behavior that no other living species can match.

## **The human superior colliculi – a small big player in the human brain?**

The superior colliculi are located at the upper brainstem of humans. In contradiction of established textbook knowledge, research in nonhuman primates through the last decade demonstrated that the superior colliculi play some role in the execution of arm movements. In our ongoing studies we found clear evidence for its role in the control of arm movements

also in healthy humans. However, the precise functional contribution of the colliculi to the processes of planning and execution and the processing of a movement's sensory feedback is still unknown. To explore this unknown territory we currently develop experimental designs that allow for event-related analyses and transfer our paradigms to the ultra-high field 9.4T scanner at the MPI for High-field Magnetic Resonance. Using tensor imaging and resting state fMRI we investigate the connectivity of the superior colliculi within the sensorimotor network. First studies in nonhuman primates have already demonstrated a connection between the functions of superior colliculi and the appearance of motor disorders like cervical dystonia. A precise functional mapping of the colliculi in living humans will not only be important for the understanding of neurological motor disorders but might also reveal that this concise structure could be good candidate regions in the framework of neuroprosthetics and brain stimulation in the future.

#### **The impact of object knowledge on visual motor control**

We grasp a screwdriver in a specific way if we are about to use it and in a very different way if we just want to put it aside. Despite of such quite obvious dependencies of visual motor control on object recognition, many researchers believe that the actual control of human grasping depends almost entirely on the direct visual information about object sizes irrespective of any stored knowledge in our memory. In contrast, we demonstrated that well established associations, build through a long-term learning process, are powerful enough to change visual motor control. Interestingly, we also observed some patients with impairments in the control of grasping who apparently exploited such associations for an individual improvement: they are better in grasping very familiar in comparison to neutral geometrical objects. Our work suggests that the role of object familiarity



Brain activity during a pointing movement can be monitored by magnetic resonance imaging. The subject gets some last instructions before the recording starts.

on the control of movements was dramatically underestimated in the past.

#### **Upper and lower limb proprioception in hereditary ataxias**

We take it for granted that we can feel our own body, position and movements of our own limbs. But soon we realize that it is pretty difficult to explore in more detail the current feedback from our body sensors. Some degenerative diseases are associated with defects of the ascending

proprioceptive pathways. Surprisingly, the functional status of upper and lower limb proprioceptive sensation has never been tested beyond neurological routine measures. In cooperation with the Department for Neurodegenerative Diseases we conduct sensitive measurements of proprioception that can provide us with new insights concerning contributing factors to the patients' devastating coordination problems.

## **SELECTED PUBLICATIONS**

Martin JA, Karnath HO, Himmelbach M. Revisiting the cortical system for peripheral reaching at the parieto-occipital junction. *Cortex* 2015; 64: 363-79.

Borchers S, Synofzik M, Kiely E, Himmelbach M. Routine clinical testing underestimates proprioceptive deficits in Friedreich's ataxia. *Cerebellum* 2013; 12: 916-22.

Borchers S, Müller L, Synofzik M, Himmelbach M. Guidelines and quality measures for the diagnosis of optic ataxia. *Frontiers in Human Neuroscience* 2013; 7: 324.

Linzenbold W, Himmelbach M. Signals from the deep: reach-related activity in the human superior colliculus. *Journal of Neuroscience* 2012; 32: 13881-8.

Borchers S, Himmelbach M. The recognition of everyday objects changes grasp scaling. *Vision Research* 2012; 67: 8-13.



**Department  
of Cellular  
Neurology**



**DEPARTMENT OF CELLULAR NEUROLOGY 116**

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Section of Dementia Research	124



## Departmental Structure



Prof. Mathias Jucker is head of the Department of Cellular Neurology.

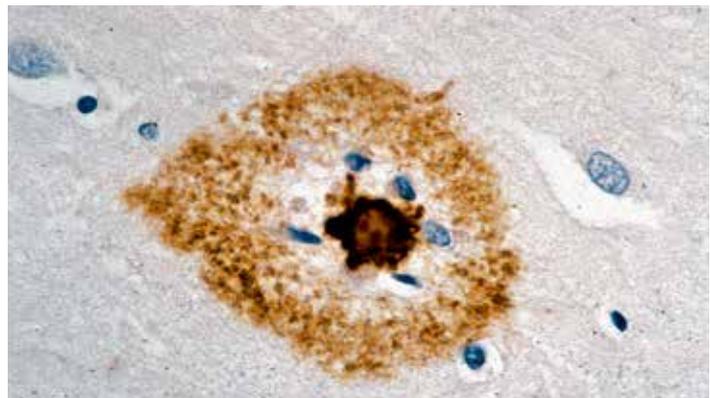
The Department is headed by Professor Mathias Jucker and was founded in 2003. The research focus is on the cellular and molecular mechanisms of brain aging and age-related neurodegenerative diseases, with a special emphasis on the pathogenesis of Alzheimer's disease and other cerebral amyloidoses. Alzheimer's disease is the most frequently occurring age-related dementia, with more than 1 million people affected in Germany. It was in Tübingen that Alois Alzheimer first described the disease to his colleagues in 1906. To mark this occasion, the Department of Cellular Neurology hosted a centennial symposium in 2006 (Alzheimer: 100 Years and Beyond). As of 2010 our department is also part of the German Center for Neurodegenerative Diseases (DZNE).

Currently our department is composed of five research groups and one core unit: The Amyloid Biology group studies the molecular mechanisms of amyloid formation using in vitro and biochemical methods. The Experimental Neuropathology group uses transgenic mouse models to analyze the pathomechanisms of Alzheimer's disease and cerebral amyloidoses.

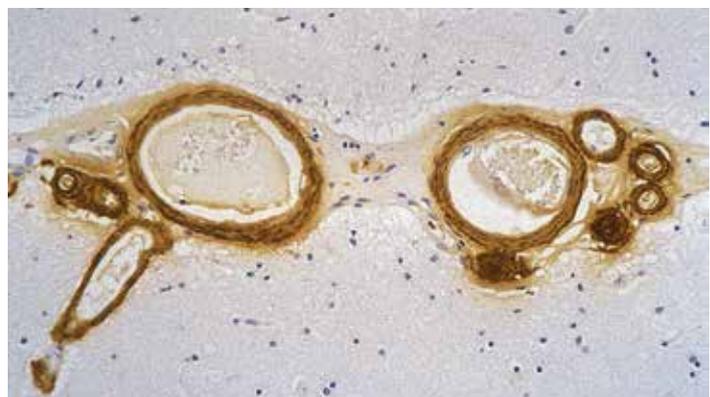
The Molecular Imaging group studies how Alzheimer's disease lesions and neurodegeneration develop over time in the transgenic mouse models using in vivo multiphoton microscopy. The Fluid Disease Biomarkers group uses immunoassays to identify early biomarkers in the cerebrospinal fluid and blood of mouse models and human subjects with Alzheimer's disease and related disorders. The Experimental Neuroimmunology group works on aspects of innate immunity in the aging brain and in neurodegenerative diseases. Finally, the core unit supports the department with mouse genotyping, ELISA measurements, and other technical and administrative activities.

We are primarily a department of basic research with a focus on preclinical investigations of disease mechanisms. To foster the translation of our research to clinical applications, we partnered with the University Clinic of Psychiatry and Psychotherapy to establish the Section for Dementia Research with its Memory Clinic. Moreover, we are coordinating the international Dominantly Inherited Alzheimer Network (DIAN) study in Germany, which aims to understand the rare genetic forms of Alzheimer's disease by longitudinal analysis of gene mutation carriers and non-mutation carrier siblings. Understanding this type of Alzheimer's disease is expected to provide important clues to the development of the more common sporadic form of Alzheimer's disease.

Our department hosts scientists from more than 10 nations, ranging from short-term fellows, master students, PhD and MD students to postdoctoral fellows and group leaders. This diversity, along with our extensive expertise in brain aging and neurodegenerative disease, creates a socially and intellectually stimulating intramural environment that is also highly competitive extramurally.



Amyloid plaque ( $A\beta$  immunochemistry) in an Alzheimer brain.



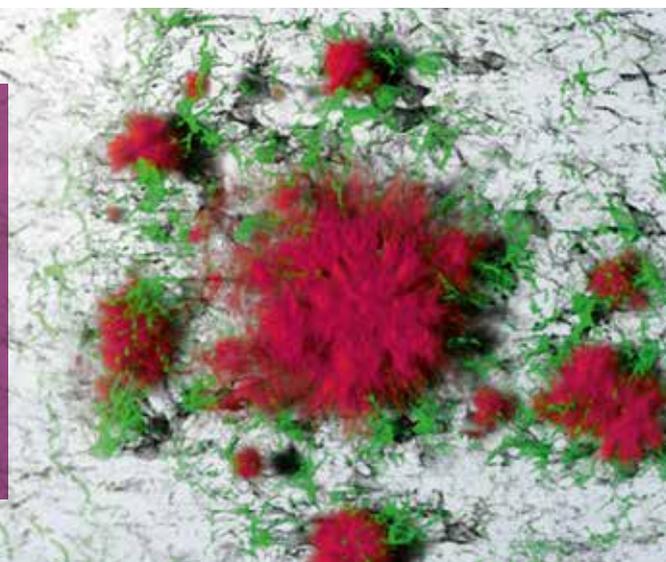
Vascular amyloid (cerebral amyloid angiopathy) in an Alzheimer brain.

# Experimental Neuropathology

Head: Prof. Dr. Mathias Jucker

Team: 18 members

Key words: cellular neurology / alzheimer's disease / cerebral amyloid angiopathy



Microglia (green) surrounding an amyloid plaque (red).

**Our objective is to understand the pathogenic mechanism of Alzheimer's disease and related amyloidoses and to develop therapeutic interventions.**

*Unser Ziel ist, den Pathomechanismus der Alzheimer-Erkrankung und verwandter Amyloiderkrankungen zu verstehen und therapeutische Interventionen zu entwickeln.*

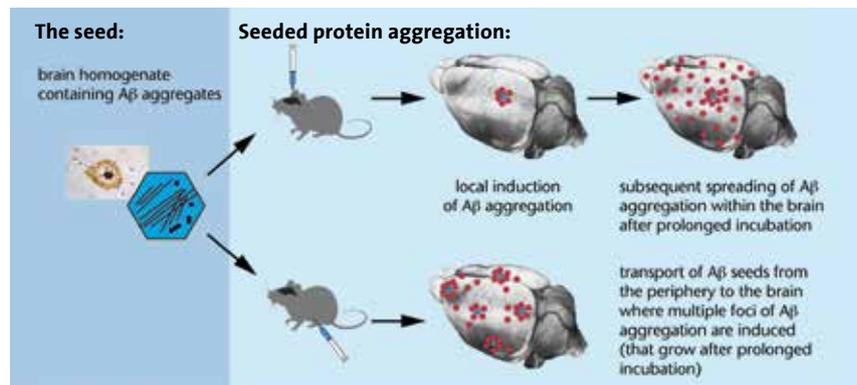
Misfolded proteins are the cause of many neurodegenerative diseases. How these proteins misfold and what the initial trigger is for the misfolding and their subsequent aggregation is, however, largely unknown. Furthermore, it remains unclear why the aging brain is a risk factor for neurodegenerative diseases.

In Alzheimer's disease aggregated  $\beta$ -amyloid ( $A\beta$ ) protein is deposited extracellularly in so-called amyloid plaques. Aggregated  $A\beta$  leads to a miscommunication between the cells and in a second stage to neuron death. The same  $A\beta$  protein can also build up in blood vessels which will cause amyloid angiopathy with potential vessel wall rupture and fatal cerebral bleedings.

In the past few years we have managed to generate transgenic mouse models that either mirror Alzheimer's pathology by developing  $A\beta$  plaques or serve as a model for cerebral amyloid angiopathy by depositing  $A\beta$  protein in blood vessels. With the help of these models we have been able to show that  $\beta$ -amyloid aggregation can be induced exogenously by inoculations of mice with brain extracts from deceased Alzheimer patients or from aged transgenic mice with  $A\beta$  deposition. The amyloid-inducing agent in the extract is probably the misfolded  $A\beta$  protein itself. Thereby, soluble proteinase K (PK)-sensitive  $A\beta$  species have been found to reveal the highest  $\beta$ -amyloid-inducing activity.

These observations are mechanistically reminiscent of prion diseases and are now used to develop new therapeutic approaches for Alzheimer's disease and other amyloidoses. First immunotherapy trials in transgenic mice are promising and show that  $\beta$ -amyloid aggregation can be reduced by targeting the initial proteopathic  $A\beta$  seeds. Microglia appear to play a crucial role in  $A\beta$  immunotherapy.

It is important to translate therapy success in mice into clinical settings. Moreover, it is essential to detect and prevent  $A\beta$  aggregation in mice and men before it leads to the destruction of neurons as seen in Alzheimer's disease. To this end we use in vivo 2-photon microscopy to track initial  $A\beta$  aggregation and analyze  $A\beta$  levels in murine cerebrospinal fluid as an early biomarker of Alzheimer's disease.



$\beta$ -amyloid containing brain extracts which are intracerebrally or intraperitoneally injected in young APP transgenic mice induce A $\beta$ -aggregation and deposition in the animals.

## SELECTED PUBLICATIONS

**Heilbronner G, Eisele YS, Langer F, Kaeser SA, Novotny R, Nagarathinam A, Aslund A, Hammarstrom P, Nilsson KPR, Jucker M** (2013) Seeded strain-like transmission of  $\beta$ -amyloid morpho-types in APP transgenic mice. *EMBO reports* 14: 1017-22.

**Jucker M, Walker LC** (2013) Self-propagation of pathogenic protein aggregates in neurodegenerative diseases. *Nature* 501: 45-51.

**Maia LF, Kaeser SA, Reichwald J, Hruscha M, Martus P, Staufenbiel M, Jucker M** (2013) Changes in amyloid- $\beta$  and Tau in the cerebrospinal fluid of transgenic mice overexpressing amyloid precursor protein. *Science Translational Medicine* 5, 194re2.

**Fritschi SK, Langer F, Kaeser S, Maia LF, Portelius E, Pinotsi D, Kaminski CF, Winkler DT, Maetzler W, Keyvani K, Spitzer P, Wiltfang J, Kaminski Schierle GS, Zetterberg H, Staufenbiel M, Jucker M** (2014) Highly potent soluble amyloid- $\beta$  seeds in human Alzheimer brain but not cerebrospinal fluid. *Brain* 137: 2909-15.

**Schweighauser M, Bacioglu M, Fritschi SK, Shimshek DR, Kahle PJ, Eisele YS, Jucker M** (2015) Formaldehyde-fixed brain tissue from spontaneously ill  $\alpha$ -synuclein transgenic mice induces fatal  $\alpha$ -synucleinopathy in transgenic hosts. *Acta Neuropathologica* 129: 157-9.

**Jucker M, Walker LC** (2015) Neurodegeneration: Amyloid- $\beta$  pathology induced in humans. *Nature* 525: 193-4.

**Ye L, Hamaguchi T, Fritschi SK, Eisele YS, Obermüller U, Jucker M, Walker LC** (2015) Progression of Seed-Induced A $\beta$  Deposition within the Limbic Connectome. *Brain Pathology* 25: 743-52.

**Maia LF, Kaeser SA, Reichwald J, Lambert M, Obermüller U, Schelle J, Odenthal J, Martus P, Staufenbiel M, Jucker M** (2015) Increased CSF A $\beta$  during the very early phase of cerebral A $\beta$  deposition in mouse models. *EMBO Molecular Medicine* 7: 895-903.

**Hefendehl JK, Neher JJ, Sühs RB, Kohsaka S, Skodras A, Jucker M** (2014) Homeostatic and injury-induced microglia behavior in the aging brain. *Aging Cell* 13:60-9.

**Ye L, Fritschi SK, Schelle J, Obermüller U, Degenhardt K, Kaeser SA, Eisele YS, Walker LC, Baumann F, Staufenbiel M, Jucker M** (2015) Persistence of A $\beta$  seeds in APP null mouse brain. *Nature Neuroscience* 18: 1559-61.

Walker LC, **Jucker M** (2015) Neurodegenerative diseases: Expanding the prion concept. *Annual Review of Neuroscience* 38: 87-103.

# Amyloid Biology

Head: Dr. Frank Baumann

Team: 4 members

Key words: molecular mechanisms of neurodegeneration

Electron micrograph of recombinant A $\beta$  fibrillized after seeding.

**We study mechanisms of neurodegeneration and the molecular nature of pathogenic protein aggregation in particular in Alzheimer's Disease. We use molecular and cell biology techniques to identify key processes and potential steps of intervention.**

*Wir untersuchen Mechanismen der Neurodegeneration sowie die molekulare Struktur von Protein Aggregaten insbesondere bei der Alzheimer-Erkrankung. Dabei nutzen wir Methoden der Molekular- und Zellbiologie, um Schlüsselschritte und Interventionspunkte zu finden.*

APP is one of the major proteins involved in Alzheimer's disease (AD) but its physiological function remains elusive. Sequential cleavage of APP by  $\beta$ - and  $\beta$ -secretase leads to generation of amyloid beta (A $\beta$ ) peptides, the major components of amyloid plaques in the brain of patients with AD.

The Amyloid Biology group has its focus in four major areas:

- (i) To determine which forms of A $\beta$  can cause protein aggregation and amyloid formation.
- (ii) To establish the subcellular localization of A $\beta$  and the mechanisms by which organell-associated A $\beta$  induces amyloidogenesis.
- (iii) To determine the environmental requirements and mechanisms underlying the self-replication of pathogenic A $\beta$  aggregates.
- (iv) Neurotoxic mechanism underlying proteopathies.

To achieve these goals, we are developing biochemical protocols to isolate different soluble and membrane-associated A $\beta$  aggregates. We determine

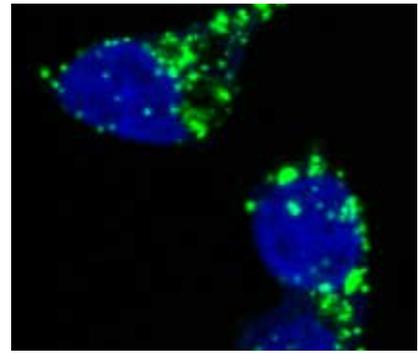
their amyloid inducing properties including structural specificities as well as toxicity with a combination of in vitro assays and in vivo genetically engineered mouse models.

A $\beta$  deposition has long been associated with neurodegeneration. However, animal models that succeeded to mimic plaque formation failed to display neurodegeneration or neuronal loss. Isolated neuronal cells are, however, very sensitive to oligomeric forms of A $\beta$  though never forming plaque like aggregates. Uptake and intracellular production of A $\beta$  may result in the accumulation of A $\beta$  also within cells and organelles. Previous reports have drawn attention to the accumulation of A $\beta$  within mitochondria. With a novel in vivo targeting approach based on previous studies we established the exclusive sorting of A $\beta$  to mitochondria and are currently investigating metabolic consequences.

Recently lipids have come into focus as a key component for aggregation of infectious prion protein aggregates. Furthermore, membranes and membrane

anchoring proved to be key features for neurotoxicity in prion diseases. To investigate whether similarly membrane association of A $\beta$  would promote both aggregation and neurotoxicity in vivo we have modified the A $\beta$  peptide. Using previous expression methods we established membrane anchored A $\beta$  with an artificial C-terminal membrane anchor. Our results indicate that A $\beta$  can indeed be stably expressed on cell surfaces both in cell culture models and in transgenic mice. Crossbreeding APP-transgenic mice demonstrated that membrane-anchored A $\beta$  promotes plaque deposition and increases neurodegeneration in vivo (Nagarathinam et al. J. Neurosci. 2013). Membrane lesions found in prion-affected mice were not replicated in double transgenic young mice which were clinically still healthy but A $\beta$ -peptides were shown to accumulate on morphologically normal neurite membranes and elicited rapid glial recognition (Jeffrey et al. Neuropathol Appl Neurobiol. 2014). Future work will employ the role of membrane-anchored A $\beta$  intermediates in the initiation of A $\beta$  aggregation and neurotoxicity.

Murine cells (blue nuclear staining) expressing on their surface GPI-anchored A $\beta$  (green).



Brain tissue from A $\beta$  laden transgenic mice has been previously shown to stimulate  $\beta$ -amyloid deposition in young transgenic mice. This process was termed seeding but little is known about the nature of the seed. We established a high throughput assay (FRANK-assay) that can identify the presence of seeds in tissue homogenates even within formalin fixed tissue (Fritschi et al. 2014). With this powerful tool we were now able to analyse defined fractions of brain tissue for the qualitative and quantitative presence of amyloid seeds. Combining differential and sucrose gradient centrifugation, we have identified distinct A $\beta$  seed-containing membrane fractions in brain homogenates from APP-transgenic mice. Potent fractions were then further characterized and analyzed for seeding activity in vivo. With this we now have conclusive data showing highly potent seeds being present in mitochondria and mitochondria associated membranes (MAMs) (manuscript under revision). The FRANK-assay is further explored to detect minute amounts of such seeds also in body fluids for diagnostic purpose.

Recently it has been hypothesized that A $\beta$  like prions can form seeds which give rise to structurally distinct aggregates which might also have an influence on spreading and pathophysiological progression of the disease in patients. For this phenomenon the term strain has been established in prion research. Similarly two A $\beta$  depositing mouse models (APP23 and APP PS1) with diverging plaque morphology have already been established. So far these could be analyzed in a labor intensive histological approach using

conformation sensitive dyes on fresh frozen brain section and analyzing individual plaques. To this end we devised in collaboration with the lab of Marc I Diamond (University of Dallas Texas) a high throughput screen based on FACS-analysis (BARCODE-assay). Different aggregated amyloid structures are identified by using a set of monoclonal antibodies with slightly different binding affinities to generate a “fingerprint” of A $\beta$ . Our method biases towards aggregate detection and away from monomer detection. The flow cytometer quantifies the amount of each antibody bound and thus creates a quantitative fingerprint

for each brain that enables binning into multivariate space, based purely on aggregate structure. In initial experiments we have determined that this method can distinguish between A $\beta$  populations from APP23 and APP PS1 mice. There is also evidence that differences can also be detected between human AD cases, in particular between familial and sporadic forms (manuscript in preparation). This method will provide a characterization of pathological protein aggregates at the molecular level of structure variation. Correlating these findings with clinical data may provide a valuable clue for the basis of disease variation.

## SELECTED PUBLICATIONS

**Baumann F**, Pahnke J, Radovanovic I, Rulicke T, Bremer J, Tolnay M, Aguzzi A. Functionally relevant domains of the prion protein identified in vivo. *PLoS One*. 2009; 4(9): e6707. Epub 2009/09/10.

**Eisele YS**, Obermuller U, Heilbronner G, Baumann F, Kaeser SA, Wolburg H, Walker LC, Staufenbiel M, Heikenwalder M, Jucker M. Peripherally applied Abeta-containing inoculates induce cerebral beta-amyloidosis. *Science*. 2010; 330(6006): 980-2.

**Nagarathinam A**, Höflinger P, Bühler A, Schäfer C, McGovern G, Jeffrey M, Staufenbiel M, Jucker M, Baumann F (2013) Membrane-anchored A $\beta$  accelerates amyloid formation and exacerbates amyloid-associated toxicity in mice. *Journal of Neuroscience* 33: 19284-19294.

**Fritschi SK**, Cintron A, Ye L, Mahler J, Bühler A, Baumann F, Neumann M, Nilsson KP, Hammarstrom P, Walker LC, Jucker M (2014) A $\beta$  seeds resist inactivation by formaldehyde. *Acta Neuropathologica* 128: 477-484.

Jeffrey M, McGovern G, Barron R, Baumann F (2015) Membrane pathology and microglial activation of mice expressing membrane anchored or membrane released forms of Abeta and mutated human APP. *Neuropathology and Applied Neurobiology* 41: 458-470.

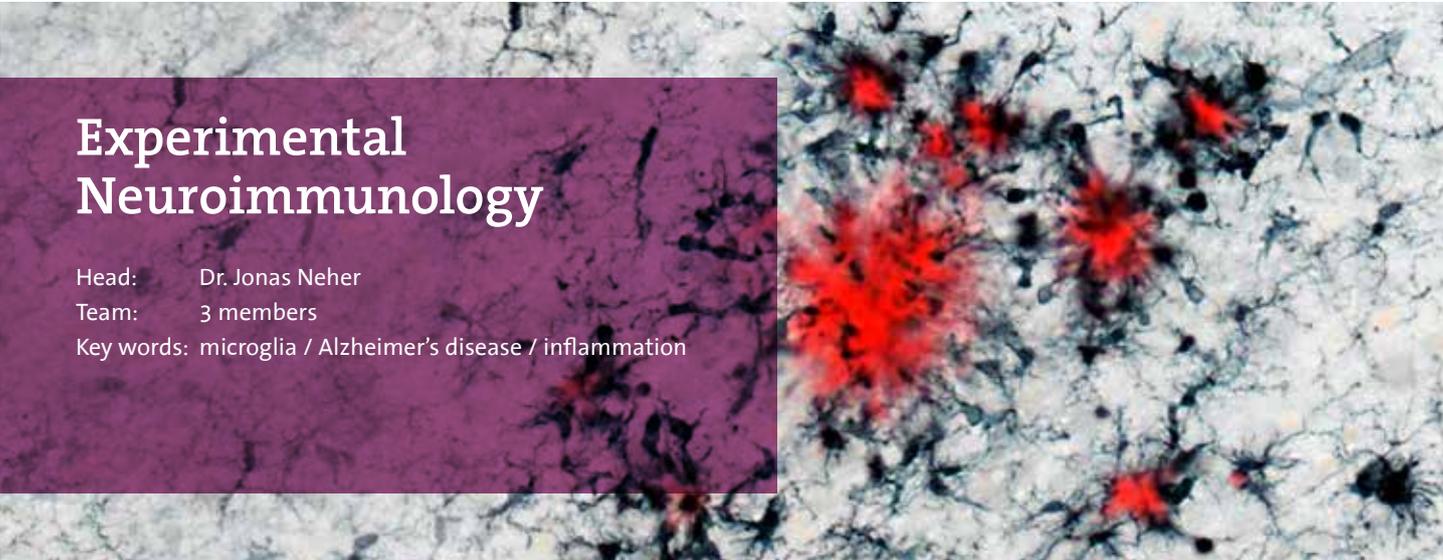
**Ye L**, Fritschi SK, Schelle J, Obermuller U, Degenhardt K, Kaeser SA, Eisele YS, Walker LC, Baumann F, Staufenbiel M, Jucker M (2015) Persistence of A $\beta$  seeds in APP-null mouse brain. *Nature Neuroscience* 18: 1559-1561.

# Experimental Neuroimmunology

Head: Dr. Jonas Neher

Team: 3 members

Key words: microglia / Alzheimer's disease / inflammation



Amyloid- $\beta$  plaques (red) surrounded by microglia (black).

**Our objective is to understand how the brain's immune system contributes to the pathogenic mechanism of Alzheimer's disease and to develop therapeutic interventions that target the immune system.**

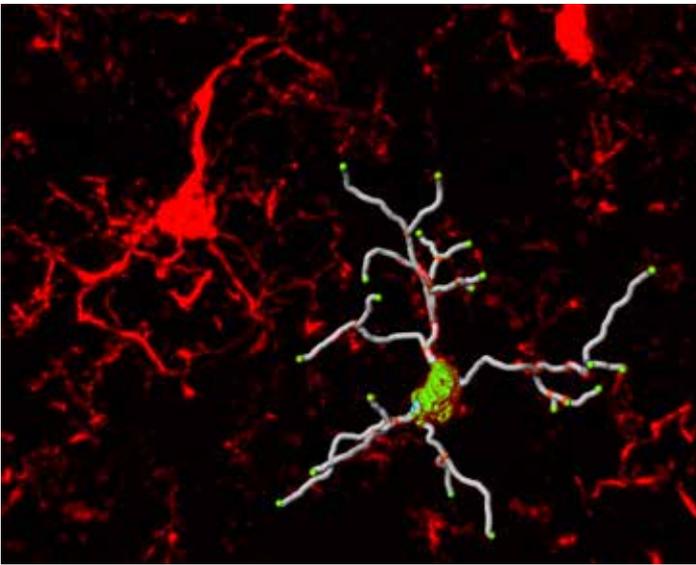
*Unser Ziel ist es zu verstehen, wie das Immunsystem des Gehirns zu dem Pathomechanismus der Alzheimer-Erkrankung beiträgt, um aus diesen Erkenntnissen therapeutische Interventionen zu entwickeln.*

It is now well established that most (if not all) neurological diseases present with an inflammatory component. These include acute conditions such as stroke as well as chronic neurodegenerative diseases such as Alzheimer's disease (AD). However, it has proven difficult to determine when the activation of the brain's immune system is beneficial or detrimental in these diseases and considerable controversy still exists in the literature. This controversy may be partially due to the fact that tissue resident macrophages (including microglia) are highly plastic cells that can adapt to their particular microenvironment. Therefore, one of our aims is to understand how the microglial activation state changes

in Alzheimer's disease. To this end we are analyzing the gene expression and epigenetic profile of microglia in mouse models and investigate how these cells adapt in response to inflammatory stimuli.

Further, it has been suggested that microglial dysfunction, i.e. the inability of these cells to perform their normal surveillance function in the brain, may contribute to the onset or progression of Alzheimer's disease. We have recently tested this hypothesis by replacing brain-resident microglia with circulating monocytes from the blood. This was possible because we initially observed that in a genetic mouse model, which allows the selective destruction of microglia, peripheral monocytes rapidly invaded the brain and completely repopulated the tissue. We used this model to replace

microglia in models of Alzheimer's disease to test whether the new, invading immune cells could prevent or alleviate pathology. To our surprise, the new immune cells were unable to improve pathological hallmarks of Alzheimer's disease. Rather, they adopted features of microglia indicating that the tissue environment dominated the function of the immune cells. We will investigate in future studies whether monocytes can indeed become microglia-like following long-term brain engraftment.



Three-dimensional reconstruction of microglia in a tissue section (cell body green, processes grey).

## SELECTED PUBLICATIONS

**Varvel NH, Grathwohl SA, Degenhardt K, Resch C, Bosch A, Jucker M, Neher JJ** (2015) Replacement of brain-resident myeloid cells does not alter cerebral amyloid- $\beta$  deposition in mouse models of Alzheimer's disease. *Journal of Experimental Medicine* 212: 1803–9.

Brown GC, **Neher JJ** (2014) Microglial phagocytosis of live neurons. *Nature Reviews Neuroscience* 15: 209–6.

**Neher JJ**, Emmrich JV, Fricker M, Mander PK, Théry C, Brown GC (2013) Phagocytosis executes delayed neuronal death after focal brain ischemia. *Proceedings of the National Academy of Sciences* 110: E4098–E4107.

# Section of Dementia Research

Head: Prof. Dr. Christoph Laske

Team: 6 members

Key words: memory clinic / alzheimer's disease / mild cognitive impairment / subjective memory complaints



**The Section for Dementia Research is run by the Department of Cellular Neurology and the University Clinic for Psychiatry and Psychotherapy. The section consists of a Research Unit and collaborates with an outpatient Memory Clinic.**

*Die Sektion für Demenzforschung wird zusammen mit der Universitätsklinik für Psychiatrie und Psychotherapie Tübingen betrieben. Die Sektion besteht aus einer Forschungsgruppe und arbeitet mit einer Gedächtnisambulanz zusammen.*



## Research Unit

### a) DIAN study

DIAN stands for “Dominantly Inherited Alzheimer Network”, the international network for dominantly inherited Alzheimer’s disease. The study was founded in the US in 2008 in order to further investigate genetic forms of Alzheimer’s disease. Individuals from families with inherited forms of Alzheimer’s disease (the autosomal dominant form or the related Abeta amyloid angiopathy) are welcome to participate in this study. These rare forms of Alzheimer’s disease are caused by mutations in one of three genes (APP, PSEN1 or PSEN2).

An autosomal dominant form of the disease is suspected if several family members are or were affected with an onset at the age of 60 years or younger. In the first phase of the DIAN study affected individuals are identified and examined via multimodal diagnostics (e.g. PET-PIB; MRI; biofluids) in regard to preclinical changes. In the second and future phase treatment trials are planned. The goal is to treat

the disease preventively already at a preclinical stage, i. e. before any symptoms appear.

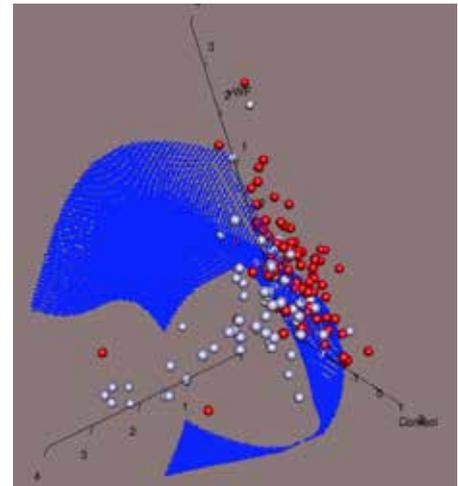
### b) DELCODE study

DELCODE (DZNE – Longitudinal Cognitive Impairment and Dementia Study) is a multicenter longitudinal observational study of the German Center for Neurodegenerative Diseases (DZNE) specifically focusing on the preclinical stage of Alzheimer’s disease. The aim of the study is to characterize the neuronal network mechanisms of cognitive adaption and decompensation. The recruitment will be via memory clinics of the DZNE sites. All DZNE sites with memory clinics will participate in DELCODE. The inclusion period is three years. Baseline and annual follow-ups are planned to cover 5 years per subject. It is planned to extend the observational follow-up per patient beyond 5 years. All subjects will undergo extensive structural and functional neuroimaging, including cognitive fMRI tasks and resting state fMRI at baseline and at follow-ups.

c) Identification and validation of new biomarkers for Alzheimer's disease  
 We aim to identify and validate new biomarkers for Alzheimer's disease using various technology platforms (ELISAs, flow cytometry, multiplex assays, mass spectrometry) and by examining a number of biofluids (blood, cerebrospinal fluid, urine, tear fluid). For example we have found that by means of three biomarkers measured in the blood (Cortisol, von Willebrand factor [vWF] and oxidized LDL-antibodies [OLAB]) Alzheimer patients can be distinguished from healthy controls with a test accuracy of more than 80% (Laske C et al., *Int J Neuropsychopharmacol* 2011). This little invasive and low-cost method may be suitable for the screening of Alzheimer patients.

### Memory Clinic

Memory disorders can be a consequence of a variety of diseases. The Memory Clinic provides early and differential diagnoses and the treatment of these disorders. Counselling of affected patients and their families is also provided. An initial visit at the Memory Clinic includes a physical, neurological and psychiatric examination. In most cases a blood sample will be taken. If indicated, a lumbar puncture to obtain cerebrospinal fluid as well as neuro-imaging (CCT or MRI), a electrocardiogram (ECG) and/or a electroencephalogram (EEG) will be performed. At a second appointment a thorough neuropsychological test of your memory will be performed by a physician and the results as well as treatment options will be discussed with you. A social worker will advice you on how to handle memory disorders in daily life. If you are interested and suitable you will be offered to participate in one of our clinical trials.



The decision surface of a computer based data-Analysis (usage of a so called "Support Vector Machine" [SVM]) for the classification of Alzheimer's disease (AD) patients (red points) compared with healthy controls (white points) by means of three biomarkers measured in the blood (Cortisol, von Willebrand factor [vWF] and oxidized LDL-Antibodies [OLAB]).

### SELECTED PUBLICATIONS

**Laske C**, Sohrabi H, Jasielec M, **Müller S**, Köhler N, Gräber S, Foerster S, Drzezga A, Mueller-Sarnowski F, Danek A, Jucker M, Bateman R, Buckles V, Saykin AJ, Martins R, Morris JC, Dominantly Inherited Alzheimer Network Dian. Diagnostic value of subjective memory complaints assessed with a single item in dominantly inherited Alzheimer's disease: results of the DIAN study. *BioMed Research International* 2015; 2015: 828120.

**Laske C**, Stellos K, Kempfer I, **Stransky E**, Maetzler W, Fleming I, Randriamboavonjy V. Increased cerebrospinal fluid calpain activity and microparticle levels in Alzheimer's disease. *Alzheimer's & Dementia* 2015; 11: 465-474.

**Laske C**, Sohrabi HR, Frost SM, López-de-Ipiña K, Garrard P, Buscema M, Dauwels J, Soekadar SR, **Mueller S**, Linnemann C, Bridenbaugh SA, Kanagasigam Y, Martins RN, O'Bryant SE. Innovative diagnostic tools for early detection of Alzheimer's disease. *Alzheimer's & Dementia* 2015; 11: 561-578.

O'Bryant SE, Gupta V, Henriksen K, Edwards M, Jeromin A, Bazenet C, Soares H, Lovestone S, Hampel H, Montine T, Blennow K, Foroud T, Carrillo M, Graff-Radford N, **Laske C**, Breteler M, Shaw L, Trojanowski JQ, Schupf N, Rissman RA, Fagan A, Barnham K, Oberoi P, Umek R, Weiner MW, Grammas P, Posner H, Martins R for the STAR-B and BBBIG working groups. Guidelines for the Standardization of Preanalytic Variables for Blood-Based Biomarker Studies in Alzheimer's Disease Research. *Alzheimer's & Dementia* 2015; 11: 549-560.

Stamatelopoulos K, Sibbing D, Rallidis LS, Georgiopoulos G, Stakos D, Braun S, Gatsiou A, Sopova K, Kotakos C, Varounis C, Tellis CC, Kastritis E, Alevizaki M, Tselepis AD, Alexopoulos P, **Laske C**, Keller T, Kastrati A, Dimmeler S, Zeiher AM, Stellos K. Amyloid-Beta (1-40) and the risk of death from cardiovascular causes in patients with coronary heart disease. *Journal of the American College of Cardiology* 2015; 65: 904-916.



Independent  
Research  
Groups



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# Laboratory for Neuro-regeneration and Repair

Head: Dr. Simone Di Giovanni

Team: 7 members

Key words: axonal signalling / spinal cord injury / axonal regeneration / neurogenesis / transcription

A spinal lesion may lead to a fragmentation of the neuronal insulating layer, myelin. We identified molecular components that counteract the inhibitors of axonal outgrowth and functional recovery.

**The MDM4/MDM2-p53-IGF1 axis controls axonal regeneration, sprouting and functional recovery after CNS injury** (Joshi and Soria et al. *Brain*, 2015)

Regeneration of injured central nervous system (CNS) axons is highly restricted, causing neurological impairment. To date, although the lack of intrinsic regenerative potential is well described, a key regulatory molecular mechanism for the enhancement of both axonal regrowth and functional recovery after CNS injury remains elusive. While ubiquitin ligases coordinate neuronal morphogenesis and connectivity during development as well as after axonal injury, their role specifically in axonal regeneration is unknown. Following a bioinformatics network analysis combining ubiquitin ligases with previously defined axonal regenerative proteins, we found a triad composed of the ubiquitin ligases MDM4-MDM2 and the transcription factor p53 as a putative central signalling complex restricting the regeneration program. Indeed, conditional deletion of MDM4 or pharmacological inhibition of MDM2/p53 interaction in the eye and spinal cord promote axonal regeneration and sprouting of the optic nerve after crush and of supraspinal tracts after spinal cord injury. The double conditional deletion of MDM4-p53 as well as MDM2 inhibition in p53 deficient mice blocks this regenerative phenotype, showing its dependence upon p53. Genome-wide gene expression analysis from ex vivo fluorescence-activated cell sorting (FACS) in MDM4 deficient retinal ganglion cells identifies the downstream target IGF1R, whose activity and expression was found to be required for the regeneration elicited by MDM4 deletion. Importantly, we demonstrate that pharmacological enhancement of the MDM2/p53-IGF1R axis enhances axonal sprouting as well as functional recovery after spinal cord injury. Thus, our results show MDM4-MDM2/p53-IGF1R as an original regulatory mechanism for CNS regeneration and offer novel targets to enhance neurological recovery.

The adult mammalian central nervous system (CNS) is unable to regenerate following axonal injury due to the presence of glial inhibitory environment as well as the lack of a neuronal intrinsic regenerative potential. Research over the past two decades has elucidated several key molecular mechanisms and pathways that limit axonal sprouting and regeneration following CNS axonal injury, including myelin or proteoglycan-dependent inhibitory signaling. More recently, accumulating evidence suggests

that the modulation of the neuronal intrinsic potential via the manipulation of selected genes in specific neuronal populations may enhance axonal regeneration in the injured CNS. More often, these are developmentally regulated pathways that contribute to locking the adult CNS neurons in a non-regenerative mode. Remarkably, deletion of phosphatase and tensin homolog (PTEN) in retinal ganglion cells (RGCs) or in corticospinal tract (CST) axons enhances mTOR activity and leads to robust axonal

regeneration after optic nerve or CST injury respectively, which is further enhanced with conditional co-deletion of SOCS3. Furthermore, modifications of the developmentally regulated neuronal transcriptional program can lead to increased axonal regeneration after optic nerve crush (ONC) or spinal cord injury (SCI) as demonstrated by the deletion of kruppel-like factor 4 (KLF4), the overexpression of p300 in RGCs as well as the overexpression of KLF7 or retinoic acid receptor  $\beta$  (RAR $\beta$ ) in corticospinal neurons.

Ubiquitin ligases and ubiquitin ligase-like proteins, including neuronal precursor cell-expressed developmentally downregulated protein (Nedd), Smad ubiquitin regulatory factor (Smurf) and murine double minute 2 and 4 (MDM2 and 4), coordinate neuronal morphogenesis and connectivity both during development and after axonal injury. Moreover, they regulate the turnover, localization and activity of a number of proteins and transcription factors involved in the axonal regeneration program, including PTEN, p300, KLFs, Smads, p21 and p53. Ubiquitin ligases and ubiquitin ligase-like proteins may therefore represent a regulatory hub controlling the regenerative neuronal response following injury. However, their role in axonal regeneration remains unaddressed. Therefore, to functionally rank ubiquitin ligase dependent control of the regeneration programme, we systematically analysed protein networks using STRING bioinformatic tool including of proteins previously described to be involved in axonal regeneration and sprouting in the CNS and the corresponding ubiquitin ligases. This had the goal to identify central protein networks that control the regeneration program that may have positive implications for functional recovery.

This analysis showed that MDM4, in association with MDM2, and p53 constitutes a central regulatory complex, potentially involved in repressing axonal regeneration. The ubiquitin ligase-like MDM4 and MDM2 can form inhibitory protein complexes with at least four key proteins involved in axonal outgrowth: Smad1/2, p300, p53. Strikingly, MDM4 and MDM2 expression is developmentally regulated in the retina reaching its maximal levels in adulthood, potentially keeping the post-injury RGC growth program in check. Therefore, MDM4 and MDM2 appear to be strong candidates for limiting axonal regeneration in the CNS, particularly in the injured optic nerve.

We investigated whether disruption of MDM4 and MDM2-dependent regulation would affect the axonal regeneration program. Indeed, we found that MDM4 and MDM2 restrict axonal regeneration after optic nerve crush. In fact, conditional MDM4 deletion in RGCs leads to axonal regeneration and sprouting of RGC axons following ONC. Additionally, conditional co-deletion of MDM4 and its target protein p53 in RGCs after ONC blocks nerve regeneration elicited by MDM4 deletion alone. Similarly, pharmacological inhibition of the interaction between the MDM4 co-factor MDM2 and p53 via the MDM2/p53 antagonist Nutlin-3a also enables regeneration after ONC, which is abolished in p53 deficient mice. Further, genome-wide gene expression analysis from a pure RGC population after conditional deletion of MDM4 showed enhancement of IGF1R expression suggesting IGF1

signaling as a downstream effector of the MDM4 deletion. Indeed, co-inhibition of MDM4 and IGF1 signalling after ONC via a specific IGF1R antagonist impairs axonal regeneration, while viral overexpression of IGF-1 in the eye enhances it. Finally, we demonstrate that MDM4/2-p53-IGF1 regulation is critical for axonal sprouting and neurological recovery after spinal cord injury. Both conditional deletion of MDM4 and Nutlin-3 delivery after spinal cord dorsal hemisection in mice enhance axonal sprouting of supraspinal descending fibers and functional recovery, which is blocked when IGF-1R signaling is inhibited.

Together, this work portrays the MDM4-MDM2/p53-IGF1R axis as a novel molecular target for axonal regeneration and neurological recovery after spinal injury.

## SELECTED PUBLICATIONS

**Joshi Y, Soria M, Quadrato G, Inak G, Zhou Z, Hervera A, Rathore K, Elnagger M, Magali C, Marine JC, Puttagunta R, Di Giovanni S.** The MDM4/MDM2-p53-IGF1 axis controls axonal regeneration, sprouting and functional recovery after CNS injury. *Brain*. 2015 Jul; 138(Pt 7): 1843-62.

**Puttagunta R, Tedeschi A, Soria MG, Lindner R, Rathore KI, Gaub P, Joshi Y, Nguyen T, Schmandke A, Bradke F, Di Giovanni S.** PCAF-dependent epigenetic changes promote axonal regeneration in the central nervous system. *Nature Comm*. 2014.

**Floriddia EM, Rathore KI, Tedeschi A, Quadrato G, Wuttke A, Lueckmann M, Kigerl KA, Popovich PG, Di Giovanni S.** p53 regulates the neuronal intrinsic and extrinsic responses affecting the recovery of motor function following spinal cord injury. *J Neurosci*. 2012 Oct 3; 32(40): 13956-70.

**Quadrato G, Benevento M, Alber S, Jacob C, Floriddia EM, Nguyen T, Elnaggar MY, Pedroarena CM, Molkentin JD, Di Giovanni S.** Nuclear factor of activated T cells (NFATc4) is required for BDNF-dependent survival of adult-born neurons and spatial memory formation in the hippocampus. *Proc Natl Acad Sci U S A*. 2012 Jun 5; 109(23).

**Puttagunta R, Schmandke A, Floriddia E, Fomin N, Gaub P, Ghyselinck NB, Di Giovanni S.** RA-RAR $\beta$  counteracts myelin-dependent growth cone collapse and inhibition of neurite outgrowth via transcriptional repression of Lingo-1. *J Cell Biol*. 2011 193,1147-1156.

**Gaub P, Joshi Y, Naumann U, Wuttke A, Schnichels S, Heiduschka P, Di Giovanni S.** The histone acetyltransferase p300 promotes intrinsic axonal regeneration. *Brain*. 2011 134, 2134-2148.



# Physiology of Learning and Memory

Head: Dr. Ingrid Ehrlich

Team: 5 members

Key words: synaptic plasticity / amygdala / fear learning / extinction / anxiety disorders

Lab member Daniel Bosch working on the patch clamp setup recording amygdala neurons in live brain slices.

**We investigate learning and memory processes using associative fear conditioning and extinction in rodents. We apply physiological techniques to decipher cellular and synaptic processes and neural circuits of the amygdala and fear-related areas. This allows us to understand how learning modifies brain circuits and how these processes may be dysregulated in anxiety disorders.**

*Wir untersuchen Lern- und Gedächtnisprozesse anhand von klassischer Furchtkonditionierung und Extinktionslernen. Dabei verwenden wir vor allem physiologische Methoden, um zelluläre und synaptische Prozesse sowie neurale Schaltkreise der Amygdala und verknüpfter Hirngebiete zu ergründen. Dies gibt Aufschluss darüber, wie Lernprozesse im Gehirn umgesetzt werden, aber auch wie eine Fehlsteuerung dieser Prozesse zu Angststörungen führen kann.*

Organisms have to continuously adapt their behavior to survive. Such experience-driven adaptations in behavior are mediated by modifications in brain function. We use classical Pavlovian learning paradigms, i.e. fear learning and extinction of fear in mice, as our model to study the mechanisms that underlie behavioral adaptation during learning and memory processes. Our goal is to elucidate the molecular, synaptic and cellular changes and the neural circuits that process fear-related information. We combine several techniques, including slice electrophysiology, optogenetics, imaging, histology, viral gene transfer in vivo, and behavioral analysis.

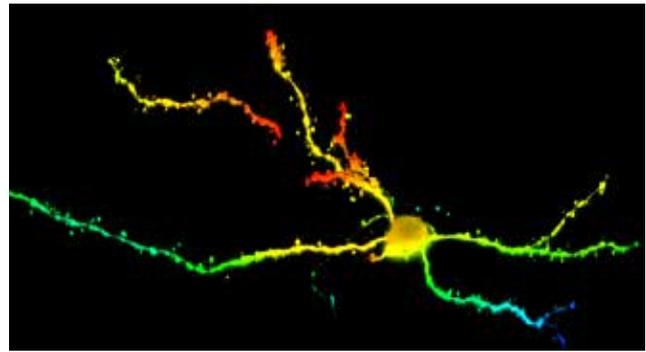
The amygdala, a highly conserved region in the temporal lobe of the brain, is a key structure for storing emotional and fear memories. Acquired fear memories can be modified by extinction learning. Here, an individual learns that certain stimuli

are not fearful anymore in a specific setting. Extinction depends on a brain network comprising the amygdala, the hippocampus (a structure important for memory and processing of spatial information) and the medial prefrontal cortex (a structure associated with the control of actions), and interactions between them. Understanding extinction is highly relevant for improving cognitive behavioral therapies used as treatment for anxiety- and other emotional disorders, because they are based on extinction learning. Emerging themes in the last years have been that fear and extinction memories are encoded by specialized, perhaps parallel networks of neurons in the engaged brain areas, whereby inhibitory mechanisms can also play a role. Our goal is to identify and investigate these networks and their learning-dependent changes.

One line of our research aims to understand the function and plasticity

of a specific inhibitory network in the amygdala, the so-called intercalated cells. It has been suggested that these cells are critically involved in extinction behavior, possibly by inhibiting the output of the amygdala and providing a break on the fear response. However, how they receive information and transfer it is incompletely understood. In a recent project, we identified a new plastic brain circuit integrating intercalated cells that becomes engaged in fear learning and memory (Asede et al., 2015). We demonstrated that intercalated cells directly receive and process information about external stimuli, and these pathways undergo plasticity upon fear and extinction learning. Once activated, intercalated cells relay information to input and output stations of the amygdala, thereby controlling incoming and outgoing activity. Our future goals are to decipher the mechanisms of intercalated cell plasticity, the interactions with

Example of an amygdala intercalated cell filled during an electrophysiological recording. Neurons are revealed using histological methods, subjected to confocal imaging, and subsequently reconstructed in three dimensions to identify their anatomical properties.



neuromodulatory systems engaged in learning, and to implement molecular tools to specifically and reversibly manipulate these cells in behaving animals to understand their function in vivo.

A second line of research investigates extinction mechanisms and extinction networks, i.e. interactions of amygdala, hippocampus, and prefrontal cortex, which is critical for understanding extinction mechanisms and return of fear. We have recently shown distinct roles for subpopulations of excitatory projection neurons in the amygdala. Two different classes of neurons, characterized by their projections to subregions of the prefrontal cortex, undergo cellular plasticity either during fear or during extinction learning (Senn et al., 2014). To understand how these neurons are activated, we employed optogenetic, targeted stimulation of prefrontal and hippocampal inputs to the amygdala. We discovered that prefrontal cortex and hippocampus innervate distinct subpopulations of neurons and microcircuits in the basolateral amygdala with distinct input properties (Hübner et al, 2014). In parallel, we started to ask which other behavioral modulations can impact extinction memories. We currently investigate the role of sleep in consolidating extinction memories. Our next goals are to understand plasticity and activity mechanisms that support extinction learning and that may be perturbed by interventions that compromise extinction, such as sleep perturbations.

A third line of research addresses development of amygdala circuits and its relationship to developmental differences in learning behavior. The ability to learn fear first emerges in juvenile animals and changes into adulthood. Extinction learning in juveniles is also different from adults. In a first step, we investigated changes in amygdala networks. We have identified a number of changes in amygdala inhibitory control of excitatory neurons that occur between infancy and adulthood. Furthermore, we have shown that this changing inhibition can modulate excitatory sensory inputs differentially during development (Bosch and Ehrlich, 2015). Our data suggest that different aspects of increased inhibitory control may

contribute to control fear specificity later in development. Our future goal is to further investigate development and function of specific inhibitory synapses, how they affect plasticity in the amygdala, and ultimately to address if changes are linked to modulation of learning behavior.

Overall, studying circuits and mechanisms of fear and extinction memory provides insights into the general principles of memory formation. On the other hand, we also gain important knowledge into mechanism that may be dysfunctional during inappropriate control of fear in conditions such as human anxiety and other neuropsychiatric or emotional disorders.

## SELECTED PUBLICATIONS

**Bosch D, Asede D, and Ehrlich I.** (in press) Ex vivo optogenetic dissection of fear circuits in brain slices. *Journal of Visualized Experiments*.

**Bosch D and Ehrlich I.** (2015) Postnatal maturation of GABAergic modulation of sensory inputs onto lateral amygdala principal neurons. *Journal of Physiology* 593 (19): 4387-409.

**Asede D, Bosch D, Lüthi A, Ferraguti F, and Ehrlich I.** (2015) Sensory inputs to intercalated cells provide fear-learning modulated inhibition to the basolateral amygdala. *Neuron* 86 (2): 541-554.

**Hübner C, Bosch D, Gall A, Lüthi A, and Ehrlich I.** (2014) Ex vivo dissection of optogenetically activated mPFC and hippocampal inputs to neurons in the basolateral amygdala: implications for fear and emotional memory. *Frontiers in Behavioral Neuroscience* 8: 64.

Wolff SBE, Gründemann J, Tovote P, Krabbe S, Jacobson GA, Herry C, Müller C, **Ehrlich I**, Friedrich R, Letzkus JJ, and Lüthi A. (2014) Amygdala interneurons subtypes control fear learning through stimulus-specific disinhibition. *Nature* 509 (7501): 453-558.

Senn V, Wolff SBE, Herry C, Grenier F, **Ehrlich I**, Gründemann J, Fadok J, Müller C, Letzkus JJ, and Lüthi A. (2014) Long-range connectivity defines behavioral specificity of amygdala neurons. *Neuron* 81 (2): 428-437.

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