

# Annual Report 2013







CENTER OF NEUROLOGY TÜBINGEN

# Annual Report 2013

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Hertie-Institut  
für klinische Hirnforschung



UNIVERSITÄTS  
KLINIKUM  
TÜBINGEN

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A close-up photograph of a man with short hair, glasses, and a goatee, wearing a white lab coat. He is looking upwards and to the right. In the background, a hand wearing a purple nitrile glove holds a test tube with a blue cap and yellow liquid. The background is a blurred laboratory setting.

# The Center of Neurology



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## The Center of Neurology in 2013

**The Center of 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 with the mission to promote excellence in research and patient care.**

Presently, the center consists of four clinical departments (Neurology and Stroke, Epileptology, Neurodegeneration and Cognitive Neurology) and one basic science department (Cellular Neurology), which has recently installed an outpatient care unit in the field of Alzheimer's disease. The clinical departments provide inpatient and outpatient care within the University Hospital, while their 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 is of course most obvious in clinical trials, which are conducted for example on the treatment of Parkinson's disease, multiple sclerosis, epilepsies and brain tumors. However, the intimate interconnection of science and patient care is of eminent importance to all areas of disease-related neuroscience. It forms the very center of the Hertie-concept and distinguishes the Center of Neurology from other institutions of neuroscientific research.

*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. Das Zentrum besteht derzeit aus vier klinischen Abteilungen: der Neurologie mit Schwerpunkt neurovaskuläre Erkrankungen, der Neurologie mit Schwerpunkt Epileptologie, der Neurologie mit Schwerpunkt neurodegenerative Erkrankungen, der kognitiven Neurologie und einer grundlagenwissenschaftlichen Abteilung, der Zellbiologie neurologischer Erkrankungen. Die letztgenannte Abteilung betreibt seit dem Jahr 2012 eine Ambulanz im Bereich der Alzheimer Erkrankungen gemeinsam mit der Universitätsklinik für Psychiatrie und Psychotherapie.*

*Die besonders enge Verknüpfung von Klinik und Grundlagenforschung ist ein fundamentaler Aspekt des Hertie-Konzepts und ein Alleinstellungsmerkmal gegenüber anderen Institutionen der Hirnforschung. Dies ist unter anderem die Grundlage für erfolgreiche klinische Studien, die am Zentrum zum Beispiel in der Therapie der Parkinson-Krankheit, der Epilepsien, der Multiplen Sklerose, von Schlaganfällen und auch in der Hirntumorbehandlung in erheblichem Umfang durchgeführt werden.*



# Facts & Figures

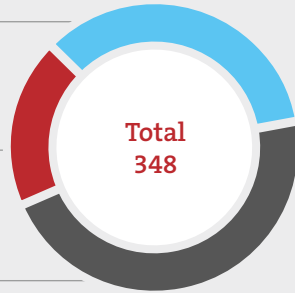
## NUMBER OF STAFF IN 2013

Center of Neurology without nursing services (by headcount)

**161**  
46 % Third party funding

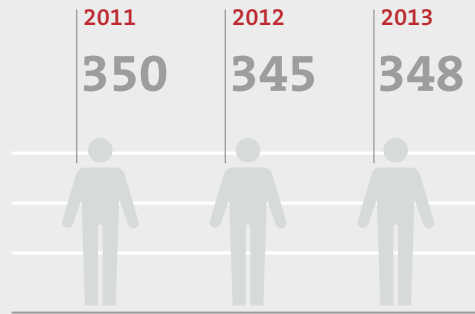
**66**  
19 % Hertie Institute

**121**  
35 % University Hospital of Neurology



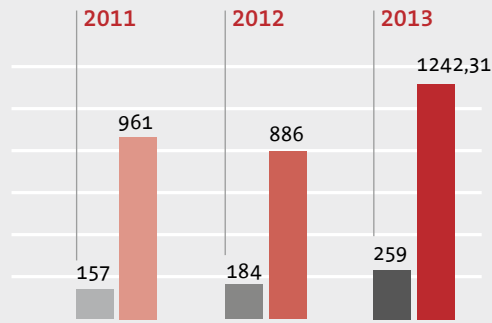
## DEVELOPMENT OF STAFF

Center of Neurology (by headcount)



## NUMBER OF PUBLICATIONS IMPACT FACTORS

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



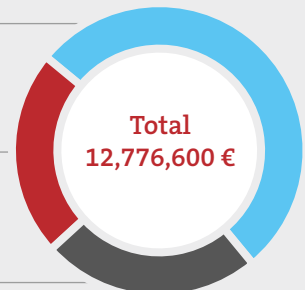
## TOTAL FUNDINGS IN 2013

Center of Neurology

**6,774,500 €**  
53 % Third party funding

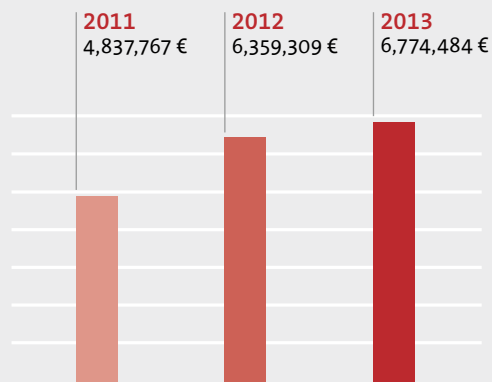
**2,900,000 €**  
23 % Hertie Foundation

**3,102,100 €**  
24 % University Hospital of Neurology



## THIRD PARTY FUNDING

Center of Neurology



## THIRD PARTY FUNDING IN 2013

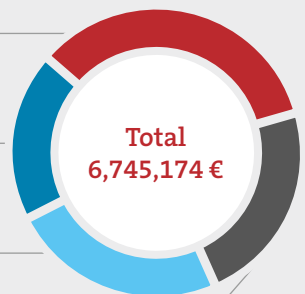
Center of Neurology

**Others: 34 %**  
2,308,416 €

**EU: 19 %**  
1,279,026 €

**BMBF: 24 %**  
1,596,240 €

**DFG: 23 %**  
1,561,492 €



# University Hospital of Neurology

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|   |           |
|---|-----------|
| <b>UNIVERSITY HOSPITAL OF NEUROLOGY</b>   | <b>10</b> |
| Clinical Care                             | 12        |
| Outpatient Clinics                        | 14        |
| Clinical Laboratories                     | 26        |
| Occupational, Physical and Speech Therapy | 30        |





## University Hospital of Neurology

### CLINICAL CARE

The clinical Department of Neurology of the University Hospital in Tübingen treats inpatients with the complete spectrum of neurologic diseases on four general wards. Patients with acute strokes are treated on a specialized stroke-unit which allows 24-hour surveillance and treatment. In addition, a specialized EEG-monitoring unit allows continuous long-term EEG recordings for patients with intractable epilepsies.

In the outpatient unit of the department, more than 12,000 (including diagnostic procedures) patients are examined and treated per year, many of them in specialty clinics which are directed by recognized specialists in the respective fields.

### PATIENTENVERSORGUNG

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

*In der neurologischen Poliklinik werden mehr als 12.000 Patienten pro Jahr ambulant betreut und Diagnosen durchgeführt, 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 4,556 patients in 2013.

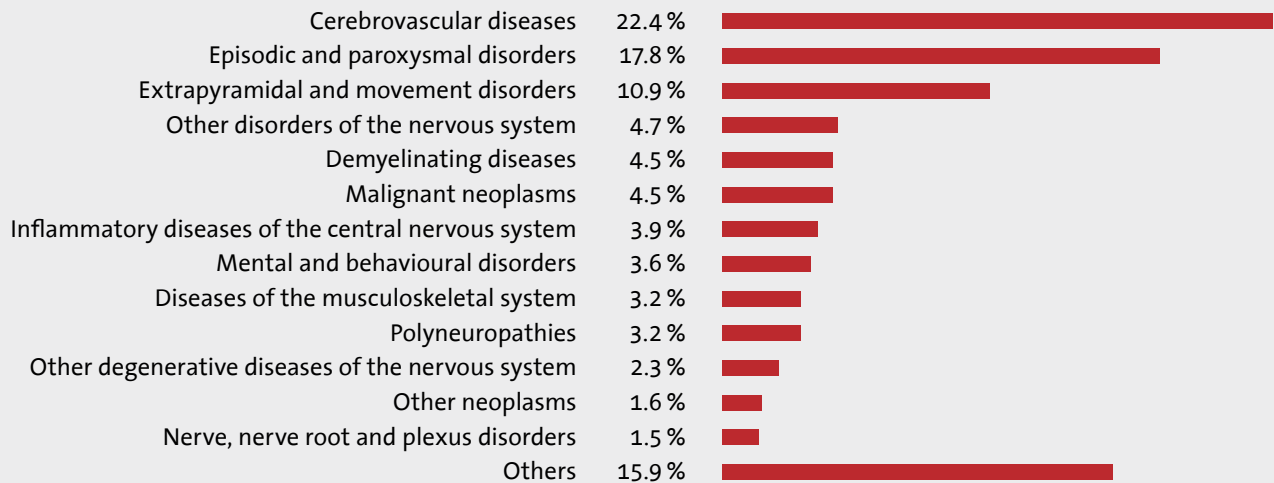
#### NUMBER OF ADMISSIONS

4,556

#### LENGTH OF STAY (IN DAYS)

5,4

#### INPATIENT DIAGNOSIS GROUPS

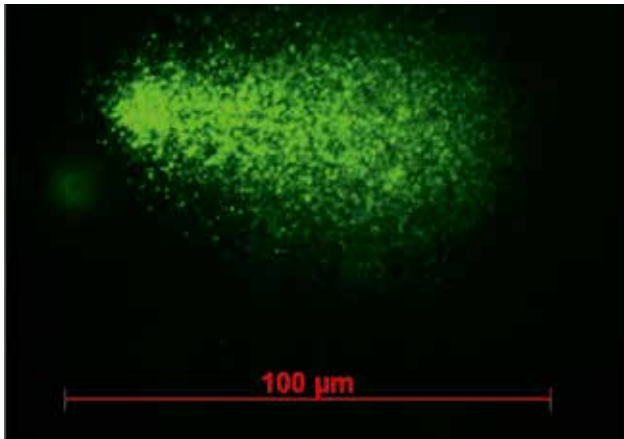


### OUTPATIENT CARE

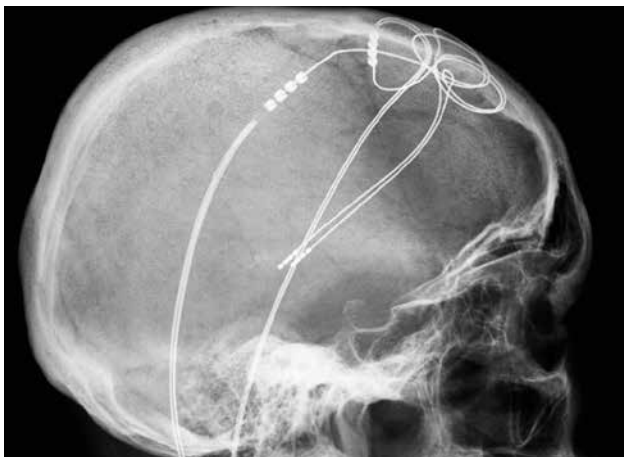
NUMBER OF CONSULTATIONS  
(including diagnostic procedures)

12,010

## Outpatient Clinics



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.



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

### ATAXIA

The ataxia clinic provides tools to discover the cause of ataxia 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 established gene panel diagnostics that allow parallel sequencing of all known ataxia genes. Therapeutic options depend largely on the underlying cause of ataxia, the genetic defect, and concomitant symptoms. In cooperation with the Center of Physiotherapy, the experts developed special exercise programs 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 for spinocerebellar ataxias (SCA) as a prerequisite for interventional trials in the future. Special emphasis is given to early onset ataxias (EOA). With support of the EUROSCAR project funded by the EU we proposed a genetic screening program to determine the frequency of genetic subtypes and to discover new genes. In Friedreich's ataxia the clinic participates in the European EFACTS project that aims to reveal the natural course of the disease and develop new biomarkers in preparation for new therapeutical approaches. The clinic is run by Dr. M. Synofzik, Dr. J. Schicks and Dr. J. Müller vom Hagen and is supervised by Prof. Dr. L. Schoels.

### 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 neurological 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 multicenter EARLYS TIM-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 highfrequency stimulation of the substantia nigra pars reticulata (SNr) as an add-on to the conventional subthalamic nucleus stimulation, was successfully accomplished and prove an effect on freezing of gait (Weiss et al., Brain, 2013). Thus we provide first means to address the major need for current therapy research due to the failure of established conventional pharmacological and neurostimulation therapies concerning these highly incapacitating axial symptoms.

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 disorders. 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 counseling 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 were seen by Dr. T. Wächter, Dr. D. Weiss, Dr. C. Mielke, and Prof. Dr. R. Krüger.

## 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 electronystagmography, measurement of 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. S. Schuh-Hofer), 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 ultrasoundguided injection techniques. Presently, the clinic is evaluating a new ultrasound-guided injection technique for the treatment of deep cervical muscles in cervical dystonia. The hospital also participates in several multicenter clinical studies 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)). The University Hospital of Neurology also contributes to different multicenter studies with the aim to evaluate and optimize pallidal DBS in dystonia patients.

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).



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 Medical Training and Rehabilitation Center.



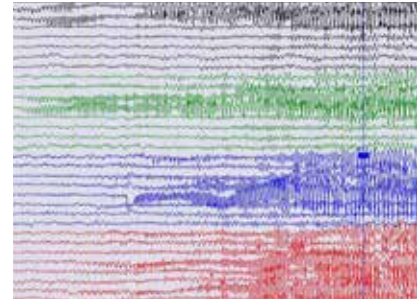
There is also a specialized ergotherapeutic service for task-specific dystonias focused on retraining (S. Wiesemeier, Therapiezentrum UKT). Two to three residents as well as host physicians are continually trained in both standardized and new injection techniques.

The tradition of expert meetings together with regional movement disorder experts has been continued. In a collaboration with four other botulinum toxin clinics in this area, a registry is presently set up which includes clinical features, life quality measures and treatment plans of approximately 1,300 patients seen regularly in these clinics. In a subgroup of dystonic patients of this registry we also collect DNA samples and capture clinical features for a European collaboration (European network for the study of dystonia syndromes, BMBS COST Action BM1101, headed by Prof. Alberto Albanese). 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. K. Freitag 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 41/42 and 45), running under the supervision of Prof. Dr. Y. Weber, Dr. N. Focke and PD Dr. T. Freilinger, includes acute care for epileptic seizures and status epilepticus, long-term complex treatment for difficult cases, and a Video-EEG-Monitoring Unit which is operated in cooperation with the Department of Neurosurgery.



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

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 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.

## 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 may either be referred by neurologists or general practitioners. 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.

Selected patients with otherwise refractory chronic headache disorders are offered access to new treatment modalities including botulinum toxin for CM or neurostimulation techniques (in collaboration with the Department of Neurosurgery), 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 Dr. N. Dammeier and Dr. S. Wolking.



# 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. The German Ministry of Education and Research (BMBF) supports a national research network for leukodystrophies (LeukoNet; [www.leukonet.de](http://www.leukonet.de)) that analyzes the natural course of the diseases and especially adult variants as an essential prerequisite for therapeutic studies. 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. Müller vom Hagen 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. 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. M. Synofzik, Dr. J. Müller vom Hagen, supervised by Prof. L. Schöls.

## NEUROIMMUNOLOGICAL DISORDERS

Patients with multiple sclerosis, immune-mediated neuropathies, and other neuroimmunological disorders are regularly seen in the outpatient-clinic for neuroimmunological diseases. Complex cases may be discussed in interdisciplinary conferences with colleagues from rheumatology, neuroophthalmology, neuroradiology, and neuropathology. The center of Neurology is certified by the German Multiple Sclerosis Association and a member of the Clinical Competence Network for Multiple Sclerosis and the Neuromyelitis Optica Study Group.

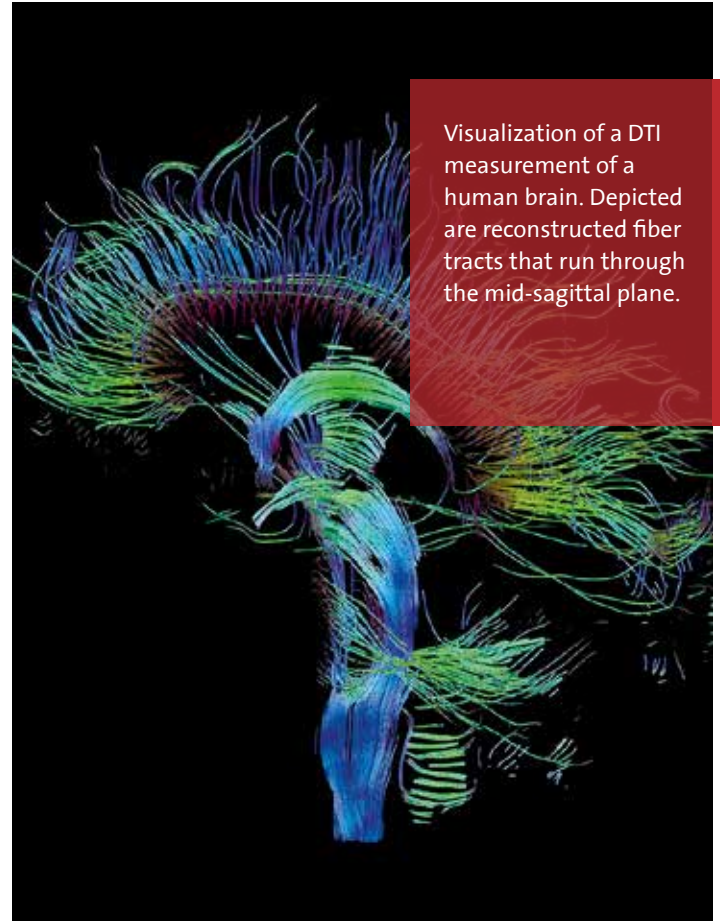
Patients with multiple sclerosis are referred from other institutions for diagnosis, follow up, or second opinion. Counselling about immunomodulatory and immunosuppressive therapy follows the guidelines by the German 'Multiple Sclerosis Therapy Consensus Group'. Standardized examination of patients is 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 copaxone. A large number of patients participates in clinical trials which explore safety and efficacy of new treatments. Clinical trials are managed by a team of study nurses including U. Küstner, C. Ruth and B. Tolle. In 2013 the clinic was run by K. Friebe and L. Zeltner under the supervision of Prof. U. Ziemann and PD Dr. F. Bischof.

## 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. Schell.

## 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

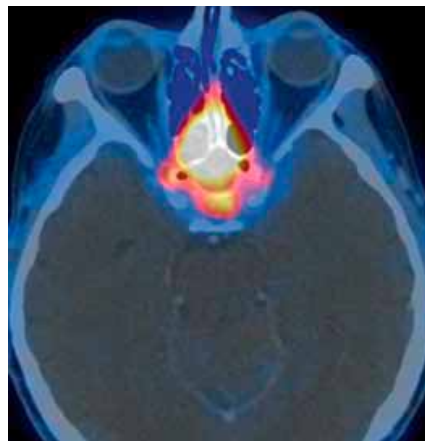
## NEURO-ONCOLOGY

This outpatient clinic sees about 180 new patients each year and all visits add up to more than 800 patient contacts. The main focuses are

- (i) monitoring of outpatients' chemotherapies,
- (ii) follow-up examinations of patients without current specific anti-tumor therapy at longer intervals, and
- (iii) evaluation of patients who have been diagnosed and treated at a community facility and are informed about further diagnostic and therapeutic options, including experimental therapies within neurooncological trials

The outpatient clinic is run by Dr. C. Braun and M. Wolf and is supervised by Prof. U. Ziemann.

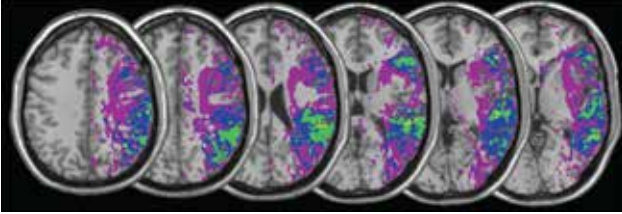
Being part of the Centre of Neurooncology Tübingen (ZNO) in the framework of the Tübingen Comprehensive Cancer Centre (CCC), the Neurooncology outpatient clinic has been rated „neurooncology module“ by the German Cancer Society (DKG). This rating is representing the highest level of excellence to be achieved. Besides the German Cancer AID (DKH) is rating the CCC as Oncology centre of excellence. This implies a thorough interaction with colleagues of the Departments of Neurosurgery, Radiooncology, Neuroradiology, Neuopathology, Pediatrics, and Oncology. Diagnostic as well as therapeutic decisions are routinely made by the Brain Tumor Board (Coordination Dr. C. Braun), taking place on a weekly basis at least. As neurooncology centre of international reputation the ZNO is represented within national and international organizations of significance. Dr. Braun is member of the following organizations: German Cancer Society (DKG), German Neuro-oncology Group (NOA), European Organisation for Research and Treatment of Cancer (EORTC). In connection with that the Neuro-oncology outpatient clinic is participating in a number of multicentre trials of the organisations mentioned above. Besides a number of local trials is carried out as well. As a study nurse MS M. Jeric is entrusted with the organization, administration of multicentre trials and specific training of the patients.



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

## 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).



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.

## 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, 24-hour Holter monitoring 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 Dr. C. Zürn (cardiologist, shared appointment by the departments of neurology and stroke, and cardiology). 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 counseling 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 years follow up of de novo Parkinson patients to better understand etiology 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 may be 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. Currently, we offer participation in studies involving a nicotine plaster in early PD, a selective adenosine A(2A) receptor antagonist

as treatment for PD patients with motor fluctuations and dyskinesia, a new COMT-Inhibitor for patients with motor fluctuations, a polyphenol with antioxidative and neuro-protective capacities in the atypical Parkinsonian syndrome Multiple System Atrophy (MSA) as well as observational studies of different medications. Moreover, close cooperation with the outpatient rehabilitation center and the establishment of a Parkinson choir guarantee the involvement of additional therapeutic approaches.

These activities are supported by the study nurses K. Gauß, C. Haaga, and N. Runge as well as the documentalist T. Heger. 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.

Appointments are scheduled daily in the outpatient clinic of the Center of Neurology. Patients are seen by Prof. D. Berg, Dr. K. Brockmann, Dr. K. Srulijes and Dr. I Wurster as well as the neuropsychologists Dr. I. Liepelt-Scarfone, Dr. S. Gräber-Sultan and Marion Thierfelder. Our Parkinson's nurse Ina Posner is engaged in the organization of the outpatient clinic, documentation, assessments and counselling of many aspects related to daily life.



## 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 options include 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. K. Karle and Dr. T. Rattay and is supervised by 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. Using this battery specific tremor subgroups have been characterized. Close cooperation with the clinic for DBS (deep brain stimulation, head Prof. R. Krüger) ensures the inclusion of this highly effective treatment option into decision making. Moreover, research on etiology and pathophysiology is pursued in national and international cooperations. Patients are seen by Dr. I. Wurster and Prof. Dr. D. Berg.

# Clinical Laboratories

## CLINICAL CHEMISTRY LABORATORY

The Clinical Chemistry Laboratory collects more than 1,500 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 analyzed on cytopsins after Giemsa or Pappenheim staining. Lysosomes within the CNS are assessed in patients with hematological diseases and intrathecal liposome therapy. 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, 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 peptide beta42, tau, and phospho-tau are measured to differentiate various forms of dementia and concentration of Interleukin-10 and CXCR-13 are determined which aids in the differential diagnosis of primary CNS lymphomas. The laboratory is supervised by Prof. W. Maetzler and PD. F. Bischof.



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 4 mobile digital and 2 stationary recording places (IT-Med). For analysis, 6 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. On 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 in 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: B. Wörner, M. Harder, R. Mahle, Fr. Vohrer (staff technicians); Prof. Dr. Y. Weber (head of the laboratory).



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

## EMG LABORATORY

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.

The laboratory is equipped with two digital systems (Dantec Keypoint G4). A portable system (Nicolet Viking Quest) is available for bedside examinations. In 2013, more than 3,000 patients were seen and more than 20,000 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 transcortical magnetic stimulation and recording of motor cortex-evoked potentials in approximately 800 patients per year.

The EMG Laboratory is organized by Mrs. J. Grimm who also performs nerve conduction studies with surface electrodes. In 2013, the EMG Laboratory was run by Dr. L. Zeltner und Dr. P. Martin under the guidance of Dr. Caroline Schell and Dr. Niels Focke.

## 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 analyzed 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

## 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 each year on more than 1,600 patients. The recordings are conducted by A. Deutsch and J. Grimm who are supervised by Dr. C. Schell and Dr. N. Focke. According to the guidelines of the German Society for Clinical Neurophysiology, the recordings are analyzed and interpreted during daily conferences visited by up to six interns.

## NEUROCARDIOLOGY LABORATORY

Cardiovascular and cerebrovascular diseases represent the leading causes of death in the Western industrialized world. This is mainly due to ischemic heart disease. The Dutch TIA Study demonstrated that patients with a transitory ischemic attack (TIA) or minor stroke have an increased cardiovascular mortality. Stroke, therefore, seems to represent an index event for cardiac diseases. Cardiovascular investigations after stroke not only identify cardio-embolic sources of cerebral events but also allow for the identification of vascular risk factors. Diseases of the heart are responsible for up to 25 % of all strokes and usually cause territorial apoplexies. After an acute stroke, cardiac investigations are urgently required to find potential cardiac causes, in order to reduce the risk of stroke recurrence within days or weeks after the first stroke.

At the University Hospital there is a department with its own neurocardiology laboratory, headed by cardiologist Dr. C. Zürn. The laboratory is fully equipped with a modern multifunction ultrasound and echocardiography machine (Acuson Sequioa 512, Siemens) including probes for transthoracic and transesophageal investigations as well



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

as abdominal and other soft tissue ultrasound (pleural, thyroid etc.). This allows to perform bedside echocardiography of stroke patients on the Stroke Unit immediately after diagnosis.

Yearly, we conduct approximately 1,500 echocardiographic examinations, including M-Mode, 2-D mode, pulse wave and continuous-wave Doppler and color Doppler investigations as well as contrast-enhanced echocardiography. The younger the patients are, the higher is the probability of identifying a cardiac cause of stroke. Younger patients are regularly examined for a patent foramen ovale and atrial septum aneurysm using a transesophageal device with contrast-enhancement. All investigations are done according to the guidelines of the German and European Societies of Cardiology.

Atrial fibrillation represents the most common arrhythmia in the elderly. Atrial fibrillation, in combination with additional risk factors, represents a very common cause of stroke. In the stroke unit, there is a completely equipped long term registration unit consisting of 24-hour ECG (Holter ECG), 24-hour ambulatory blood pressure measurement, and in cooperation with the cardiology unit of the department of internal medicine, 7-day event recorders and implantable event recorders. Yearly, well over 1000 24-hour (Holter) ECGs and 800 24-hour ambulatory blood pressure measurements are recorded. In high-risk patients event recorders are implanted to accelerate the detection of atrial fibrillation.

## NEUROSONOLOGY LABORATORY

The neurosonology laboratory is equipped with two Color-coded Duplex sonography systems: a Toshiba Aplio and a Philips Epiq7. Furthermore we have two portable CW/PW Doppler systems: a DWL Multi-Dop pro and a DWL Multi-Dop T digital. The neurosonological examinations are performed by the ultrasound assistants Mrs. Nathalie Vetter and Mrs. Yvonne Schütz under the supervision of Dr. Sven Poli, consultant stroke neurologist and neurointensivist.

The laboratory consists of a unit in the outpatient department of the Dept. of Neurology mainly for non-acute or elective ultrasound examinations of in- and outpatients as well as a mobile unit located directly on our Stroke Unit allowing the full range of neurosonological examinations at the bedside immediately after admission.

Routine diagnostic tests include Duplex imaging of carotid, vertebral, and subclavian arteries, as well as the Circle of Willis (with and without contrast). Furthermore functional testing for vertebral steal, bubble test for 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 routinely performed.

Each year, approximately 4,000 examinations of extracranial arteries and approximately 3,000 transcranial Doppler or color-coded Duplex exams are conducted in the laboratory.



**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.

## TRANSCRANIAL B-MODE SONOGRAPHY LABORATORY

The method to visualize morphological changes of the brain parenchyma in neurodegenerative disorders has been pioneered by Prof. G. Becker and Prof. D. Berg and is being continuously advanced and extended in its application by the group of D. Berg. Patients with various movement and neurodegenerative disorders come from all over Germany and far beyond to receive additional diagnostic information by this supplementary neuroimaging tool, which is especially helpful in the early and differential diagnosis of diseases. Regular teaching courses attended by medical doctors and scientists from all over the world are continuously overbooked. Meanwhile the method is being applied in many countries on all continents.

# 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 2013, approximately 1,000 patients were seen.

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, occupational training for writer's cramp in dystonia patients, and counselling of spouses and relatives.



## PHYSIOTHERAPY

All neurological inpatients with sensory or motor deficits, movement disorders, pain syndromes, and degenerative spinal column disease are allocated to individualized physiotherapy. Currently 10 physiotherapists are working within the "TherapieZentrum" 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 2013 approximately 2,500 patients were seen.



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 five speech therapists is the treatment of patients with dysphagia (approximately 1,000 patients in 2013).

Every acute stroke patient receives a bedside and, if necessary, a videoendoscopic or videofluoroscopic 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. Additionally, in 2013 approximately 500 patients with aphasia and dysarthria received an intensive speech- and language treatment. The aim of the speech therapy with these patients is to improve their communication ability.



**The Hertie  
Institute for  
Clinical Brain  
Research**





**HERTIE INSTITUTE FOR CLINICAL BRAIN RESEARCH (HIH) 32**





## The Hertie Institute for Clinical Brain Research (HIH)



**Hertie-Institut**  
für klinische Hirnforschung

**In 13 years of its existence, the Hertie Institute has grown to more than 350 employees of all levels, from technicians to PhD students to full professors. Outstanding achievements of the institute are discoveries related to the molecular, genetic and physiological basis of a number of major neurologic diseases.**

The institute presently consists of four clinical and one basic science department: the Departments for Neurology and Stroke, for Epileptology, for Neurodegenerative Disorders and for Cognitive Neurology all share inpatient related research as well as patient care, while the Department of Cellular Neurology has been a pure basic science department, focusing on Alzheimer's disease. In the year 2012 it has recently installed an outpatient care unit in the field of Alzheimer's disease.

The institute is home to a total of 28 research groups, 25 of them within the aforementioned departments, three as independent junior research groups. The first of these independent groups, which has been established in 2006, has successfully passed its evaluation by the scientific advisory board of the Hertie Institute as an independent group status. In the year 2013 the head of this independent research group accepted the offer of a chair in restorative neuroscience at Imperial College in London, but he still runs his independent research unit at HIH. In 2013, scientists at the Center of Neurology have obtained more than 6.0 million Euro in third party funding and have published 250 papers in peer reviewed journals. As a primary care institution, all clinical departments together treat patients with the complete spectrum of neurological diseases. To strengthen the interactions



between clinical and basic research the “Hertie Grand Rounds” have been established as novel seminar series that reflects an initiative to allow both, clinicians and basic researchers, to comprehensively discuss exemplary diseases of our research focuses at the Center of Neurology and Hertie Institute. Silke Jakobi has become the new head of communications in October 2012. She is responsible for all HIH communication and public outreach activities. She will also support the fundraising activities.

Since 2012 the new research building for the “Werner Reichardt Centre for Integrative Neuroscience (CIN)” on the Schnarrenberg neuroscience campus, which also houses HIH groups, has been completed, and the scientific work has begun. The construction for the new building of the Tübingen site of the “German Center for

Neurodegenerative Diseases (DZNE)” within the Helmholtz Association is making progress as well as the setting up of DZNE Tübingen.

Finally, the Hertie Institute for Clinical Brain Research and the entire Neuroscience Community in Tübingen set up the Hertie Lecture in Brain Research. The first Hertie Lecture in Brain Research will initiate a yearly series of highly visible neuroscience lectures and will act as a crystallization core for neuroscientists and the public in Tübingen. The entire Neuroscience Community in Tübingen has actively participated by nominating internationally renowned scientists of the highest level as candidates for speakers.

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)

*13 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.*

*Seine Arbeitsschwerpunkte 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.*

*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. Als Einrichtungen der Primärversorgung versorgen alle Abteilungen zusammen Patienten aus dem gesamten Spektrum neurologischer Erkrankungen. Um die Interaktion zwischen der Grundlagenforschung und klinischer Forschung zu stärken wurde im Jahr 2013 die Seminarserie „Hertie Grand Rounds“ ins Leben gerufen. Diese Seminarreihe soll Grundlagenforschern und Ärzten die Gelegenheit bieten, sich zu unseren im wissenschaftlichen Fokus stehenden Haupterkrankungen auszutauschen, um damit einen Brückenschlag zwischen Forschungsergebnissen und klinischer Praxis zu schaffen. Das HIH verstärkt seine Kommunikationsaktivitäten: Silke Jakobi ist seit Oktober 2012 als Leiterin Kommunikation hierfür verantwortlich und wird sich auch in die*

*Fundraising-Aktivitäten einbringen. Im Herbst 2013 öffnete das Hertie-Institut für klinische Hirnforschung seine Türen für die Öffentlichkeit: Wissenschaftler stellten aktuelle Forschungsergebnisse vor und führten durch die Labore. Außerdem fanden Vorträge über neue Entwicklungen aus Forschung und Therapie zur Multiplen Sklerose, Alzheimer, Epilepsie und Parkinson statt. Der Tag der offenen Tür wurde außerordentlich gut von der breiten Öffentlichkeit angenommen.*

*Das HIH, ein Modellprojekt für Public Private Partnership, hat auch im Jahr 2013 mehr als 6,0 Millionen Euro an Drittmitteln eingeworben und 250 Veröffentlichungen in wissenschaftlichen Fachzeitschriften publiziert. Diese Zahlen belegen u. a. die wissenschaftliche Leistungsfähigkeit des Zentrums. Die wichtige Rolle, die das HIH im Leben der Universität Tübingen spielt, wurde auch durch die intensive Beteiligung am erfolgreichen Konzept der Universität im Exzellenz-Wettbewerb deutlich. Im Jahr 2013 wurde gemeinsam mit den weiteren neurowissenschaftlichen Einrichtungen Tübingens die Hertie Lecture in Brain Research initiiert. Im Rahmen dieser jährlich stattfindenden Vorlesung wird ein international renommierter Neurowissenschaftler nach Tübingen geladen. Die erste Vorlesung findet im Januar 2014 statt.*



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.

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 einen leistungsabhängigen Gehaltszuschlag für alle Mitarbeiter. Um der Größe des Hauses gerecht zu werden und um Erfahrungen aus den letzten zehn Jahren einfließen lassen zu können, wurde im Jahr 2012 die Governance des HIH überarbeitet und im Jahr 2013 implementiert. 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 neuro-regenerativen Prozessen des Rückenmarks beschäftigt, wurde im Frühjahr 2006 eingerichtet und 2010 aufbauend auf einer erfolgreichen internationalen Evaluierung in eine selbständige Arbeitsgruppe umgewandelt. 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 mit Fokus auf der Untersuchung synaptischer Plastizität im Drosophila-Modell wurde Ende 2013 international evaluiert und bis Ende 2014 verlängert.

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, welche große Fortschritte macht, ermöglicht die langfristige Sicherung von Forschungsmitteln und führt zu einer erheblichen Stärkung des neurowissenschaftlichen Standorts. Nach einer dreijährigen Bauphase konnte im Frühjahr 2012 der Neubau des Werner-Reichardt Zentrums für Integrative Neurowissenschaften (CIN), das direkt gegenüber dem HIH liegt, bezogen werden. Die Forschungsgruppen des HIH haben auch in diesem Gebäude ihre Arbeiten mittlerweile aufgenommen.

*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.**

Patients are referred from all over southern Germany as well as the neighboring countries according to the clinical and scientific expertise of the Department, including complex neurovascular diseases (ischemia, intracranial hemorrhage, vasculitis, vascular malformations, rare neurovascular diseases such as endovascular lymphoma, paraneoplastic coagulopathy, or reversible cerebral vasoconstriction syndrome), neuroimmunology (multiple sclerosis, myasthenia gravis and others), and brain tumors. Specialized teams in stroke medicine (stroke unit and 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.



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



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, five Research Groups exist that are active in brain networks and plasticity (Prof. Dr. Ulf Ziemann), stroke and neuroprotection (Dr. Sven Poli), neuroimmunology (PD Dr. Felix Bischof), neuro-oncology (Prof. Dr. Ulrike Naumann) and speech disorders (Prof. Dr. Hermann Ackermann). The Research Groups are located in the immediate proximity of the clinical setting 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 is focused 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, neuroophthalmology, 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.

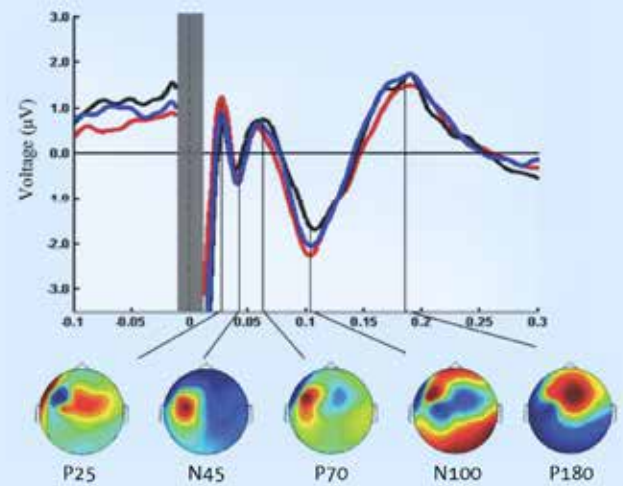
# Neuroplasticity

## Brain Networks & Plasticity (BNP) Laboratory

Head: Prof. Dr. Ulf Ziemann

Team: 8 members

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



**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. Interest in our group focuses on understanding principles of neural plasticity in the human cortex. In particular, we are interested 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. Das prioritäre Interesse unserer Arbeitsgruppe liegt darin, das Verständnis der Grundlagen von Plastizität der Hirnrinde auf systemneurowissenschaftlicher Ebene weiter zu verbessern. Im Besonderen sind wir darin interessiert zu verstehen, welche Mechanismen von Plastizität Lernen im gesunden Gehirn, und Wiedererlernen verlorengangener 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

A first project aimed at improving our understanding of the physiological underpinnings of TMS-evoked EEG potentials:

Combining transcranial magnetic stimulation (TMS) and electroencephalography (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). It is thought that inhibitory neurotransmission through GABA-A receptors (GABAAR) modulates early TEPs (< 50ms after

TMS), whereas GABA-B receptors (GABABR) play a role for later TEPs (at around 100ms after TMS). However, the physiological underpinnings of TEPs have not been clearly elucidated yet. Here, we studied the role of GABAA/B-ergic neurotransmission for TEPs in healthy subjects using a pharmaco-TMS-EEG approach. In a first experiment, we tested the effects of a single oral dose of alprazolam

(a classical benzodiazepine acting as allosteric positive modulator at  $\alpha 1$ ,  $\alpha 2$ ,  $\alpha 3$  and  $\alpha 5$  subunit-containing GABAARs) and zolpidem (a positive modulator mainly at the  $\alpha 1$  GABAAR) in a double-blind, placebo controlled, crossover study. In a second experiment, we tested the influence of baclofen (a GABABR agonist) and diazepam (a classical benzodiazepine) vs. placebo on TEPs. Alprazolam and diazepam increased the amplitude of the negative potential at 45ms after stimulation (N45) and decreased the negative component at 100ms (N100), whereas zolpidem increased only the N45. In contrast, baclofen specifically increased the N100 amplitude.

These results provide strong evidence that the N45 represents activity of  $\alpha 1$ -subunit containing GABAARs, while the N100 represents activity of GABAARs. Findings open a novel window of opportunity to study alteration of GABAA-/GABAB-related inhibition 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**

Another project has just started and will investigate the possibility to increase the size effect of non-invasive brain stimulation (cortico-cortical paired associative TMS) by concurrent brain polarization by transcranial direct current stimulation (tDCS), according to the following rationale and project plan: 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 supplementary motor area (SMA) and primary motor cortex (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.

Here we propose 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 SMA-M1 connection to strengthen task-dependent increases in 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. In a second step, successful protocols will be applied to elderly healthy subjects with an expected reduced potential for plastic change, with the ultimate translational aim to apply these protocols (in an extension of this project) to stroke patients with the intention to improve function of their paretic hand.

#### **Sonification of arm/hand movements to improve neurorehabilitation of stroke patients.**

Finally, we are currently starting a first clinical neurorehabilitation trial, using

sonification of hand/arm movements to improve rehabilitation of hand/arm function in stroke patients. We will use sensors attached to the hand and arm that allow real-time sonification (translation into music) of arm movements in 3D-space. We expect that musical encoding of arm movements will help to improve motor control of impaired arm movements in stroke patients. We plan, together with Prof. Altenmüller at the Hannover Medical School (MHH) to recruit 90 stroke patients with arm/hand paresis into a randomized study with three arms: arm movement training in 3D space (1) with sonification using classical music; (2) with sonification using pop music; (3) without sonification.

The patients will be training in 15 sessions á 20 minutes over a period of 3 weeks. We will test performance in several standardized motor tests and evaluate quality of life in a standardized questionnaire prior to and after the training period. In addition, EEG will be used to explore intervention-induced changes in cortico-cortical networks.

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# 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

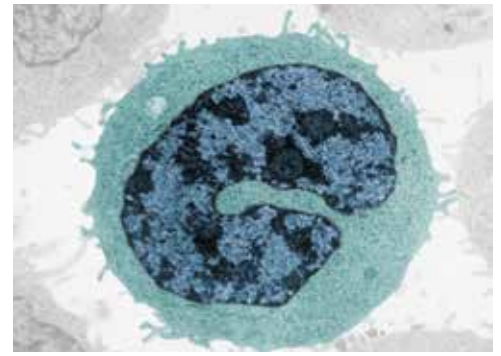
**Work in this group is focused on the role of the immune system in inflammatory diseases of the central nervous system (CNS) and stroke and covers the whole spectrum from basic science to clinical science and translation into innovative treatments. Biochemical and cellular techniques as well as animal 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.*

Lately, we assessed whether CNS-directed autoimmunity can be suppressed by intravenous injection of neural stem / progenitor cells (NSPC), which were genetically modified to overexpress the immunomodulatory cytokine Interleukin-10 (IL-10) and which have the ability to directly migrate into the CNS and to replace damaged CNS cells. 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 and time-lapsed phase contrast microscopy.

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.

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.

N-glycosylation of CD4+FoxP3+ regulatory T cells was increased during the induction-phase of EAE and this increased N glycosylation was related to a higher immunosuppressive capacity of regulatory T cells. In parallel, we assessed surface glycosylation of immune cells in patients with MS and discovered that Interferon beta-1a treatment leads to reduced alpha-2,3-sialylation of non-activated helper T cells. Alpha-2,3-sialylated residues were predominantly expressed by effector T cells while only a small fraction of regulatory T cells displayed alpha-2,3-sialylated residues. T cells

without alpha-2,3-sialylation displayed a reduced capacity to proliferate after polyclonal stimulation. Collectively, these data indicate a new mechanism of action of Interferon beta-1a in RRMS.

As a possible new treatment option for patients with progressive multifocal leukoencephalopathy (PML), an often devastating inflammatory disease of the CNS, we assessed efficacy and immunological consequences of continuous infusions of the CD4 T cell stimulating cytokine interleukin-2.

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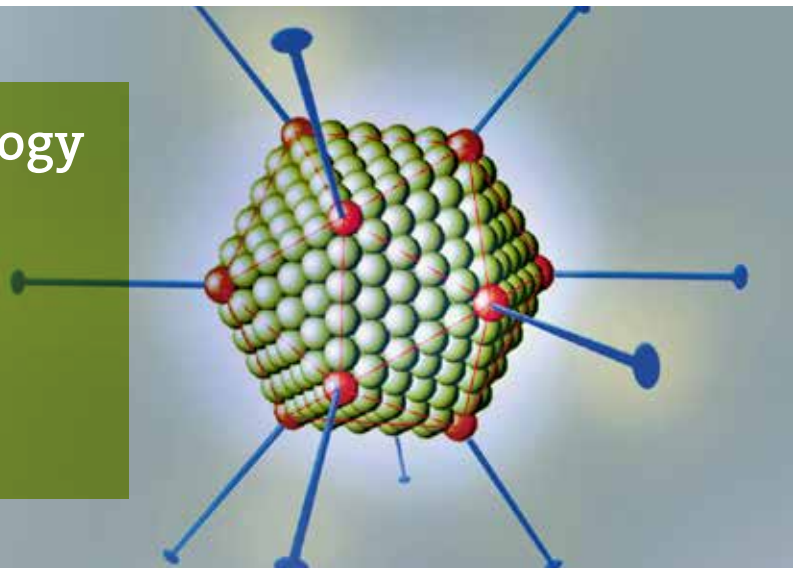
*\*shared senior authorship*

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# Molecular Neuro-Oncology

Head: Prof. Dr. Ulrike Naumann  
Team: 5 members  
Key words: brain tumor / glioblastoma / virotherapy / gene therapy



In mice, the oncolytic adenovirus Ad-Delo3-RGD is capable of inducing death of glioblastoma cells.

**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, destructive 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 functional and therapeutic effects of natural substances, cancer associated transcription factors, secreted cellular proteins and so called “oncolytic” viruses.**

*Die Arbeitsgruppe für Molekulare Neuro-Onkologie befasst sich mit Fragestellungen zur Tumorbiologie 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; nach operativer Entfernung können Sekundärtumoren innerhalb kurzer Zeit sogar in der bislang gesunden Gehirnhälfte entstehen. GBM hindern zudem Immunzellen daran, Krebszellen 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 verschiedenen 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 Entwicklung „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 associated with the GBM malignant characteristics meaning that GBM are mainly cell death resistant, possess immunosuppressive function and show a highly invasive and migratory growth. We set our research to get more information concerning the immunology, the molecular and cell biology of GBM. To know the biology of GBM in detail is important for the development of novel therapeutic strategies, a second section of our research.

Drivers of GBM motility include cytokines, protein modifiers altering the extracellular matrix, cytoskeleton members and regulators of adhesion. Inhibiting migration by novel therapeutic strategies might therefore be an important treatment strategy for GBM. In collaboration with Prof. Mittelbronn (Frankfurt) we have shown that the neuropeptide processor carboxypeptidase E (CPE) plays a central role in tumor cell motility. Reduced CPE in a cell death resistant GBM cell line and lower 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 was associated with worse prognosis. CPE expression reduced, whereas inhibition enhanced

GBM cell motility. Decreased migration following CPE expression was paralleled by altered cellular morphology, promoting more stable adhesion contact sites to the ECM. 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 extracts for complementary cancer therapy. We could demonstrate that mistle lectins (ML) enforce immune cells to attack and to kill GBM cells. Beside its immune stimulatory effect, mistle extracts mitigated GBM cell motility, paralleled by decreased expression of genes known to push and by enhanced expression of genes known to delimitate cancer progression. Treatment of GBM with mistle extracts also delayed tumor growth in mice. ML, 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 ML are able to inhibit GBM-induced neovascularization and if these lectins, especially the recombinant ML I, works in synergy with chemo- or radiotherapy.

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, NF $\kappa$ B activation was mandatory for Ad-CTS-1 induced cell death. Our results were in contrast to other groups who demonstrated that activation of NF $\kappa$ B protected GBM cells. This has important implications for the role of NF $\kappa$ B as a player involved in

tumor progression and should also be kept in mind when using NF $\kappa$ B-specific inhibitors in the therapy of cancer, especially in the therapy of GBM. In our recent project we are interested to unravel the role of I $\kappa$ B $\alpha$ , an atypical inhibitor of NF $\kappa$ B known to be a transcription factor per se, but also a modulator of NF $\kappa$ B transcriptional activity, in the growth and cell death resistance of GBM cells.

Oncolytic adenoviruses (OAV) that replicate selectively in tumor cells and not in normal cells are used as 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



Extracts from the mistletoe plant reduce tumor growth in mice.

have demonstrated that in vitro OAV works synergistically with the GBM standard chemotherapeutic temozolomide (TMZ). In vivo in a mouse model using highly resistant GBM stem cells, intratumoral injection of OAV induced tumor lysis and prolonged survival of tumor bearing mice.

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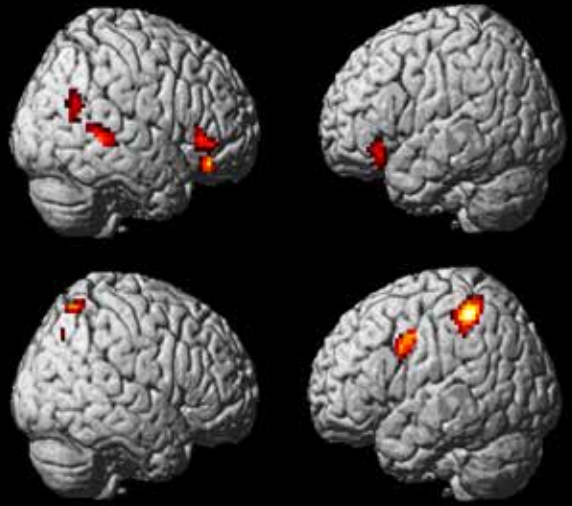
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# Neurophonetics

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 synthetic 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 on a previous functional magnetic resonance imaging (fMRI) study in a group of blind subjects (Dietrich et al. 2013a, Hertrich et al. 2013a), a training experiment is currently under way to further support the notion that the recruitment of a “visual” strategy underlies both in early – as well as late-blind subjects the acquisition of this exceptional skill (Dietrich et al. 2013b). Against the background of our preceding fMRI as well as magnetoencephalography (MEG) investigations, we assume, more specifically, that blind people use auditory-afferent information via an audiovisual pathway (pulvinar) to record prosodic speech events within right-hemispheric visual cortex. Presumably, this signal-related event structure is then transmitted to the frontal speech processing network via subcortical structures and rostral

parts of the supplementary motor area (pre-SMA) – a region considered an important timing interface (Hertrich et al. 2013b). We now have begun to apply the technique of Transcranial Magnetic Stimulation (TMS) in healthy and in blind subjects to further determine in how far the various brain regions involved in time-critical aspects of speech processing contribute (in a causal manner) to ultra-fast speech perception (in cooperation with the Brain Networks & Plasticity (BNP) Lab, see above).

## **Studies in Neurophonetics**

Following an invitation by Wiley’s Interdisciplinary Reviews, the scope of the – relatively new – research area “Neurophonetics” as been delineated in a review paper (Hertrich & Ackermann 2013). Furthermore, a dissertation project could be finished in 2013 that investigated the neural underpinnings of the perception of the categorical vowel length contrast in German subjects, based upon psychoacoustic tests and MEG measurements (Tomaschek et al. 2013).

## **Speech motor deficits in disorders of the cerebellum**

Cerebellar disorders may give rise to a distinct syndrome of speech motor deficits, called ataxic dysarthria. In cooperation with L. Schöls, M. Synofzik and T. Lindig, 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 analysed so far. Reduced speaking rate and voice irregularities were found specifically related to ataxia in other domains (Brendel et al. 2013). In SCA-patients, by contrast, articulatory problems emerged as a predictor for ataxia severity.

## **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 paleoanthropological 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
- (ii) subsequent “vocal-laryngeal elaboration” of cortico-basal ganglia circuitries, driven by human-specific FOXP2 mutations.

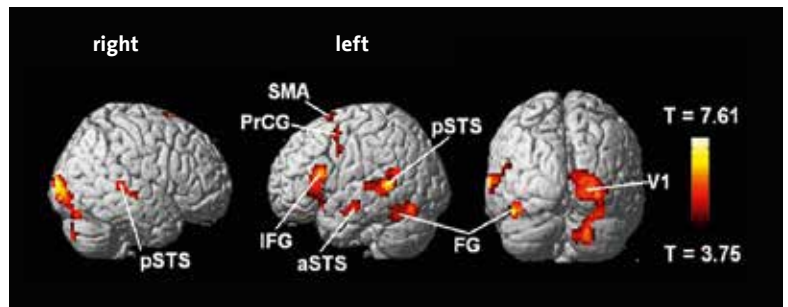
A more extensive representation of laryngeal muscles within the basal ganglia should have allowed for the deployment of the vocal folds – beyond sound generation (“voice box”) – as an “articulatory organ” which can be pieced together with orofacial gestures into holistic “motor plans”, controlling syllable-sized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

- (i) elucidates the deep entrenchment of articulate speech into a nonverbal matrix of vocal affect expression (emotive prosody) which “gestural-origin theories” fail to account for, and
- (ii) points at age-dependent interactions between the basal ganglia and their cortical targets similar to vocal learning in songbirds.

Thus, the emergence of articulate speech – often considered a sign of human superiority within the animal kingdom – appears to have involved the “renaissance” of an ancestral organizational principle (“evolutionary tinkering”). An elaborated account of this model has been accepted as a target article in *Behavioral and Brain Sciences* (Ackermann H, Hage SR, Ziegler W. Brain mechanisms of acoustic communication in humans and nonhuman primates: An evolutionary perspective) and will be printed together with 30 commentaries and a response of the authors.

\* cooperation with E. Zrenner and A. Bernd, Center for Ophthalmology, University of Tübingen

\*\* cooperation with S. Hage, Department of Biology, University of Tübingen, and W. Ziegler, Clinical Neuropsychology Research Group, Munich



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”.

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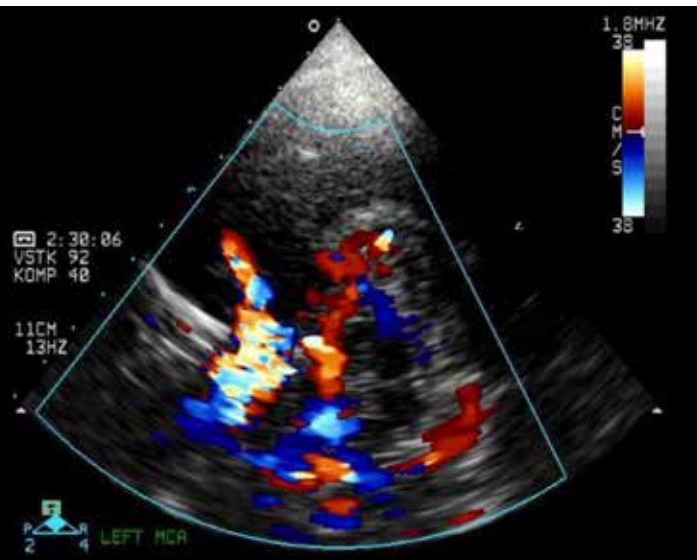
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# Stroke and Neuroprotection Laboratory

Head: Dr. Sven Poli

Team: 6 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.*

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Department  
of Neurology  
and  
Epileptology



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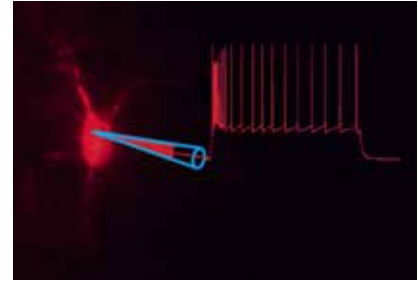


## Departmental Structure



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 41/42 and 45), running under the supervision of Prof. Dr. Y. Weber, 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 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. Schell), 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 and
- (iii) the molecular function, pharmacology and localization of ion channels and transporters, which are membrane proteins that regulate neuronal excitability
- (iv) unraveling 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

The Experimental group has been complemented in 2013 by the research group of PD Dr. T. Freilinger. Their work is focused on understanding the molecular pathophysiology of primary headache disorders with a special focus on migraine (iv).

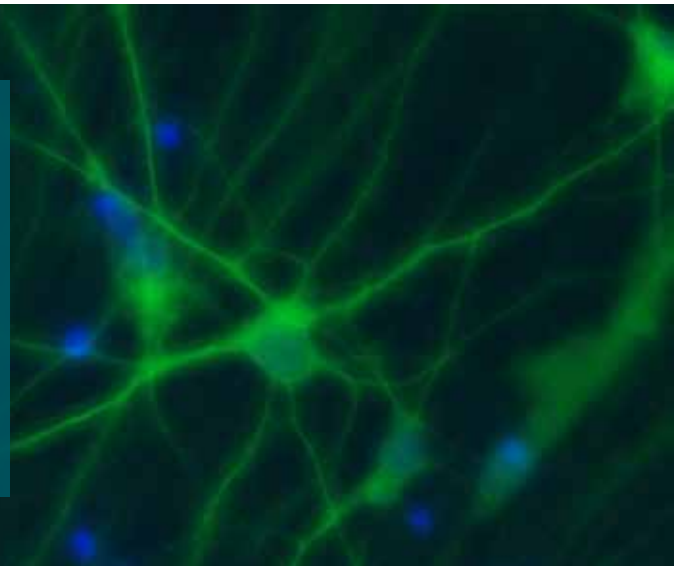
For electrophysiological recordings of neuronal activity in brain slices, a glass micropipette with a very fine tip (left) is brought in tight contact with a neuronal cell under microscopic control (middle). Recorded neurons are labelled with fluorescent dyes (red) to identify them after recordings; 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: 21 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) and international (FP6: Epicure, ESF: EuroEPINOMICS, FP7: EpiPGX) research networks confined to the recruitment of large cohorts of affected individuals and/or families and their genetic analyses. As an example, within the EuroEpinomics consortium we recently identified mutations in GRIN2A encoding the NR2A subunit of the NMDA receptor in two large independent cohorts comprising the whole spectrum of genetic focal epilepsies (Lemke et al, 2013).

Detected genetic variants are subjected to functional analysis in different heterologous systems. Using an automated two-voltage clamp system in *Xenopus laevis* oocytes, we showed that newly detected GABA(A) receptor variants reduce GABA-induced currents, which can explain the occurrence of seizures via reduced inhibition in brain. Furthermore, our analysis of seven KCNQ2 mutations associated with severe epileptic encephalopathy with pharmacoresistant



seizures and mental retardation suggested a dramatic loss-of-function with prominent dominant-negative effects on WT channels as a common pathophysiological mechanism of these mutations (Orhan et al, 2013). Expressing a number of SCN2A mutations affecting the sodium NaV1.2 channel in a mammalian cell line revealed a gain-of-function pathomechanism (Lauermann et al. 2013).

Functional implications of selected mutations are further examined in neuronal expression systems, such as transfected murine primary neurons and genetically-altered animal models. Electrophysiological methods, including single cell patch clamp or multielectrode array (MEA) technique to analyze neuronal network activity are employed. Such studies revealed for instance a hyperexcitability of single neurons and neuronal networks caused by two disease-causing KCNQ2 mutations (Füll et al., in preparation). We further 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 NaV1.1 channel expressed in inhibitory neurons and our findings indicate dysinhibition as a mechanism of seizure generation in this model. To better understand these mechanisms we also assessed network dysfunction on MEAs, in thalamocortical field recordings and by using Ca<sup>2+</sup> imaging of the hippocampus (Hedrich et al. submitted). In contrast, in a SCN2A mouse model, which is currently analyzed in cooperation with D. Isbrandt (Hamburg) and H. Beck (Bonn), epilepsy is driven by an intrinsic hyperexcitability of excitatory pyramidal neurons (ongoing work by Liu et al.). We also started collaborations on in vivo multi-electrode array analysis with C. Schwarz (Systems Physiology group) and on 2-photon Ca<sup>2+</sup> imaging of the

cortex with O. Garaschuk (Inst. Physiology II). Furthermore, in cooperation with the groups of T. Gasser and S. Liebau (Institute of Neuroanatomy), we have been generating induced pluripotent stem (iPS) cells from fibroblasts and keratinocytes of patients with epilepsy and differentiating them into neurons in order to establish human disease models to examine mechanisms of epileptogenesis.

An additional research interest is structural and functional brain imaging. We are establishing novel methods, in particular the use of EEG combined with functional MRI, high-density EEG and magnetoencephalography (MEG). The combination of these methods may emerge as strong tool to localize epileptic activity and identify pathological networks. We also intend to use these methods to understand pathological networks

in genetically determined epilepsies and correlate the molecular defects to network dysfunction. Moreover, we also use fMRI to characterize and better understand these effects, in particular episodic memory processes generated in the hippocampus.

The newly established research group of PD Dr. T. Freilinger aims at understanding the molecular pathophysiology of primary headache disorders with a special focus on migraine. As a monogenic model disease we are studying familial hemiplegic migraine (FHM), which is caused by mutations in cerebral ion channels and transporters. Complementary to ongoing genetic studies in this field, a major focus will be the multimodal clinical and electrophysiological characterization of a newly generated transgenic mouse model for FHM.

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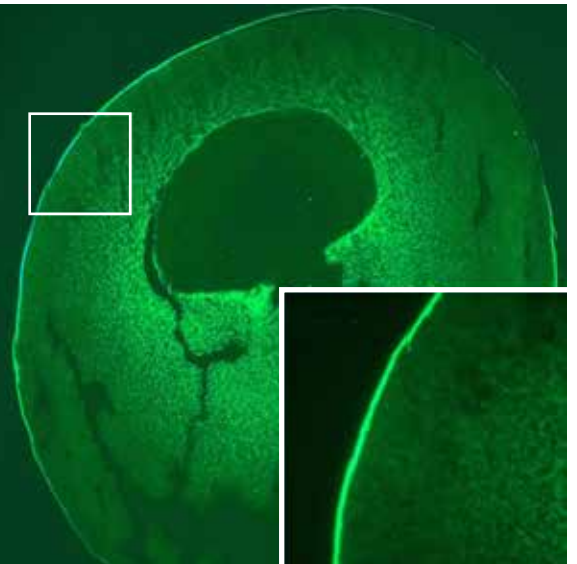
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# Clinical Genetics of Paroxysmal Neurological Diseases

Head: Prof. Dr. Yvonne Weber

Team: 9 members

Key words: paroxysmal neurological diseases / epilepsies / dyskinesia



Paroxysmal neurological disorders include a broad spectrum of clinical entities such as migraine, episodic ataxia or myotonia. The research group is focused on the clinical genetics of epilepsies and paroxysmal dyskinesias, paroxysmal neurological disorders with overlapping clinical and pathophysiological features.

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 but only 1-2% are monogenic with a single mutation leading to the clinical syndrome. Most of the genetic epilepsies follow a complex-genetic trait in which only a combination of genetic changes lead to the phenotype. Important groups of genetic epilepsies are the following:

- (i) idiopathic generalized epilepsies (IGE) like the absence epilepsies with childhood (CAE) and juvenile onset (JAE) or juvenile myoclonic epilepsy (JME)
- (ii) the idiopathic focal epilepsies such as the Rolandic epilepsies, nocturnal frontal lobe epilepsy (ADNFLE) or familial temporal lobe epilepsies (TLE)

- (iii) the benign syndromes of early childhood such as the benign familial neonatal (BFNS), infantile (BFIS) and the infantile-neonatal (BFINS) forms and
- (iv) the epileptic encephalopathies such as the Dravet syndrome (SMEI, severe myoclonic epilepsy of infancy) or Othahara syndrome.

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)
- (ii) kinesigenic dyskinesia (PKD)
- (iii) exertion-induced dyskinesia (PED)

The observed episodic dyskinesias include choreatic, ballistic, athetotic and dystonic features. They can be induced by different stimuli: In PNKD by stress or alcohol but not by movements, in PKD by sudden voluntary movements, and in PED after prolonged periods of exercise (10-30 min) in the exercised muscle groups.

PNKD has been associated with idiopathic generalized epilepsies with mutations found in the potassium channel gene *KCNMA1*, PKD with benign familial infantile seizures (BFIS)

with mutations found in *PRRT2* (proline rich transmembrane protein), and PED with different forms of absence epilepsies (mutations found in *SLC2A1* coding for the glucose transporter type 1, *Glut1*).

*Glut1* is the most relevant glucose transporter of the brain since it delivers glucose across the blood brain barrier. The *Glut1* syndromes include a variety of clinical features with a huge variability in phenotypes. The first mutations in PED were described in 2008. The mutations lead to a functionally relevant reduction in the glucose uptake which can well explain the episodic character of the symptoms under exertion (Weber et al. 2008). Mutations in *SLC2A1* were also found in early-onset absence epilepsy (EOAE, Suls et al. 2009) and childhood absence epilepsy (CAE, Striano et al. 2012). All *Glut1* syndromes respond well to a ketogenic diet which bypasses the defective glucose metabolism by providing ketones.

The syndrome of benign familial infantile seizures (BFIS) is characterized by a cluster of seizures with an onset between 3 and 12 months of age. The seizures can be treated well by common antiepileptic medications or disappear spontaneously. Most of the patients never develop seizures in adult ages and have a normal psychomotor development. Up to 40% of patients also suffer from PKD which can be also treated by

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

common antiepileptic drugs. Since many years, it was clear that both syndromes are linked to a huge region on chromosome 16 (Weber et al. 2006, Weber et al. 2008) but an underlying gene could not be detected for a long time. Recently, mutations in the gene *PRRT2* were described as the main cause of BFIs, ICCA and PKD by our and many other groups (Schubert et al. 2012, Becker et al. 2013). The resulting protein might be functionally relevant in the vesicle synaptic metabolism of neurons.

We are furthermore interested in the genetics of epileptic encephalopathies. A trio sequencing approach has been applied by the EuroEPINOM-IC-RES consortium (see Experimental Epileptology group) as a valuable tool for detecting the underlying genetic causes of these severe disorders. De novo loss-of-function variants were detected in *CHD2* (encoding chromo-domain helicase DNA binding protein 2) in a cohort of severely affected children with a fever-sensitive myoclonic epileptic encephalopathy very similar to Dravet syndrome. The functional relevance of *CHD2* haploinsufficiency was assessed in a zebrafish *in vivo* model system, in which knocking down *chd2* revealed epileptiform discharges similar to seizures in affected persons and altered locomotor activity (Suls et al., 2013).

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# 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 HHH)

Team: 3 members

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

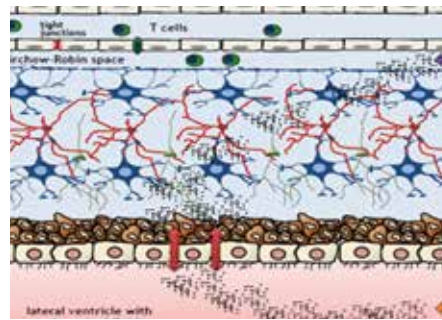
Primary and secondary inflammatory processes are playing a role in nearly all brain pathologies. As endogenous neural stem cells supply the brain throughout life with new functional cells, it is important to verify the effect of inflammatory processes that include e. g. the up-regulation of cytokines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

## Epilepsy-associated alterations of *in vitro* neuronal network activity

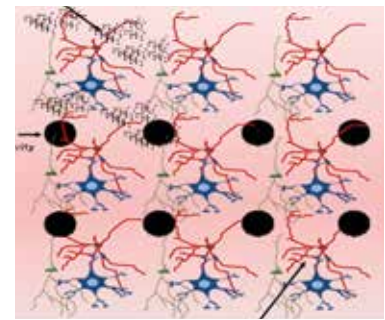
The impact of epilepsy-associated mutation in genes encoding for ion channels on neuronal network activity is currently under investigation.

## 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 (ivNNA) recorded by multielectrode arrays measuring synchronously bursting neural populations. Functionally relevant substances in pathologically altered CSF were biochemically identified, and ivNNA was partially recovered by pharmacological intervention. When considering



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.

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. 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 directly result in VTEs.

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**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. 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, under the supervision of Prof. L. Schöls and Prof. R. Krüger) 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, dementias and restless legs syndrome, dystonias, motor neuron diseases, ataxias, spastic paraplegias, and neurogenetic disorders allows highly efficient patient management. The equally close interaction of clinicians with basic and clinical scientists within the Hertie Institute for Clinical Brain Research, on the other hand, allows truly translational research. This innovative concept includes active education and training of scientific and clinical junior staff. In 2013, the clinical department was named as one of Germany's Top Ten hospital departments in Parkinson's Disease by the Magazine Focus.

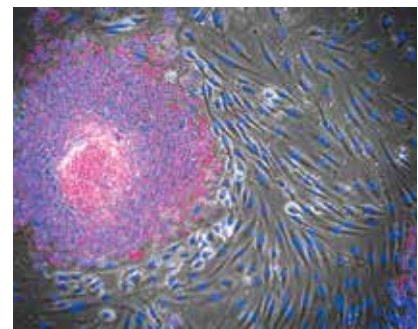
Research is currently organized within 7 research groups, headed by group leaders. The group of Prof. T. Gasser investigates the genetic basis of Parkinson's disease and other movement disorders with classic positional cloning and also high throughput array and next generation sequencing techniques. 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. Some members of the group of Prof. R. Krüger develop

and test novel stimulation paradigms of deep brain stimulation, while others are interested in fundamental mechanisms of neurodegeneration in PD, with a particular focus on mitochondrial function and dysfunction. Prof. P. Kahles group (section of Functional Neurogenetics) investigates also fundamental aspects of neurodegeneration in PD, while Prof. L. Schöls and his team (Section for Clinical Neurogenetics) focusses on clinical and fundamental aspects of inherited ataxias, spastic paraplegias, motor neuron diseases and other rare neurogenetic conditions. 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 focusses on neurogeriatrics and gait disorders in Parkinson's disease and dementias.

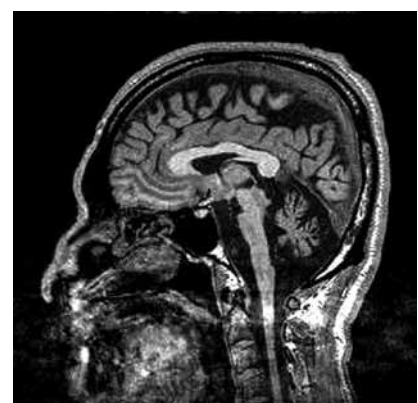
Thus, the wide spectrum of activities in the department covers all aspects from basic research to highly competent care of patients with Parkinson's disease and other neurodegenerative diseases.



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 embryonal connective tissue (blue) from mice).



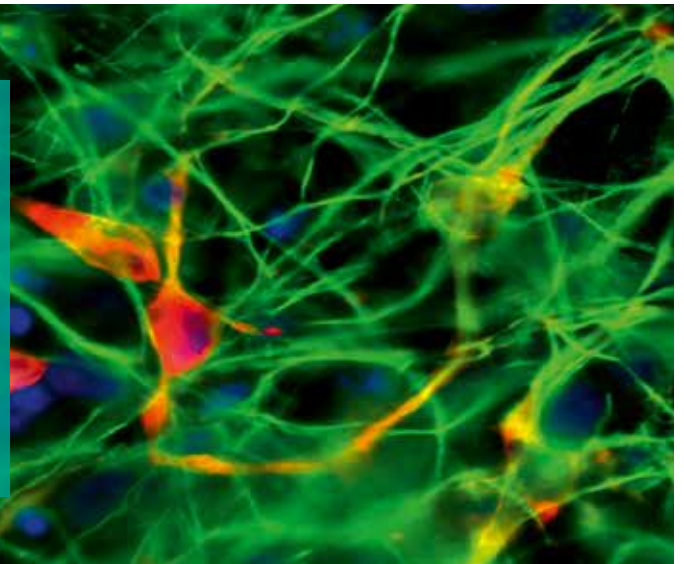
Both, fundamental mechanisms of neurodegeneration in Parkinson's disease and the effects of deep brain stimulation are investigated in Professor Krüger's group.

# Parkinson Genetics

Head: Prof. Dr. Thomas Gasser

Team: 12 members

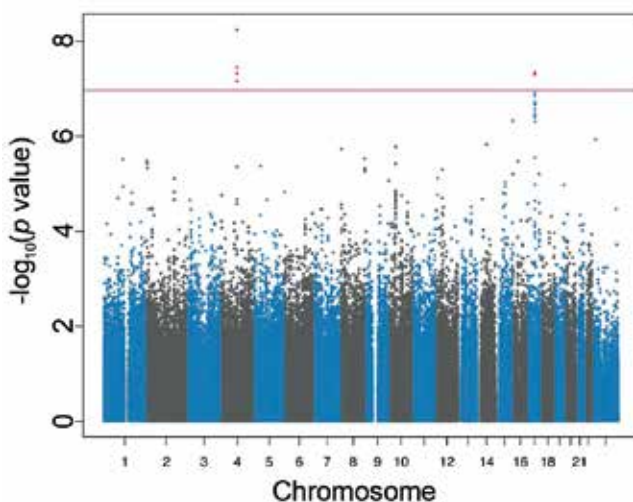
Key words: parkinson's disease / genetics / association studies / GWAS / mutation / induced pluripotent stem cells



**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 an international consortium, 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 beeinflussen. Innerhalb eines großen internationalen Konsortiums arbeiten wir mit modernen Hochdurchsatzmethoden verbunden mit genauen klinischen Analysen daran, diese genetische Varianten zu identifizieren.*

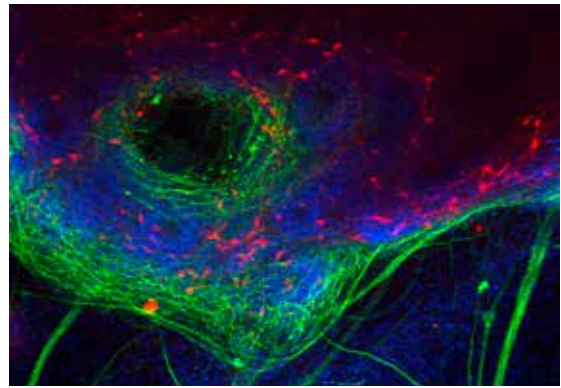
Specific mutations in some genes can cause rare inherited forms of Parkinson's disease (PD). Mutations in the LRRK2-gene, causing the most prevalent autosomal-dominant form of PD, was discovered by us and collaborators in 2004 (Zimprich et al., Neuron 2004). A contribution of more common genetic sequence variants, often called single nucleotide polymorphisms (SNPs), in the etiology of the much more common sporadic (= non-familial) is now equally well established.



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.

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 Network, NGFN2, in a collaboration with the laboratory for neurogenetics of the National Institutes of Health (NIH). This landmark study, which used material from more than 5,000 patients and 8,000 control individuals, identified two major genetic risk loci for sporadic PD: SNCA, encoding  $\alpha$ -synuclein and MAPT, containing the gene for the microtubule associated protein tau (Simon-Sanchez et al., Nat Genet 2009). Both genes had already been known to play an important role in familial PD and frontotemporal dementia, and the findings have now been confirmed by numerous other studies.

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.



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., Lancet 2011; Nalls et al., submitted). These variants can also influence the course of the disease (Brockmann et al., Mov Disord 2013).

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.

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 damage a specific population of neurons and lead to disease. Until recently, studies on gene function have only been possible in animal and cellular models, which often just have provided a rather artificial environment, not capturing the specific features of human

neurons. The revolutionary technology of “reprogramming cells” into so-called “induced pluripotent stem cells” (iPSC) has opened up a whole new research area: The iPSCs 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 been able to demonstrate that iPSC-derived neurons from patients with LRRK2-mutations have specific abnormalities which can be rescued by correcting the gene mutation using highly specific molecular tools (Reinhardt et al., Cell Stem Cell, 2013).

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# Dystonia

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

Team: 5 members

Key words: dystonia / torticollis / genetics / botulinum toxin



An artist's depiction of a dystonic syndrome (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 led by Prof. Schöls, on international collaborations but also on the work of Dr. E. Lohmann, who is presently working at the University of Istanbul, supported by a Margarete von Wrangell-stipend. As Turkey is a country with a high rate consanguinity, the prevalence of hereditary recessive diseases is greatly increased. Building on an existing cohort of patients with dystonia from consanguineous families in Turkey, detailed phenotyping and a thorough work-up of the families will provide the basis for future genetic analyses.



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## Functional Neurogenetics

Head: Prof. Dr. Philipp Kahle

Team: 10 members

Key words: parkinson's disease / amyotrophic lateral sclerosis / frontotemporal dementia / synuclein / RNA-binding proteins / mitochondria / signal transductions

We are elucidating the molecular mechanisms of neurodegeneration and physiological roles of genes linked to Parkinson's disease (PD) 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, fly and mouse models, and patient-derived biomaterials.

In the past year, we completed a structure/function study on novel DJ-1 variants discovered in Dutch patients with early-onset PD by the group of

Peter Heutink (now DZNE Tübingen). While the A179T substitution showed no defects, the P158del mutation led to protein destabilization and loss of neuroprotective functions of DJ-1 (Rannikko et al. JNC). In the course of these studies, we started to collaborate with Poul Henning Jensen (Univ. Aarhus, Denmark) in the context of the EU Training Consortium NEURASYN. We used their cell culture model of MSA and demonstrated neuroprotective effects of DJ-1 in this model. Moreover, we found that the cell death receptor Fas plays a role in MSA a-synucleinopathy (Kragh et al. PONE).

We were active in contributing antibody expertise to a local biomarker study on

serum levels of IgG against a-synuclein in dementia patients (Koehler et al. PONE), and provided transgenic mouse tissue for validation of studies on a-synuclein oligomers (Fagerqvist et al. JNC; Fagerqvist et al. AMYLOID) by the group of Martin Ingelsson (Univ. Uppsala, Sweden).

Finally we helped the group of Andreas Zell at the Institute of Bioinformatics (Univ. Tübingen) to develop a computational model of dopaminergic neurons within the frame of the German National Genome Research Network NGFNplus. Flux balance analyses were performed for several PD risk pathways, including oxidative stress, neurotoxins, and a-synuclein mismetabolism (Büchel et al. BMC Neurosci.).

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# Clinical Neurogenetics

Head: Prof. Dr. Ludger Schöls

Team: 12 members

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



20  $\mu$ m

Fragmented mitochondria in fibroblasts of a patient with OPA1 mutations causing defects of mitochondrial fission and fusion dynamics.

**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 2013).

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 (leucocaphalopathy with brainstem and spinal cord involvement and increased lactate) (Synofzik et al. Orphanet J 2013, Schicks et al. Neurology 2013a, Schicks et al. Neurology 2013b).

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 2013 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* 2013, 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* 2013). 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* 2013).

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* 2013), DDHD1 causing SPG28 (*AJHG* 2012), GBA2 causing SPG46 (*AJHG* 2013), 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* 2013). 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* 2013, epub).

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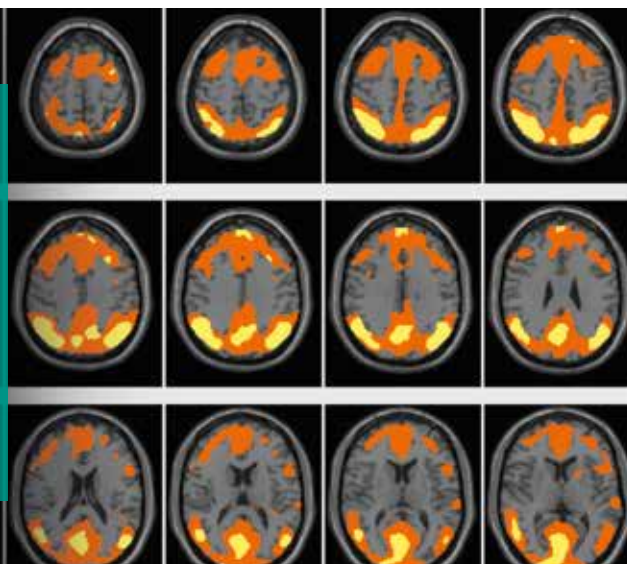
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# Clinical Neurodegeneration

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.**

*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.*

## 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 has expanded its involvement in a number of mono- and multicenter clinical studies, phase II to IV, for all stages of PD.

### Atypical Parkinsonian syndromes

Major effort has been put in the characterization of progressive supranuclear palsy (PSP) by use of clinical, biochemical and neuroimaging parameters. As an example Richardson-type PSP-patients were found to have thalamic and frontal hypometabolism in FDG-PET examinations, compared to parkinsonism-type patients who tended to have putaminal hypometabolism. These findings are the basis for a better understanding of these subgroups, and for future therapeutic interventions.

### Dementias with Lewy-bodies

With the demand for an early, individualised, 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 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.

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# Functional Neurogeriatrics

Head: Prof. Dr. Walter Maetzler

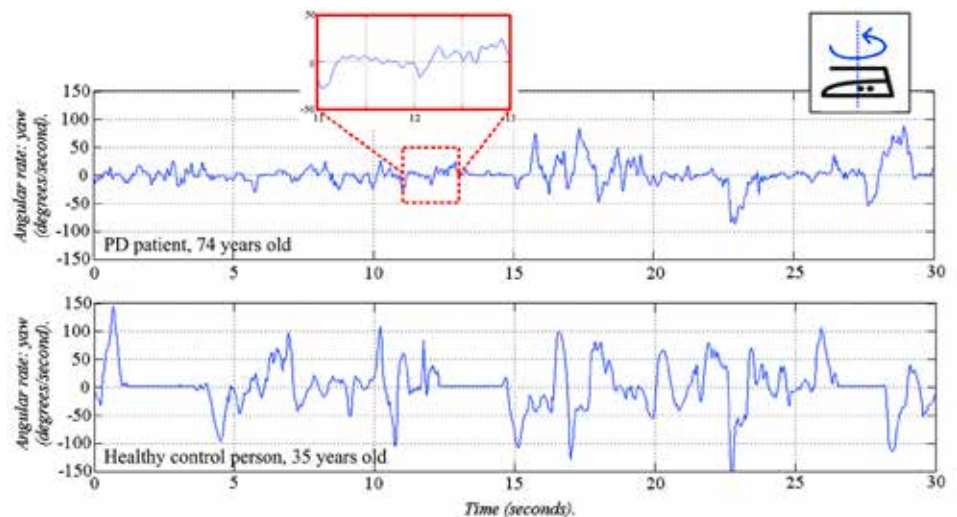
Team: 9 members

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

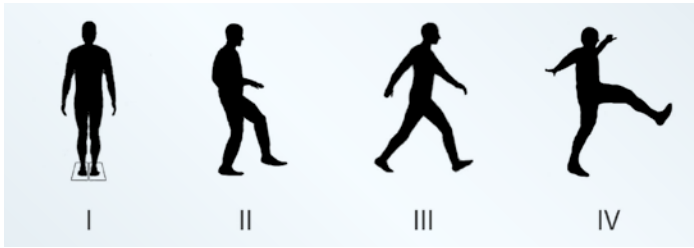
The functional neurogeriatrics group is dedicated to investigations of movement control of the elderly and individuals with prodromal and early stages of neurodegenerative disorders such as Parkinson's disease. Quantitative ambulatory assessment of axial and distal movements is performed with state-of-the-art 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, Domingos, et al., 2013). 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.

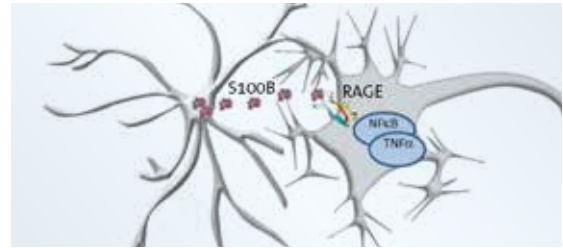
Our group is centrally involved in the development of a multimodal sensor information system for individuals with Parkinson's disease which exactly focuses on self-empowerment of the users. This system will be used in the home environment of patients with Parkinson's disease, and will be modular, extendible, adaptive and minimally obtrusive. Partners from England, Germany, Norway and Portugal contribute to this EU-funded ICT project (FP7, SENSE-PARK).



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).

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. Since there are many factors contributing to a patient's quality of life, it is essential for clinicians and scientists to measure these as objectively as possible (Maetzler, 2014). In the EU-funded ITN project Moving beyond we aim at defining a conceptual framework that will quantitate quality 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., 2012; Maetzler, Nieuwhof, et al., 2013).

#### Effects of Protein clearance deficits on 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 Bio-bank. In this context, our group aims at investigating the association of biochemical markers – such as markers of protein clearance – with symptoms and signs of Parkinson's disease.

Protein clearance is critical for the maintenance of the integrity of neuronal cells, and there is accumulating evidence that in Parkinson's disease impaired protein clearance fundamentally contributes to functional and structural alterations eventually leading to clinical symptoms. Particular focus is put on amyloid precursors such as APP (Vijayaraghavan et al., 2013), and astrocytic and microglial responses (Sathe et al., 2012).

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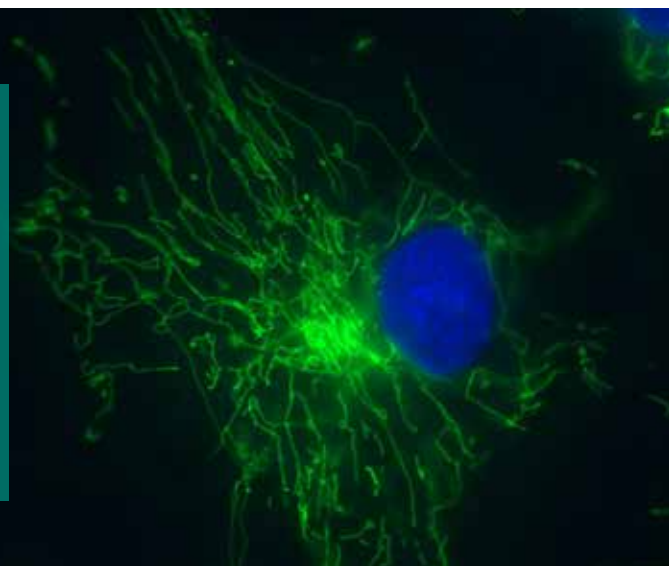
# Functional Neurogenomics and Deep Brain Stimulation

Head: Prof. Dr. Rejko Krüger

Team: Functional Neurogenomics: 11 members

Deep Brain Stimulation: 10 members

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) and the 'E64D' mutation in the DJ-1 gene (Hering et al., 2004). 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., 2010). 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 et al., in press). 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. 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., 2013; Abstract).

The Deep Brain Stimulation Group represents the clinical focus on advanced stages of Parkinson's disease, dystonia and tremor. Here the interdisciplinary BrainStimNet Tübingen (Neurosurgery, Prof. Gharabaghi; Psychiatry, Prof. Plewnia; Ethics, Prof. Wiesing) developed into one of the leading centers in Germany during the last 5 years. This is reflected by the integration into large international multi-center studies, where Tübingen substantially contributed to the EarlyStim Study, that revealed a significant gain in quality of life in PD patients with early motor fluctuations and will change treatment algorithms for PD in the future (Schüpbach et al., 2013). Moreover the group translated electrophysiological

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



recordings in a rat model of PD (Breit et al., 2006), that showed pathological hyperactivity of the substantia nigra pars reticulata (SNr) into a novel treatment concept with stimulation of the SNr in PD patients with severe gait impairment (Weiss et al., 2011). The first randomized controlled trial implemented a novel paradigm of concomitant stimulation of the (conventional) subthalamic nucleus (STN) and SNr and provided first proof of concept that freezing of gait can be successfully treated with this novel stimulation approach (Weiss et al., 2013). Current clinical research projects aim at the electrophysiological characterization of freezing of gait and the definition of biomarkers of freezing of gait in order to develop novel treatment algorithms to address this still major unmet need in the treatment of advanced PD.

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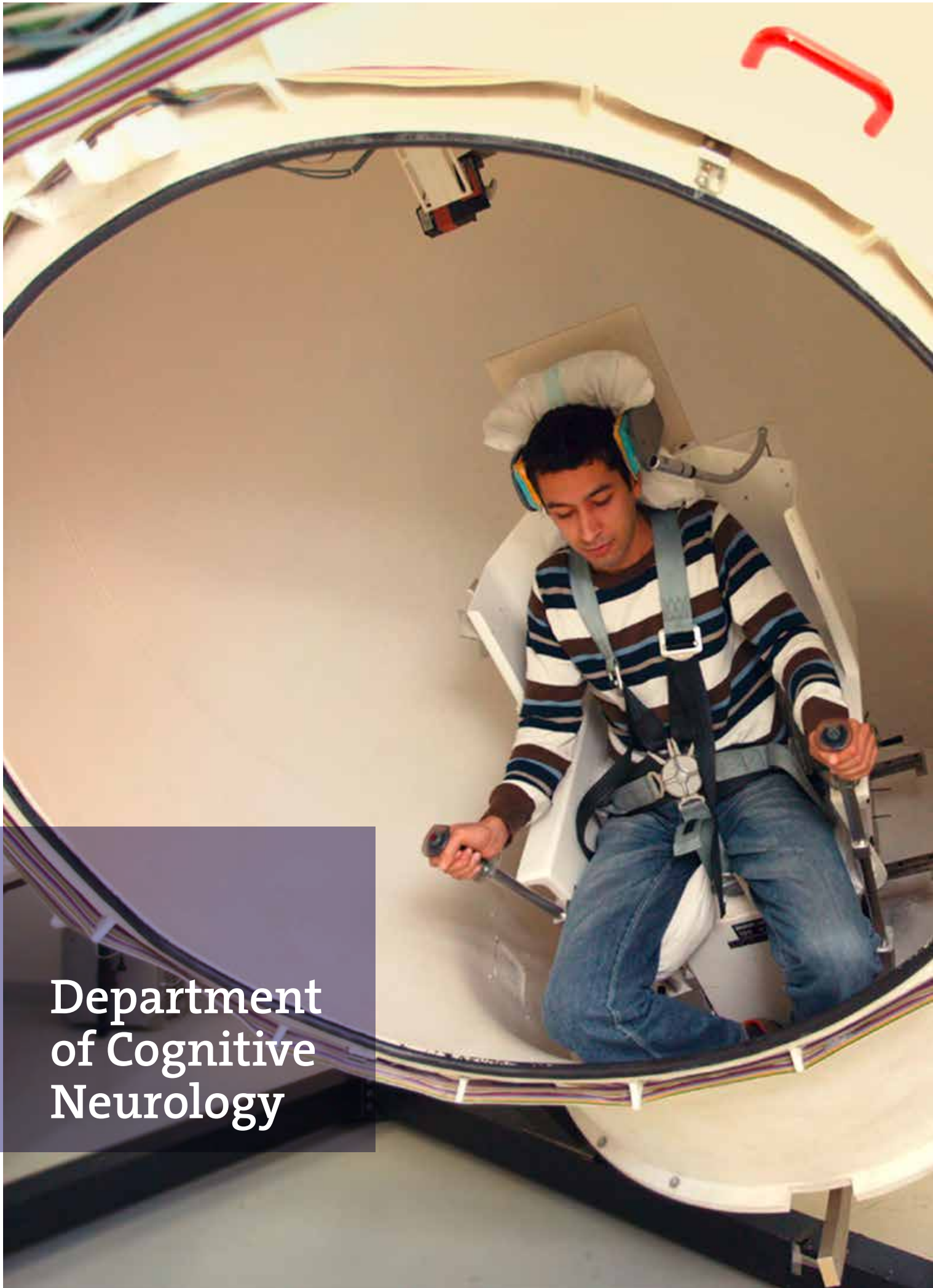
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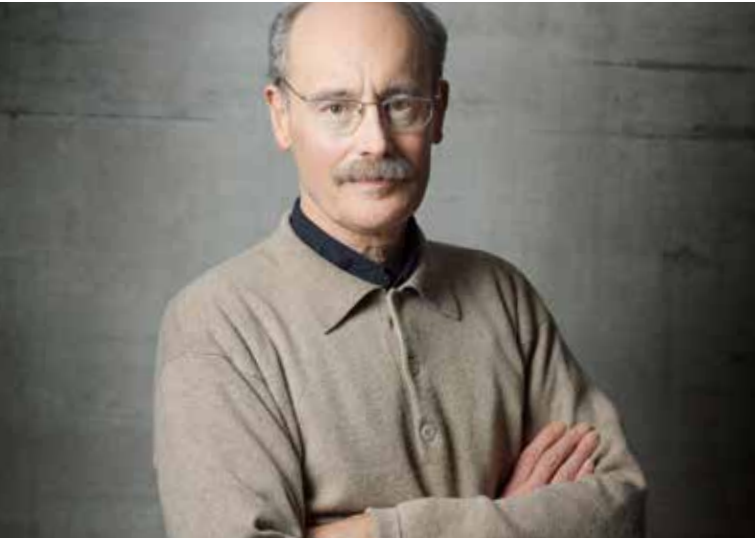


Department  
of Cognitive  
Neurology



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## Departmental Structure



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

The Department of Cognitive Neurology (DCN) 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 DCN 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 on Neuropsychology associated with a professorship for neuropsychology both taken over by Hans-Otto Karnath. In summer 2008 the Section on Computational Sensomotrics, 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 “Werner Reichardt 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 DCN.

The DCN is devoted to research on the basis of higher brain functions and their disturbances due to disease of the nervous system. To this end, the DCN 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.

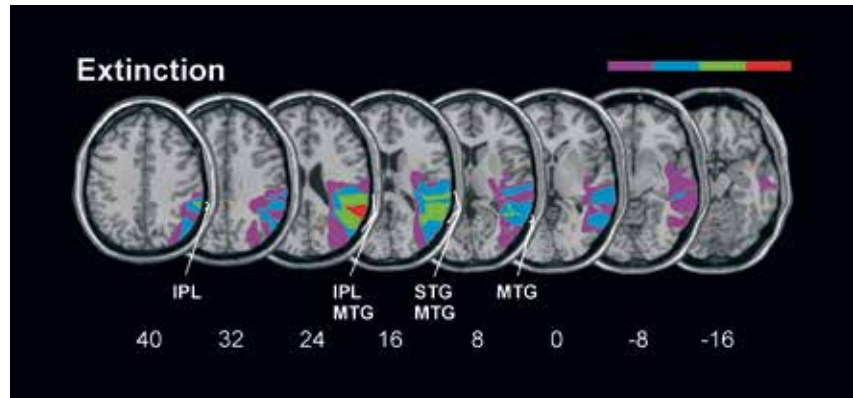


The displayed system allows the application of external mechanical perturbations to the body in order to study the motor control during complex walking.



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 recording 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. 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. In close collaboration with the interdisciplinary centers for magnetoencephalography and magnetic resonance imaging (MRI) at the Medical Faculty, functioning imaging experiments are carried out that tie up the behavioral experiments on patients with brain lesions, on the one hand, and experiments on animal models, on the other hand.

Several members of the DCN are currently engaged in an initiative to set up a Collaborative Research Center (SFB) on "Perception-Action and Social Interaction – Neural Encoding and Computations." This initiative is coordinated by Martin Giese, leader of the research group 'Computational Sensomotrics' at the DCN and the pre-proposal will be evaluated in May 2014. Many members of the DCN are also part of the ongoing excellence cluster "Werner Reichardt Centre for



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 behavioural studies to resolve questions concerning the anatomy and the underlying mechanisms of extinction.

Integrative Neuroscience (CIN)", which is now in its second funding period. The CIN is coordinated by Peter Thier. In October 2013 the DFG-funded trans-regional Research Unit (FOR1847) 'The Physiology of Distributed Computing Underlying Higher Brain Functions in Non-Human Primates' was granted and will be fully operational in March 2014. It brings together research groups from Göttingen, Marburg, Frankfurt and Tübingen working with non-human primates. The Research Unit is coordinated co-jointly by Peter Thier and Stefan Treue of the German Primate Center Göttingen.

All members of the DCN contribute significantly to research-oriented teaching at the Graduate Training Center 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: 21 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 or 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, 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 and ultimately to put the simulation theory to a critical test, our lab is carrying out experiments on premotor

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.



cortical area F5. In a nutshell, these experiments show that this particular area has access to streams of information which are obviously very important for the evaluation of actions of others such as information on the operational distance between actor and observer or the subjective value, the observed action has for the observer. Moreover, recent work of the lab clearly shows 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.

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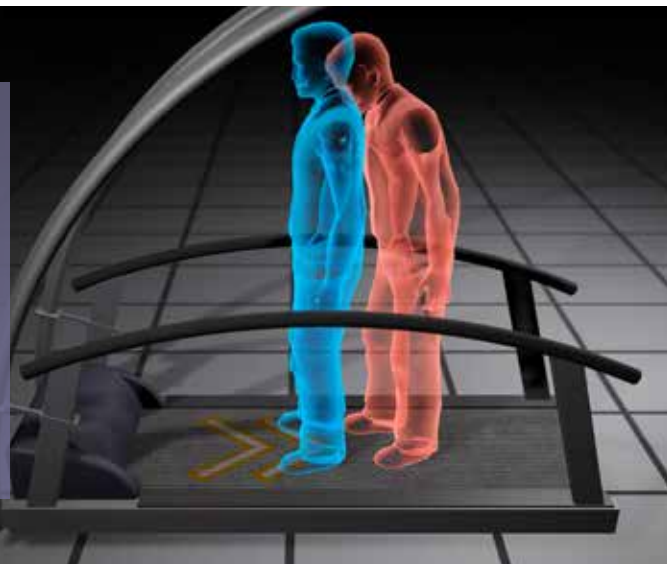
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# Computational Sensomotrics

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 for Computational Sensomotrics 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 mechanisms of action processing, and 3) biologically-inspired technical applications and biomedical engineering. 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.**

*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 Bewegungsrepräsentation; 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.*

## **Clinical movement control and rehabilitation**

Many neurological disorders, such as cerebellar ataxia, are associated with characteristic movement deficits. Their detailed quantitative characterization can help to understand underlying neural mechanisms and 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.

In recent projects, we were able to show that physiotherapeutic training induces beneficial long-term effects in cerebellar ataxia patients, contrasting with the common opinion that cerebellar patients do not profit from motor training. Using computer games (Kinect and Wii system) we devised (in collaboration with L. Schöls, Dept. Neurodegeneration) novel training paradigms for children suffering from cerebellar ataxia at different levels of severity. Developing advanced mathematical methods for the analysis of coordination patterns, we were able to identify training-induced motor improvements in complex movement sequences that transfer to other motor tasks, indicating efficient generalization of the underlying control mechanisms. In collaboration with D. Timmann, (University Clinic Essen) we were able to examine the role of the cerebellum in the interaction of cognitive and motor tasks, as well as the influence of specific cerebellar lesion sites on such dual-task performance. In addition, we currently examine the influence of non-invasive stimulation on complex whole-body motor learning tasks (in cooperation with D. Timmann, and with U. Ziemann, Dept. Vascular Neurology) in order to explore

the potential benefits for the support of motor rehabilitation.

### Neural mechanisms of action perception and representation

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 and electrophysiological experiments with non-human primates in the group of P. Thier (Dept. Cognitive Neurology), we investigate underlying detailed neural circuits in premotor cortex (area F5). For example, contrasting with established general ideas, we were able to show that mirror neurons in premotor cortex represent visual view parameters and do not show adaptation for repeated stimulus presentation. New results show also that mirror neurons represent the hierarchical temporal structure of actions while they can show extreme degrees of invariance against shape information. Based on such experimental results we develop physiologically-constrained neural models for the visual recognition of actions, and its relationship to motor representations, that recognize actions from real videos. These models demonstrate that action semantics and the perception of causality might be accounted for by relatively simple learning-based neural mechanisms.

A second central topic is the processing of social communication signals, such as bodily and facial movements, and underlying neural mechanisms. 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, investigating critical spatio-temporal features and underlying role of movement primitives. Stimuli generated by such methods are also useful for the study of the perception of emotions and social signals in psychiatric patients. In collaboration with S. Park (Vanderbilt University,

Nashville) and A. Sekuler (McMasters University, Canada), we were able to show characteristic deficits in perception of emotional body expressions of schizophrenia patients. Collaborating with A. Fallgatter (Clinic of Psychiatry), we have investigated neural correlates of the processing of such stimuli using Near Infrared Spectroscopy (NIRS).

### Biologically-inspired technical applications and biomedical engineering

Many technical systems require highly flexible and accurate representations of complex human movements. Exploiting machine learning approaches for the learning of hierarchical dynamical models combined with game engines, we were able to develop highly realistic models for the behavior of interacting humans that are used to study emotion perception, and which we plan to use also in monkey experiments for the study of the mirror neuron system. In several European projects, similar models are exploited in order to improve the control of humanoid robots. Most recent work in this domain has added a probabilistic semantic layer to such models. In collaboration with H.-O. Karnath (Division of Neuropsychology), we have successfully exploited such semantic models to detect automatically errors



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.

in the execution of complex manipulation actions that are characteristic for different sub-forms of apraxia.

In the domain of biomedical engineering we currently develop a mobile and low-cost (< 5000 EUR) motion analysis system (based on Kinect sensors), which will allow to quantify movement changes and rehabilitation outcomes, including also preclinical motor symptoms of movement disorders, e.g. in cerebellar ataxia and Parkinson's disease.

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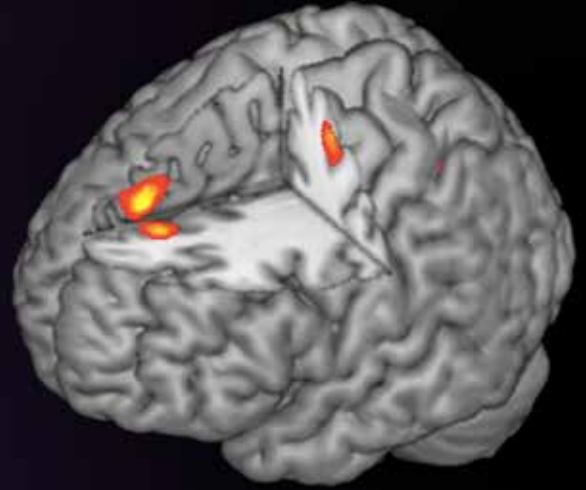
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# Neuropsychology of Action

Head: Dr. Marc Himmelbach

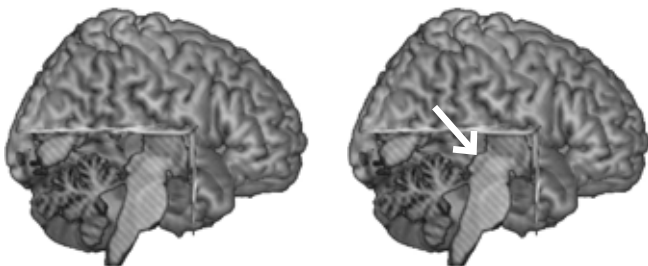
Team: 7 members

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 behavioural 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.

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

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



### 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 proprioceptive deficits on visuomotor coordination in neurological patients

We take it for granted that we can feel our own body, the position and movements of our own limbs. But soon we realise that it is pretty difficult to explore in more detail the current feedback from our body sensors. Surprisingly, this is also true for experimental and clinical measures of proprioception. There are very few investigations of the influence of an impaired proprioceptive input on visually guided movements in patient populations using trustworthy measurements of proprioception. The widely used clinical screenings are

very insensitive, whereas more precise measures require unacceptable examination durations and procedures. We established technically simple, but nevertheless sensitive and reliable procedures to elucidate proprioceptive impairments where these were previously overlooked. These procedures allow us to really determine the role of proprioceptive deficits in the occurrence of visuomotor disorders. In collaboration with the Department for Neurodegeneration we examine the proprioceptive status of patients suffering from hereditary ataxias to determine the impact of such a specific sensory disorder on the general status of these patients.

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# Oculomotor Laboratory

Head: Prof. Dr. Uwe Ilg

Team: 5 members

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



**We analyze the details of eye movements in various conditions as an ideal example of goal-directed behavior. We ask whether these details are different in people playing routinely video games compared to people who do not play. In several studies we were able to show that video game players (VGP) have shorter latencies in general compared to non-players and a better control of the allocation of attention. However, we did not find differences in the correctness of eye movements and in the speed of changing the locus of attention. We are able to separate the effects of video game play from the regular effects of age.**

*Wir untersuchen die Details von Augenbewegungen, die ein ganz hervorragendes Modellsystem für die Untersuchung von ziel-gerichtetem Verhalten darstellen. Dabei interessieren wir uns vor allem für die Frage, ob wir Unterschiede zwischen Menschen, die mehr als eine Stunde am Tag am Computer spielen, und Nichtspielern erkennen können. Wir können in einigen Studien belegen, dass Computerspieler im Allgemeinen kürzere Latenzen an den Tag legen und über eine bessere Kontrolle der Ausrichtung der Aufmerksamkeit verfügen. Wir finden allerdings keinen Unterschied in der Genauigkeit der Blickbewegungen und in der Geschwindigkeit der Aufmerksamkeitsverlagerungen. Wir sind in der Lage, die Einflüsse der Computerspiele von den altersbedingten Einflüssen zu trennen.*

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!**

We applied a very simple oculomotor task. Our subjects have to perform a cognitively driven saccade to the

mirror position of a visual target (anti-saccade). In some cases, the subject is not able to suppress the gaze shift towards the target, triggered by a reflexive shift of attention towards the target. These saccades are called pro-saccades. As illustrated in Figure 1, the fast visual orienting responses are generated by the superior colliculus in the midbrain whereas the cognitively driven anti-saccades are mediated by the frontal eye field in the frontal cortex. So the frequency of erroneous pro-saccades can be used as a measure for the strength of the executive control function of the frontal cortex upon the midbrain circuit.

As basis for our experiment we tested a total of 55 subjects aged 15 to 31 years. All subjects were classified as VGPs and non-players in regard to 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 erroneous pro-saccades had about 100 ms shorter reaction times than the correctly executed anti-saccades. More strikingly, the reaction times of both 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 is between 350 and 400 degrees/second. In other words, if the eyes could rotate without limitations, a complete rotation would occur within one second. As reported by others, pro-saccades reach higher peak velocities than anti-saccades. Surprisingly, both types of saccades of VGPs reach higher peak velocities as gaze shifts executed by non-VGPs.

To examine the cognitive control function, we examined the frequency of erroneous pro-saccades 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



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.

rates than subjects with longer latencies. Despite this general relationship, we fail to find an increased amount of errors in VGPs compared to non-players. Since the frequency of pro-saccades 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.

### Speed of shifting the spotlight of attention?

The decrease of reaction times in VGPs may be attributed to faster attentional processing. To test this hypothesis, we designed an experiment in which subjects had to report the identity of a specific visual target presented in the array of a total of 12 items (see Figure 2). We varied the cue leading times between 0 and 600 ms. We examined 116 subjects, 63 identified as VGPs and 53 as non-players, 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. Interestingly, we found no difference

in the optimal cue leading time between VGPs and non-player. Therefore, our data do not support the hypothesis that VGPs are able to shift their spotlight of attention faster compared to non-players.

### Suppression of an already planned movement

In certain conditions, it is important to suppress an already planned action triggered by a specific stimulus. Initially, we asked our subjects (n=80) to press the space bar as fast as possible upon the appearance of a green rectangle. As expected, VGPs showed shorter reaction times as non-players. In our second paradigm, in 50% of the trials, the green target changed to red at five different stimulus onset asynchronies (SOA 16 to 320 ms). The color red indicated that the space bar should not be pressed! Now, the latencies in the still present Go-trials (frequency of 50% of all trials) are significantly increased compared to the latencies obtained in the first paradigm. VGPs have significant shorter latencies. From our behavioral data at different SOAs we were able to calculate the stop signal reaction time (SSRT) for each subject, i.e. the minimal lead for a successful suppression of the key press. We found mean SSRT of approx. 250 ms with no difference between VGPs and non-player.

### Differences in latencies between subjects

In the course of our experiments, we observed the natural scatter of saccadic latencies. We asked whether the individual value of saccadic latency of a given subject is determined by the processing time of the early visual system. We compared saccadic latencies obtained in two different saccade paradigms (regular and gap) with the latencies of visual-evoked potentials (VEP). We emphasized the latency of the p100 component. Our data revealed a highly significant correlation of the saccade latencies obtained in both paradigms. However, there was no correlation between saccadic latencies and p100 latencies suggesting that differences in eye movements are not determined by the processing time of the early visual system

### Effect of age

In the course of our studies, we had the possibility to compare the anti-saccade parameters in subjects with very different age (total of 334 subjects ranging from 15 to 80 years). We found that the saccadic peak velocities are not affected by age. The latencies of pro- and anti-saccades drop from young to adults and slowly increase again towards elderly subjects. The frequency of pro-saccades showed a similar dependency of age.

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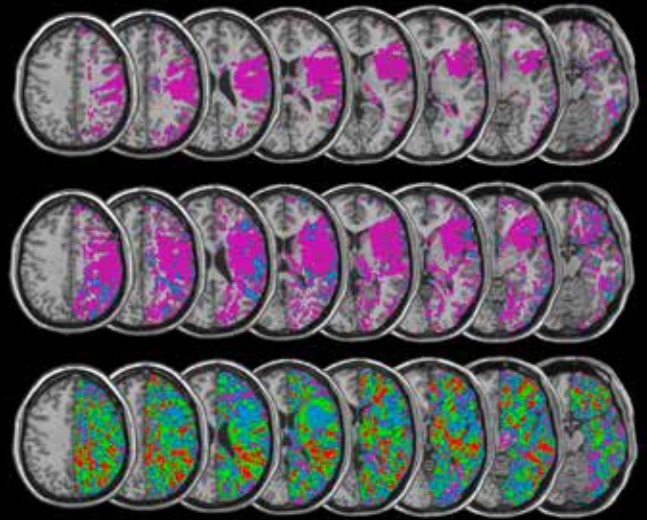
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# Neuropsychology

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

Team: 23 members

Key words: cognitive neuroscience / neuropsychology



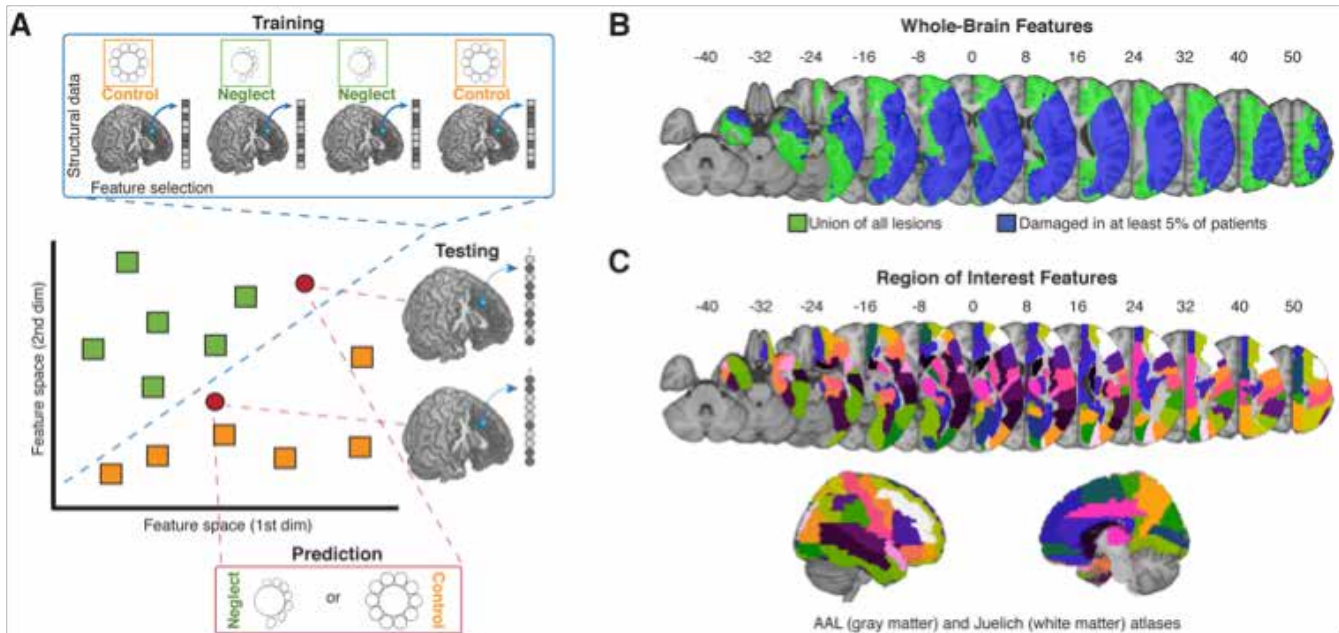
Evaluation of methods for detecting perfusion abnormalities after stroke in dysfunctional brain regions.

**The Section for 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 Division 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 Division 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 are 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.



We employed a multivariate pattern analysis (MVPA) procedure for using brain injury maps to predict the presence or absence of cognitive functions (Smith et al., PNAS 2013: 1518-1523).

MVPA involves both training and testing a predictive model. The training procedure employed machine-learning algorithms (support vector machines; SVMs) to construct a model of how distributed patterns of lesion data are consistent in indicate the presence or absence of spatial neglect. The constructed model is then tested on new data (i.e., not used to train the model).

This procedure was repeated for each individual in our dataset (N = 140), meaning each individual was tested on a model that was built independently of that individual's data. The average of those predictions is the predictive power of the model.

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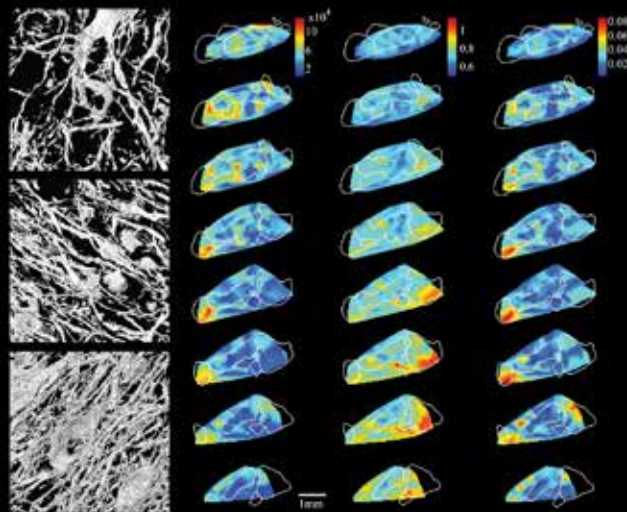
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# Functional Neuroanatomy Laboratory

Head: PD Dr. Fahad Sultan

Team: 4 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 activity related 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 (with laser confocal microscopy) the neuropil of subcortical regions 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. Verschiedene Bildgebungsverfahren erlauben Details auf die unterschiedlichsten Ebenen zu visualisieren. Funktionelle Bildgebungsverfahren beinhalten zahlreiche Verfahren, die uns helfen die Funktion des Nervensystems zu verstehen. Diese Verfahren funktionieren entweder dadurch, dass sie Aktivitäts-abhängige 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 auf rechnerbasierten Modellierungen. In diesem Labor verwenden wir Bildgebungsverfahren, die uns die Visualisierung der komplexen molekularen und zellulären Architektur des Gehirns auf mikroskopischer Ebene erlauben und auch weit-verteilte Hirnnetzwerke (elektrischer Stimulation mit fMRT) auf der meso- bis makroskopischen Ebene. 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. Imaging quantitative traits typical for the human cerebellar dentate

Compared to most mammals the cerebellar hemispheres are expanded in size in primates and even more so in apes and humans. A long-standing division of the primate dentate into an evolutionary older dorsal thin-folded (microgyric) and a newer ventral thick-folded (macrogyric) half based on dentate morphology has recently been linked to the emergence of a major projection from the cerebellum to the prefrontal cortex, and hence also to the development of related prefrontal specific human abilities (e.g. executive functions). 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. From these observations, we propose that the crucial enlargement of the human cerebellum was along the medio-lateral dimension and that the folding of the dentate has the function to ensure less convergence from neighboring parasagittal stripes. In future studies we want to relate the remarkable folding of the dentate to underlying cellular and molecular processes in order to better understand the mechanisms that underlie the emergence of folds in the ape and human dentate.

### 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.

#### 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. In-vivo imaging of the monkey brain's connectivity

Electrical stimulation, combined with functional magnetic resonance imaging (es-fMRI), has become an important tool to study the functional properties of spatially distributed neuronal networks of the brain (Logothetis et al., 2010; Sultan et al., 2012). Electrical stimulation of the gateway of the cerebellar output, the deep cerebellar nuclei (DCN), leads to reliable transsynaptic responses in the neocortex. There is a striking difference between our previous results by electrical stimulation of striate and extrastriate neocortex and our current DCN stimulation results. Differences in inhibition probably constitute a crucial factor. Stimulation of the neocortex revealed the presence of strong inhibition, thus preventing the

propagation of electrically-induced activation over multi-synaptic pathways. Stimulating the 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. In conclusion, both the topography of the stimulation effects as well as its emphasis on temporal precision is in full accordance with the notion of cerebellar forward model information modulating cerebro-cortical processing.

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# Systems Neurophysiology Laboratory

Head: Prof. Dr. Cornelius Schwarz

Team: 10 members

Key words: neocortex / multiple neuron recordings / active touch / cognitive control / perception / sensory detection / sensory discrimination



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 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. 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 Großhirnkrankungen übertragbar sein werden (Alzheimer, Parkinson, Schizophrenie und Depression).*

## Associative coupling in the neocortex

The overarching goal of our work is to understand the operating principles of the neocortex, a unique brain structure, which mainly evolved in mammals. There is clear evidence that the neocortex, in the broadest sense, endows the subject with cognitive capabilities. The big mystery is, how the vast diversity of neocortex-dependent behaviors are generated by a structure that shows nearly identical neural architecture across species (mouse, rat, monkey, human) and functional systems (sensory, motor, cognition): The neocortex is a quasi-two dimensional sheet of neural tissue, which is composed of repetitive neuronal elements and network connections. Even an expert can hardly decide on

the basis of a microscopic image of neocortex, whether it was prepared from a mouse, rat, monkey or human.

The generality of 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 in animals and humans. 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.

To verify this hypothesis, we firstly need to clarify how signals are represented within neocortical networks and what role the confusing multitude of neuronal components plays (e.g. the six neocortical layers, or the various types of excitatory and inhibitory neurons). Second, it must be resolved how separate areas are linked and whether the link and concurrent signal processing make use of the same neural elements and activities, or whether they can be separated.

This research therefore 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 and combine it with behavioral observation at highest precision.



Active tactile exploration of the environment using vibrissae.

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 tactile representations, how they are formed into a percept, and how they are coupled to motor representations to optimize tactile exploration. In addition, we have begun to study coupling of other areas, e.g. sensory and so-called higher cortical areas, during decision making.

Regarding the close similarity of neocortex in animals and humans it is very

likely that basic scientific knowledge that we gain in animals can be generalized very easily to understand also principles of function or dysfunction in humans and patients. Of course, future applications for the better of humans suffering from neocortical diseases such as Alzheimer's and Parkinson's disease, schizophrenia, or depression, need future progress in applied and translational neuroscience. However, before this can happen, a thorough understanding of the bases of neocortical function has to be reached. This is the purpose of our research.

Beyond the goal to understand the function of the neocortex we have started to direct our research toward

possible future applications. We work toward the establishment of cortical sensory neuroprostheses, that in the future might help those patients, who lost a sense due to a disease of the central nervous system. A major problem is that percepts produced by electrical activation of cortical networks depend very much on the sensory and behavioral context. Our solution to this problem is to establish intelligent implants that measure neural activity to assess information about contexts (i.e. the associative state of the cortical tissue to be activated) and use this to increase precision with which sensory signals can be imprinted into central neuronal structures and reach perception.

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Department  
of Cellular  
Neurology





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## Departmental Structure



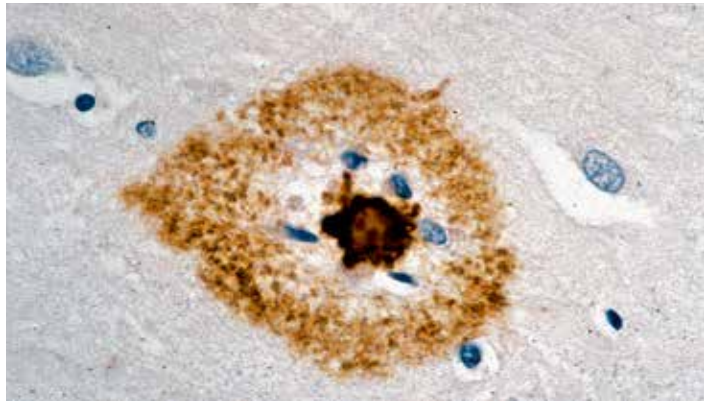
Prof. Mathias Jucker is head of the Department of Cellular Neurology.

The Department 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 cerebral amyloid angiopathy. Alzheimer's disease is the most frequently occurring age-related dementia with more than one million people affected in Germany. It was in Tübingen that Alois Alzheimer described the disease in 1906 for the first time to his colleagues.

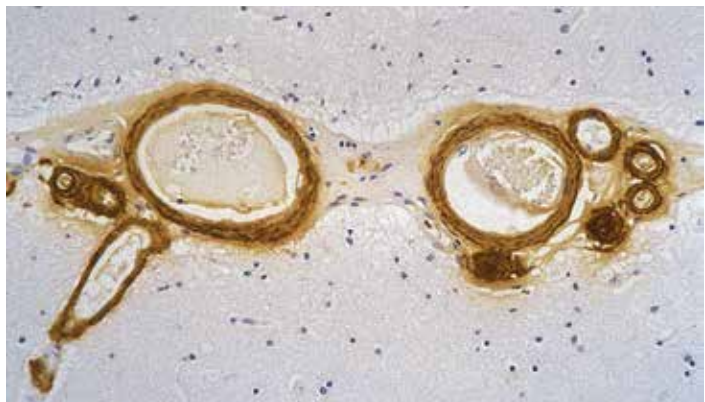
Currently our department is composed of four research groups and one core unit: The group of Molecular Biology studies the processing and metabolism of the pathogenic proteins that are involved in Alzheimer's disease and related dementias. The Neuropathology group uses primarily transgenic mouse models to study the pathomechanisms of Alzheimer's disease and cerebral amyloid angiopathies. The Neuroimmunology group works on aspects of innate immunity in the aging brain and neurodegenerative diseases with a special focus on therapy. Finally, the group of Molecular Imaging studies

how Alzheimer's disease lesions and neurodegeneration develops over time using in vivo multiphoton microscopy. The core unit supports the department primarily with mouse genotyping, ELISA measurements, and other technical and administrative support.

Our department hosts scientists from more than 10 nations ranging from short-term fellows, master students, PhD/MD students to postdocs and group leaders. To bridge our basic and preclinical research towards clinical applications we run the Section of Dementia Research, that includes the memory clinic, in collaboration with the University Clinic of Psychiatry. The goal is to build a department with expertise in brain aging and age-related neurodegenerative disease that is extramurally highly competitive and intramurally socially attractive for co-workers.



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



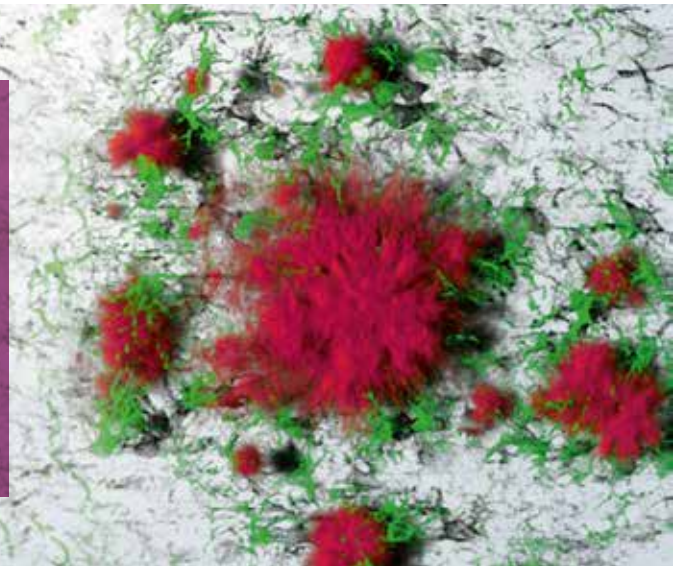
Vascular amyloid (cerebral amyloid angiopathy) in an Alzheimer brain.

# Alzheimer's Disease

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.*

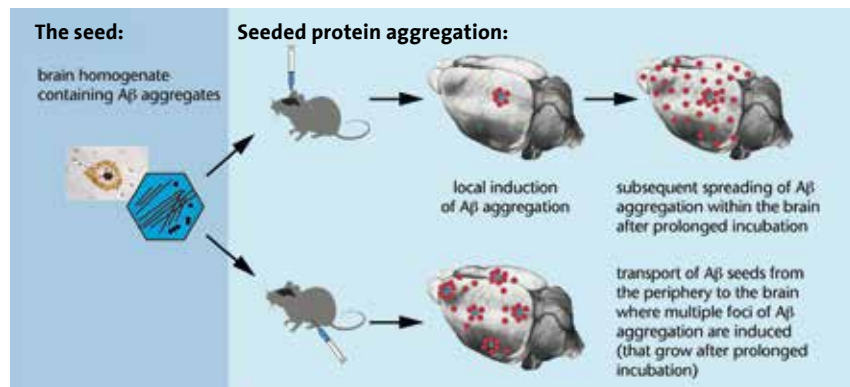
Misfolded proteins are the cause of many neurodegenerative diseases. How these proteins misfold and what the initial trigger is for the misfolding and their subsequent aggregation is, however, largely unknown. Furthermore, it remains unclear why the aging brain is a risk factor for neurodegenerative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

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(\*co-first author, \*\*co-corresponding author)

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# Molecular 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 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 bei der Alzheimer-Erkrankung. Dabei nutzen wir Methoden der Molekular- und Zellbiologie, um Schlüsselschritte und Interventionspunkte zu finden.*

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

- (i) Processing and cellular function of the amyloid precursor protein (APP)
- (ii) Identification of essential factors causing protein aggregation and amyloid formation
- (iii) Uptake, accumulation and spreading of protein aggregates
- (iv) Neurotoxic mechanism underlying proteopathies.

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 amyloidbeta (A $\beta$ ) peptides, the major components of amyloid plaques in the brain of patients with AD. In addition, also a c-terminal fragment (AICD) is liberated whose cellular function is not yet understood. In the past the group has concentrated on processes that regulate APP processing with the aim

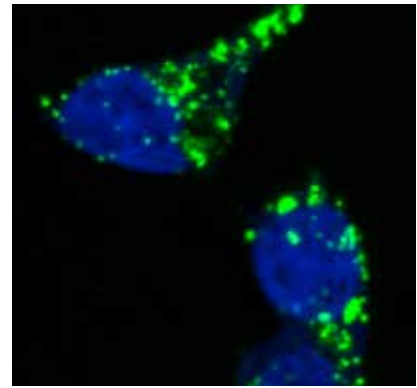
to find new pathways to decrease the amount of A $\beta$  peptides as a therapeutic strategy for AD. Profound insights into the mechanism of Gleevec, a known tyrosine kinase inhibitor, were gained.

Recently another protein Bri2 came into focus. APP and Bri2 are both genetically linked to specific forms of dementia. Mutations in the Bri2 gene cause Familial British Dementia (FBD) and Familial Danish Dementia (FDD). One similarity is that both APP and Bri2 have to undergo proteolytic processing to liberate small aggregation-prone peptides. These are in turn the building blocks of extracellular deposits formed either as plaques or around blood vessels in brains of patients. Like in AD models a recently established FDD-model recapitulates the main histologic features. Recent in vitro results suggested a direct interaction of Bri2 with APP which lead to altered APP processing and decreased A $\beta$  secretion. Our further indepth analysis revealed a new mechanism of interaction.

In agreement with previous results we found that overexpression of Bri2 leads to a significant decrease in total secreted A $\beta$  and an increase in other c-terminal fragments. Furthermore, we could identify the upregulation of secreted insulin degrading enzyme (IDE), a major degradation protease, under these conditions. This was independent of the processing of Bri2 and also worked in a truncated version of the protein. We suggest now that Bri2 might act as a receptor that regulates IDE levels which in turn affects A $\beta$  levels by influencing APP metabolism. Therefore the regulation of IDE activity may be a new promising therapeutic approach.

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.

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



To understand this discrepancy we started to investigate uptake and spreading of A $\beta$  in cultured cells. Preliminary results suggest a prominent role of APP in the process of uptake yet it remains unclear whether it acts as a receptor or whether APP associated modifications have an indirect influence on internalization. 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.

Transgenic mouse brain tissue 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 that can identify the presence of seeds in tissue homogenates. With this powerful tool we are now able to analyze defined fractions of brain tissue for the qualitative and quantitative presence of amyloid seeds. The assay is further explored to detect minute amounts of such seeds also in body fluids for diagnostic purpose.

A $\beta$  aggregation has been studied extensively *in vitro*. 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. Preliminary results

indicate that A $\beta$  can indeed be stably expressed on cell surfaces both in cell culture models and in transgenic mice. Membrane anchored A $\beta$  also promotes plaque deposition *in vivo* when coexpressed with soluble A $\beta$  in mice. Future work will utilize this model to understand the role of membrane-anchored A $\beta$  intermediates in initiation of A $\beta$  aggregation and neurotoxicity.

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# 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 outpatient Memory Clinic and a Research Unit.**

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



## 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.

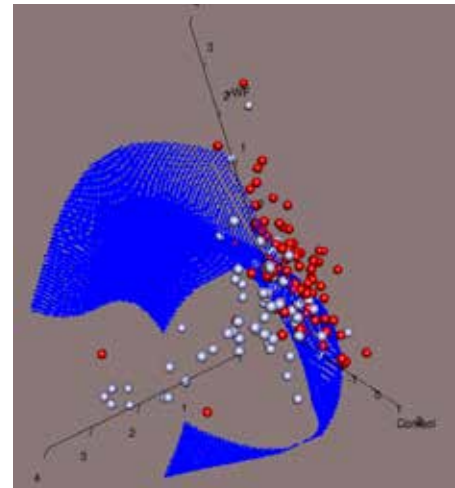
## Research Unit

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 pre-clinical stage, i. e. before any symptoms appear.



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]).



#### 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.

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# Laboratory for Neuro-regeneration and Repair

Head: Dr. Simone Di Giovanni

Team: 8 members

Key words: synaptic plasticity / spinal cord injury / stroke / 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.

**Axonal regenerative failure being a major cause of neurological impairment occurs following central nervous system (CNS), but not peripheral nervous system (PNS) injury. Importantly, PNS injury triggers a coordinated regenerative gene expression program not found after CNS injury such as spinal cord lesions, whose core molecular substrate remains elusive. Here we show that epigenetic pathways mediated by PCAF-dependent acetylation of H3K9 at the promoters of key regeneration associated genes are triggered by a conditioning peripheral sciatic nerve lesion but not by a spinal dorsal column injury. Next, we found that NGF-MEK-ERK retrograde axonal signaling trigger PCAF-dependent regenerative gene reprogramming. Finally, PCAF is required for conditioning mediated axonal regeneration and PCAF overexpression promotes axonal regeneration across the inhibitory environment of the injured spinal cord. This work provides evidence that a specific epigenetic code regulates the capacity for axonal regeneration of CNS neurons providing a novel epigenetic-based target for regenerative therapies for clinical spinal injury.**

**PCAF-dependent epigenetic changes promote axonal regeneration in the central nervous system** (Puttagunta et al., Nature comm, in press)

Initially after a peripheral nerve injury rapid ion fluxes increase, followed by a rise in cAMP levels, axonal translation occurs, phosphorylation retrograde cascades activate transcription factors, gene expression is induced and finally regeneration occurs. However, the final link between axonal injury-induced retrograde signaling and the regulation of essential regenerative gene expression remains elusive. As epigenetic changes are a rapid and dynamic way to translate external stimuli into targeted We believed that a positive retrograde signal initiated by PNS injury could relax the chromatin

environment surrounding specific promoters and allow for gene expression; however, a negative signal following CNS injury may restrict promoter accessibility and inhibit gene expression.

Given that DNA methylation did not show a clear pattern associated with axotomy, we investigated whether key histone modifications would be specifically enriched on established critical genes for the regenerative program in DRG neurons. Of all histone modifications that correlate with active gene transcription (H3K9ac, H3K18ac, H3K4me2) or gene repression (H3K9me2 and H3K27me3) that were screened, H3K9ac and H3K9me2 and H3K27me3 were enriched compared to IgG on most promoters, but only H3K9ac and H3K9me2 were found

differentially enriched at GAP-43, Galanin and BDNF promoters, consistently correlating with early and sustained increased expression following SNA (1-7 days). In contrast, SCG-10, whose gene expression is unaltered after 24h and only modestly increased following 3 and 7 days SNA, did not show an enhancement of H3K9ac or PCAF at its promoter. Given that a preconditioning lesion (SNA preceding DCA) activates the regenerative capacity of the CNS, we questioned if a PNS epigenetic signal overrides a CNS signal. We observed an increase in the gene expression of these genes following preconditioned DCA versus DCA alone, which correlated with an increase in PCAF at these promoters. Furthermore, a broader picture of post-axotomy H3K9ac promoter enrichment

is depicted by regeneration-associated (Chl1, Lgals, L1cam, CAP-23 and SPRR1a), axonal growth (ATF3 and Bcl-xL), housekeeping (ribosomal unit 18S) genes or axonal structure (NF-L) genes. Importantly, these experiments show that H3K9ac, a marker of actively transcribing genes linked to the enhancement of gene expression, is selectively enriched on the promoters of GAP-43, Galanin and BDNF, but not on the promoters of other SNA induced genes such as SPRR1a, ATF3 and HSP27, suggesting that their common regulation maybe linked to their importance in regeneration.

We then turned our attention to understanding whether retrograde signaling following SNA plays a role in this positive chromatin remodeling. Immediately following peripheral injury, pERK levels rise in the injured axon and ERK signaling modules are retrogradely transported to the DRG cell body, where we show that global PCAF and H3K9ac levels rise. In adult primary DRG cultures, nerve growth factor (NGF), an activator of ERK signaling and neurite outgrowth, increased expression of PCAF and H3K9ac, while the ERK kinase (MEK) inhibitor, PD98059 (PD), prevented PCAF and H3K9ac induction. NGF induces PCAF expression, nuclear localization and activation of acetyltransferase activity specifically by threonine phosphorylation at its histone acetyltransferase domain. In L4-L6 DRGs, SNA induced the expression of nuclear PCAF and PCAF threonine but not serine phosphorylation. This correlated with an increase of pERK in DRGs, as well as nuclear PCAF translocation and acetylation of H3K9, all of which are dependent on ERK activation following SNA. As suspected, inhibition of ERK activation following SNA decreased gene expression as well as PCAF and H3K9ac at the promoters of GAP-43, Galanin and BDNF. These data present the first link between retrogradely transported PNS-injury related signals and epigenetic modifications at the promoters of specific established regenerative genes.

As a preconditioning lesion is able to induce neurite outgrowth in primary adult DRG neurons cultured on permissive (laminin) or non-permissive (myelin) substrates, we tested whether increased PCAF expression by adeno-associated virus could also drive neurite outgrowth. Indeed, neurite outgrowth increased on laminin and myelin by PCAF overexpression in DRGs as well as another CNS primary culture, cerebellar granule neurons. In ex vivo experiments, the inhibition of PCAF activity by Garcinol was able to significantly limit neurite outgrowth on both laminin and myelin as well as repress H3K9ac induced by SNA. Correspondingly, PCAF  $-/-$  mice provided full abolishment of neurite outgrowth induced by SNA in ex vivo cultured DRG neurons. Additionally, SNA-dependent neurite outgrowth in ex vivo cultured DRG neurons was blocked by ERK inhibition via delivery of PD at the nerve stump phenocopying PCAF loss of function experiments.

To validate these observations in vivo, we studied regeneration of ascending

sensory fibers following a preconditioning lesion (SNA 7 days prior to DCA) in the absence of PCAF and found that PCAF is required for regeneration induced by a conditioning lesion and for the expression of GAP-43, Galanin and BDNF in DRGs. Importantly, axonal tracing in SCI experiments in a cohort of PCAF  $-/-$  mice and strain matched controls showed that PCAF  $-/-$  mice did not display any abnormalities or overt phenotype in axonal tracing or regarding the lesion site (data not shown). Finally overexpression of PCAF promotes regeneration across the injured spinal cord.

We have shown the first systematic study of various epigenetic modifications revealing specifically that increased H3K9ac and PCAF as well as decreased H3K9me2 at the promoters of GAP-43, Galanin and BDNF are due to retrogradely induced pERK activation of PCAF leading to essential gene activation, which is sufficient to mimic the regenerative response assembled by a conditioning lesion, thus driving regeneration in the CNS.

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# Physiology of Learning and Memory

Head: Dr. Ingrid Ehrlich

Team: 8 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 study 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.*

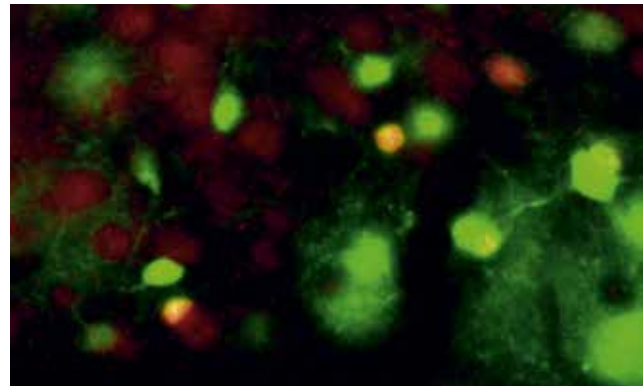
Organisms continuously adapt their behavior to survive. Such experience-driven adaptations are mediated by modifications in brain function. We study changes in brain function by investigating fear conditioning and extinction of fear in mice, a powerful model for associative learning and memory. Our goal is to elucidate the molecular, synaptic and cellular changes in 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 brain structure in the temporal lobe, is a key structure for storing fear memories. Fear memories can be modified by extinction, where an individual learns that certain stimuli are not fearful anymore in a

specific setting. Extinction depends on a larger brain network comprising the amygdala, the hippocampus (a structure important for different forms of memory and processing of spatial information) and the medial prefrontal cortex (a structure associated with the control of actions), as well as interactions between them. To date, some of the strongest links between neural plasticity and behavioral learning come from studies of fear memory in the amygdala. While plastic changes of sensory inputs (from thalamus and cortex) to excitatory cells in the amygdala are well understood, plasticity may also occur at other inputs and amygdala inhibitory elements, thus, encoding memories in parallel and distributed networks. Our goal is to identify and investigate these networks and their learning-dependent changes.

One line of research aims to understand properties and function of a specific inhibitory network in the amygdala, the intercalated cells. These cells have recently received much attention, as they are active during fear behavior and are critical for extinction, possibly providing a break for the fear response. However, their function and connectivity is poorly understood. We have discovered novel wiring principles of intercalated cells that suggest that they process information

Fluorescent image of the amygdala of transgenic mice expressing green fluorescent protein (green) in inhibitory interneurons, costained with the calcium binding protein Calretinin (red) which weakly labels excitatory neurons and some interneurons (yellow).



about external sensory stimuli during learning and are part of a novel network that inhibits input and output nuclei of the amygdala. Our future goals are to decipher the mechanisms of intercalated cell plasticity and to develop molecular tools to specifically manipulate these cells to understand their function in vivo.

A second line of research investigates interactions of amygdala, hippocampus, and prefrontal cortex at the level of individual cells, which is critical for understanding extinction mechanisms and why fear memories can come back after extinction. Toward this end, we employ optogenetic, targeted stimulation of prefrontal and hippocampal inputs to the amygdala. We discovered that prefrontal cortex and hippocampus innervate distinct subpopulations of neurons in the basolateral amygdala with distinct input properties. Our next goals are to address the physiological impact of these inputs, whether they converge or diverge onto individual cells, and if the synaptic communication between these brain areas changes in animals after fear and extinction learning.

A third line of research addresses the 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. We have identified a number of changes in amygdala inhibitory networks that

occur between infancy and adulthood. Our future goal is to elucidate the underlying mechanisms, how these changes affect plasticity in the amygdala, and ultimately to see if they are linked to differences in learning behavior.

Studying circuits and mechanisms of fear and extinction memory not only provides us with insights into general principles of memory formation, but also into neural dysfunction during inappropriate control of fear in conditions such as human anxiety and other neuropsychiatric disorders.

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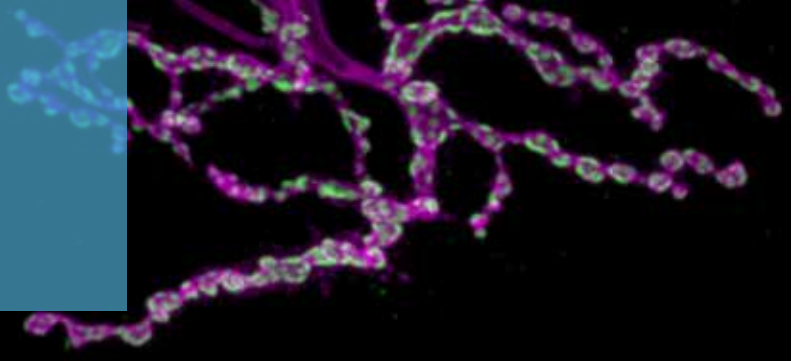
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# Synaptic Plasticity

Head: Dr. Tobias Rasse

Team: 13 members

Key words: drosophila / synapse / axonal transport /  
in vivo imaging



**Our research focuses on regulatory mechanisms controlling neuronal communication. We want to unravel the nature of neuronal communication breakdown that leads to neurodegeneration. Drosophila larvae are used to trace individual neuronal contacts (synapses) to detect structural modifications in them that lead to changes in larva and adult fly behaviour. A time course study of cellular alterations occurring during progression of neurodegeneration provides a valuable insight into the mechanisms underlying biological processes of loss of neuronal connections and cell death.**

*Im Verlauf vieler neurodegenerativen Krankheiten sterben Nervenzellen und Nerven-Zellkontakte werden aufgelöst. Um dies näher zu untersuchen, bedienen wir uns einer selbst entwickelten Methode, die es uns erlaubt, in lebenden Fruchtfliegenlarven die Bedingungen zur Bildung von synaptischen Verbindungen zu untersuchen. Diese Erkenntnisse sollten es uns langfristig ermöglichen, Strategien zu entwickeln, um neurodegenerative Krankheiten ursächlich anzugehen, und zwar vom Ort des Informationsverlustes her: der Synapse.*

## In vivo imaging

Recently we described a microscopy technique that enables the analysis of synapse assembly in intact *Drosophila* larvae. The assay is useful for studying the dynamics of formation of new synapses. Using this technique we were able to prove that the disruption of active zone development is accompanied by abnormal postsynaptic development. While the formation of synapses is a comparatively slow biological process, axonal transport and the delivery of cargo occurs much faster.

Recent modifications of the imaging setup allow for the monitoring of both fast and slow processes. To analyze stability, turnover and redistribution of proteins possibly involved in stabilizing synapses fluorescence recovery after

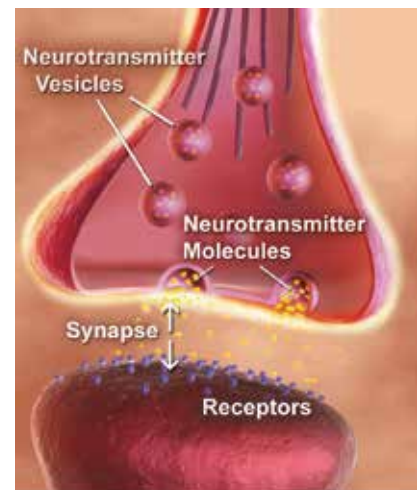
photo-bleaching (FRAP) or photo-activation can be combined with in vivo imaging. Two prerequisites are required for applying this method to study the turnover of any protein of interest. The protein subjected for analysis must be tagged with two different fluorophores, e.g. GFP and mCherry, and has to be expressed in two independent transgenic constructs. Flies carrying GFP constructs are crossed with mCherry carriers to give a progeny expressing two fractions of the protein of interest: the mCherry-tagged protein and the other one labeled with GFP. The mCherry signal can be selectively bleached, while the GFP fluorescence will serve as a reference. This type of bleaching does not affect the function of the protein as it was exemplified previously by examining a GFP-tagged glutamate receptor.

Alternatively, local protein turnover, transport and delivery can be determined directly by tracing a photo-labeled population of proteins. Thereby, photo-labeling can either be achieved by photo-activation, i.e. the conversion of non-fluorescent protein such as photoactivatable-GFP into the activated fluorescent state by illumination with 400 nm laser light, or by photo-conversion, i.e. the switching of a convertible fluorescent molecule (i.e. dendra2). The photo-conversion is preferable since it allows us to trace non-converted and converted protein populations simultaneously. We are currently establishing a new transgenic stock and microscopy assays to measure protein turnover at the NMJ directly.



Abnormal synaptic terminal growth in a neuromuscular junction. Neuronal membranes (magenta) and postsynaptic densities (green) were visualized with antibodies against HRP and the Glutamate receptor subunit IIC.

In normal cells, synapses mediate the communication between neurons. A loss of these neuronal connections is closely linked with neurodegenerative disorders.



### Behavioral Assays

We have a custom-build software Animal tracer coordinates quantification of subtle changes in larval locomotion. It provides a functional readout of synaptic pathology at the neuromuscular synaptic terminal. Using this software, we showed that the motoneuron-specific expression of a mutated protein initially identified in hereditary spastic paraplegia patients is sufficient to impair *Drosophila* larval locomotion. We found that these pathological impairments in locomotion are correlated with the size and age of *Drosophila*, something analogous to observations in human patients.

### High-throughput screening

*Drosophila melanogaster* has been extensively used for genetic screens. Moreover it has also emerged as an efficient whole animal model for high-throughput drug screens. The great advantage of using *Drosophila* in such screens is the possibility of testing and characterizing drugs that can take effects only in the context of a multi-cellular organism to target neurodegenerative disorders.

### Future directions

Synapse formation and maturation critically depends on kinesin-based fast axonal transport. Kinesin motors mediate the anterograde transport of synaptic cargoes along microtubule tracks. The depletion of axonal transport cargoes from synapses

contributes to the pathology associated with both neurodegenerative and neurodevelopmental disorders such as hereditary spastic paraplegia (HSP), Morbus Parkinson (PD), Frontotemporal dementia (FTD), Fragile X Syndrome, Angelman's Syndrome, Autism, Down's Syndrome, Rett's Syndrome and Schizophrenia. Synaptic cargoes that are inadequately transported in neurodegenerative and neurodevelopmental disorder include: mitochondria, RNA, proteins important for stability and dynamics of the cytoskeleton and structural proteins important for the formation of new active zones.

Our laboratory is interested in the mechanisms that regulate intracellular transport. Furthermore we want to understand how synapses are affected in a background compromised for supply of transport cargoes. Our research aims to clarify whether a synaptic "deficit" is caused by direct defects in the molecular motors or by induced disturbances in microtubule tracks.

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