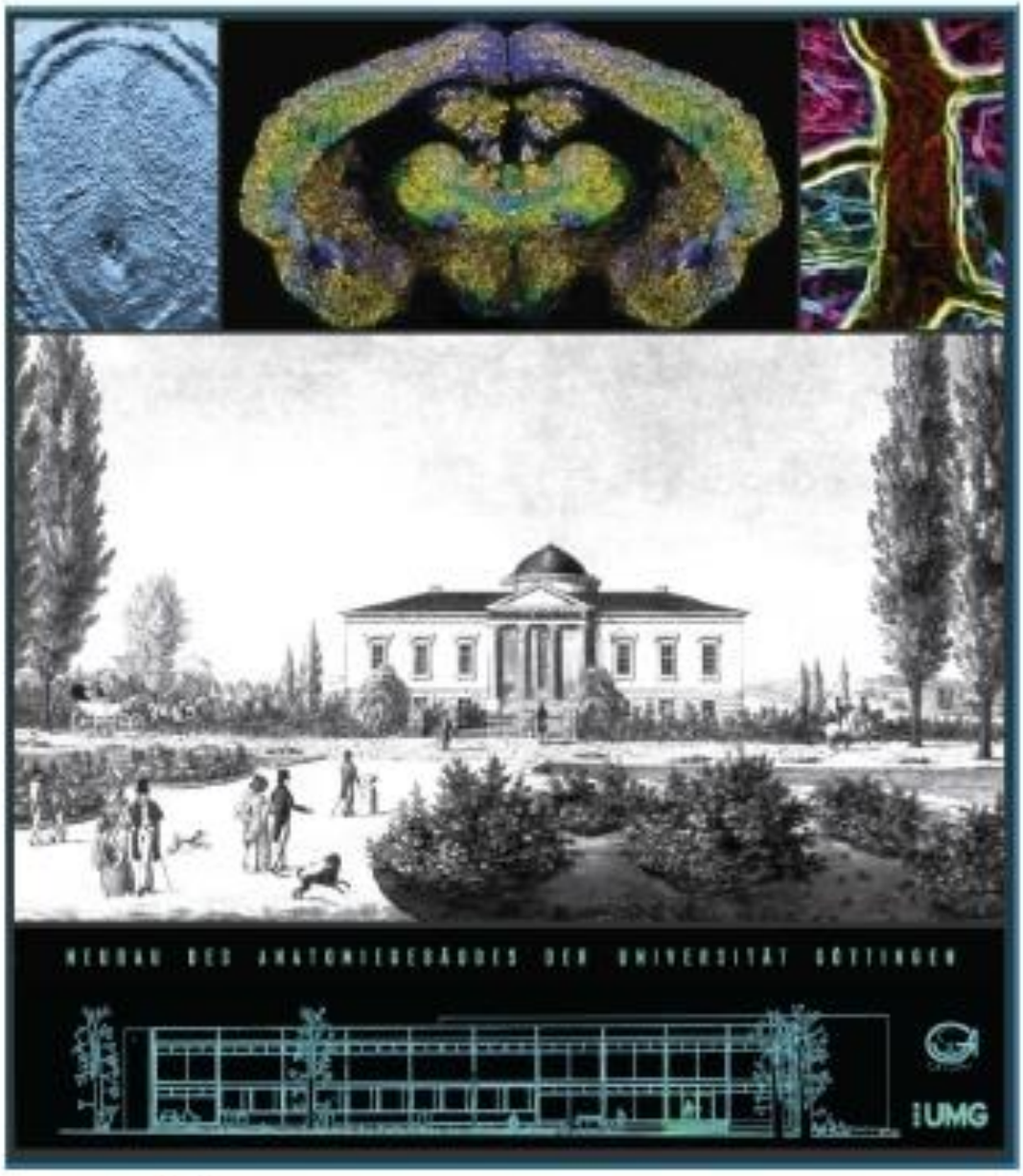
ANATOMISCHE GESELLSCHAFT



111TH ANNUAL MEETING

GÖTTINGEN| 2016, SEPTEMBER 21–24

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Authors (alphabetical order)

Vortrag 1:

Titel: A new concept for vertebrate eye morphogenesis, impacting on optic fissure formation and fusion

Autoren/Adressen: Priska Eckert (University Freiburg), Stephan Heermann (University Freiburg); stephan.heermann@anat.uni-freiburg.de

Abstract:
Optic cup formation is a dynamic process. Two epithelial domains are secondarily integrated into the cup by a bilateral flow over the distal rim. A prematurely arrested flow, induced by targeted expression of BMP4, results in a partially ectopic neuroretina and a coloboma, being a persisting optic fissure. In this project we aim at the relation between the bilateral flow and the development and the consecutive fusion of the optic fissure.

We make use of in vivo time-lapse imaging of zebrafish embryos.

Addressing the optic fissure fusion, we find that the contact in between the fissure margins is established in the upper third of the fissure. Here, the presumptive neuroretina and retinal pigmented epithelium are separated. Investigating the relation between the bilateral flow and optic fissure formation, we find that the flow is occurring also through the fissure, moving the border in between the two epithelia into the correct position. Notably, optic fissure formation is likely is a result of the flow. We propose that, in addition to BMP4, the bilateral flow is facilitated also by other factors and that a pathologically altered flow is resulting in coloboma of an own entity. We started investigating additional factors facilitating the flow and with it optic fissure formation and closure.

Taken together, the bilateral flow is important for optic fissure formation, including the exact localization of the fissure margins, facilitating the onset of fusion. We propose that factors negatively affecting the bilateral flow result in an own entity of coloboma.

Vortrag 2:

Titel: The DMDD project: phenotype of e14.5 mouse embryos derived from 60 knock-out lines that produce embryonically lethal offspring

Autoren/Adressen: Stefan Geyer (Medical University of Vienna), Lukas Reissig (Medical University of Vienna), Julia Rose (Medical University of Vienna), Dorota Szumska (Wellcome Trust Centre for Human Genetics, Oxford), Robert Wilson (The Francis Crick Institute Mill Hill Laboratory), Tim Mohun (The Francis Crick Institute Mill Hill Laboratory), Wolfgang Weninger (Medical University of Vienna); stefan.geyer@meduniwien.ac.at

Abstract:
The project “Deciphering the Mechanisms of developmental disorders” aims at generating and phenotyping prenatally lethal embryos of mouse lines with novel gene knock outs. The project has already produced and published interesting phenotype information on E14.5 embryos of around 60 lines. Examples are to be presented in this presentation.

Embryos were harvested at E14.5 at the Sanger Institute and “High resolution episcopic microscopy” (HREM) data with voxel sizes of 3 x 3 x 3 µm3 were produced from at least 3 homozygous mutants and 1 control embryo per line. The data were analysed on high-end workstations operating specially adapted Osirix software.

We exemplarily show phenotype features and provide analysis on the effect of these features on the developmental progress of the embryos. Gross to subtle defects on tissue level are described, which can be expected to be causal for embryo lethality. Comprehensive phenotype information is made available at www.dmdd.org.uk.

DMDD is an excellent source for searching for knock out lines that could be used as models to research human pathologies and for researching the role genetic factors play during organogenesis.

Vortrag 3:

Titel: Genetic and epigenetic signature of reelin and the cell adhesion molecule l1 (l1cam) in brain development and neuropsychiatric disease

Autoren/Adressen: David Lutz (Universitätsklinikum Hamburg-Eppendorf), Melitta Schachner (Rutgers University), Michael Frotscher (Universitätsklinikum Hamburg-Eppendorf); david.lutz@zmnh.uni-hamburg.de

Abstract:
Migrating neurons form laminated brain structures under the control of Reelin, which activates signaling via binding to canonical lipoprotein receptors. Why Reelin attracts neurons and at the same time arrests their migration to form layers has remained an unresolved question.

We used a variety of molecular in vitro and in vivo approaches, including in utero electroporation, and transgenic mice to study the mechanisms of Reelin action as a ‘go and ‘stop’ signal for migrating neurons as well as to investigate a novel interaction between Reelin and the non-canonical receptor L1CAM during brain development.

We found that a single amino acid in Reelin allows the protein to cleave non-canonical and canonical receptors, promoting or repressing neuronal migration during brain development. Mutation in this amino acid unfolds a fatal phenotype in mice, characterized with abnormal brain architecture, tremor, epileptic seizures, ataxia and lack of motor control. Moreover, Reelin interacts with and cleaves L1CAM thereby generating a proteolytic fragment, which enters the nucleus to regulate gene expression. Mice with a mutation preventing generation of this proteolytic fragment develop hydrocephalus owing to improperly anchored ependymal cilia and display impaired working memory. Genetic profiling of these mutant mice revealed a huge range of affected genes modulating ciliogenesis, cytoskeleton stability and consolidation of long-term memory.

Our study elucidates fundamental novel roles of Reelin in brain organization and establishes the genetic and epigenetic basis of the Reelin substrate L1CAM in the pathogenesis of hydrocephalus and mental retardation.

Vortrag 4:

Titel: There is no mesenchymal-to-epithelial-transition (met) while somatic cell reprogramming to ipscs

Autoren/Adressen: Stefanie Raab (University Tübingen), Moritz Klingenstein (University Tübingen), Alexander Kleger (University Ulm), Stefan Liebau (University Tübingen); stefanie.raab@uni-tuebingen.de

Abstract:
Reprogramming of somatic cells to iPSCs with the overexpression of transcription factors is a well-established method. In the developing embryo Epithelial-to-Mesenchymal-Transition (EMT) occurs. This process describes the travelling of cells from the early epiblast to form the endoderm and mesoderm. This process can also be reversible, then called MET and occurs e.g. in the reprogramming of mesoderm derived fibroblasts to iPSCs.

Human keratinocytes and fibroblasts were transduced with a lentivirus to undergo iPSC formation. RNA samples were taken at different time points and expression analysis using Fluidigm technologies was performed. For the analysis of the protein expression while reprogramming we used Western blot and immunofluorescence techniques. Genes involved in the development of the three germ layers as well as important genes of the pluripotency network were selected.

We see increased expression at day 18 of genes representative for primitive streak and mesendoderm. Keratinocytes show the same expression profile like fibroblasts in the reprograming process. However, on protein level we cannot see this specific RNA expression profile neither on keratinocyte nor on fibroblast reprogramming.

During reprogramming somatic ectodermal cells, here keratinocytes from plucked hair, show similar gene expression profiles like mesodermal cells. Therefore, they transit through a similar state to get pluripotent. We can also show that independent of the reprogramming source, cells need to acquire an MET-like intermediate state which can just be seen on RNA level short before entering the pluripotent state.

Vortrag 5:

Titel: Glioma cells on the run: characterization of fast migrating glioma – “guerilla” – cells

Autoren/Adressen: Vivian Adamski (UKSH, Campus Kiel), Kirsten Hattermann (CAU Kiel), Michael Synowitz (UKSH, Campus Kiel), Janka Held-Feindt (UKSH, Campus Kiel); Vivian.Adamski@uksh.de

Abstract:
The malignant brain tumor glioblastoma multiforme (GBM) is composed of heterogeneous cell populations that are responsible for its incurability. GBMs partly obtain their aggressiveness by tumor subclones that infiltrate into the surrounding neuropil (“guerilla” cells) with the potential to rebuild the tumor. Thus, we characterized “guerilla” cells in solid tumors and investigated the influ-ence of chemotherapeutic treatment on migration.

We isolated fast migrating glioma cells from 19 solid tumors of different malignancy grades (II – IV), and characterized them by qPCR concerning migration related genes and stem cell markers. In addition, we assayed the migratory behavior upon temozolomide treatment in glioblastoma cell lines.

Analysis of gene expression profiles of solid tumors revealed differences not only in “guerilla” cells compared to not-“guerilla” cells, but also in terms of malignancy grades revealing an increased expression of migration related genes in glioblastomas than in astrocytomas and recurrences. These observations were mimicked in the expression analysis of neural stem cell markers. By treatment of glioblastoma cell lines with subletal doses of temozolomide, we observed different responses in stimulated cells concerning their migratory potential resulting in a suppressed, equal or enhanced migration. Beyond that, we identified gene expression patterns comparable to those observed in isolated “guerilla” cells.

The expression of migration related genes in “guerilla” cells is not only regulated in a progression-dependent manner, but these cells are possibly also characterized by stem cell-like features. Hence, especially “guerilla” cells have to be addressed by therapeutic strategies.

Vortrag 6:

Titel: Impact of forced trefoil factor family peptide 3 (tff3) expression on growth, viability, migration and tumorogenicity of human retinoblastoma cell lines

Autoren/Adressen: Maike Busch (University of Duisburg-Essen, Medical Faculty), Jan Große-Kreul (University of Duisburg-Essen, Medical Faculty), Claudia Winter (University of Duisburg-Essen, Medical Faculty), Stefanie Pikos (University of Duisburg-Essen, Medical Faculty), Harald Stephan (University of Duisburg-Essen), Nicole Dünker (University of Duisburg-Essen, Medical Faculty); maike.busch@uk-essen.de

Abstract:
Trefoil factor family (TFF) peptides have been shown to effect cell proliferation, apoptosis, migration and invasion of normal cells and various cancer cell lines. In the literature, TFF peptides are controversially discussed as tumor suppressors and potential tumor progression factors. In the study presented, we investigated the effect of recombinant, transient and stable lentiviral TFF3 overexpression on growth, viability, migration and tumorogenicity of different human retinoblastoma (RB) cell lines.

Effects of TFF3 overexpression on RB cell growth and viability were revealed by WST-1 and TUNEL assays as well as BrdU and DAPI cell counts. A broad spectrum caspase inhibitor and capase-3 immunocytochemistry were used to investigate the involvement of caspases in TFF3 mediated apoptosis induction. Effects of TFF3 overexpression on RB cell tumorogenicity and migration were analysed using in ovo chicken chorioallantoic membrane(CAM)and soft agarose assays.

Recombinant TFF3 significantly lowered retinoblastoma cell viability and increased apoptosis levels. Transient TFF3 overexpression likewise significantly increased apoptosis. Stable, lentiviral TFF3 overexpression lowered RB cell viability, proliferation and growth and significantly increased cell death in RB cells. Blockage experiments and capase-3 immunocytochemistry revealed the involvement of caspase-3 in TFF3 induced apoptosis in RB cells. Besides, TFF3 overexpression influenced anchorage independent growth and migration and significantly decreased the size of tumors forming from RB cells.

Our study demonstrates that forced TFF3 expression exerts a significant pro-apoptotic, anti-proliferative, and tumor suppressive effect in retinoblastoma cells, setting a starting point for new additive chemotherapeutic approaches in the treatment of retinoblastoma.

Vortrag 7:

Titel: Targeting of tail-anchored proteins to different subcellular compartments is controlled by interplay of tail polarity and hydrophobicity of the transmembrane domain.

Autoren/Adressen: Joseph L Costello (University of Exeter), Ines G Castro (University of Exeter), Fatima Camoes (University of Aveiro), Tina Schrader (University of Exeter), Doug McNeall (Hadley Centre), Silvia Gomes (University of Aveiro), Evdokia-Anastasia Giannopoulou (EMBL Hamburg), Vivian Pogenberg (EMBL Hamburg), Nina A. Bonekamp (University of Aveiro), Daniela Ribeiro (University of Aveiro), Matthias Wilmanns (EMBL Hamburg), Michael Schrader (University of Exeter), Markus Islinger (University of Heidelberg); markus.islinger@medma.uni-heidelberg.de

Abstract:
Tail-anchored (TA) proteins are characterized by a single transmembrane domain (TMD) followed by a short C-terminal amino acid sequence. Their functional domains, by contrast, are highly divergent but always exposed to the cytosolic side of individual organelles such as mitochondria, the endoplasmic reticulum and peroxisomes. Since TA proteins are involved in important physiological tasks such as viral defense, organelle interaction and biogenesis it is crucial to understand how these proteins are precisely targeted to individual subcellular compartments in order to ensure their correct function.

Mutational analysis of a set of model TA proteins was used to monitor the influence of the tail-sequence on protein localization

We showed that an increasing positive charge in the C-terminal tail controls targeting to mitochondria and peroxisomes, the latter by interaction with the peroxisomal membrane protein shuttle receptor Pex19. A closer look on protein sequences, however, revealed that the targeting is influenced by an additional factor – the hydrophobicity of the TMD. Subtle alterations in those physicochemical parameters can shift TA protein targeting between organelles and explain why individual organelles can share a subset of TA proteins. Training of a support vector machine classifier using data for TMD hydrophobicity, tail charge and cellular location enabled us to build a statistical model that is able to predict the probability of a protein to be targeted to mitochondria, peroxisomes and the ER.

Experimental verification of the predicted allocation of uncharacterized candidate TA proteins revealed that this classification allows successful prediction of the location of mammalian TA proteins.

Vortrag 8:

Titel: Membrane-binding of lkb1 and its activation by phospholipids is essential for its function in cell cycle control and tumour suppression

Autoren/Adressen: Giada Dogliotti (Universirty of Regensburg), Olga Panichkina (Universirty of Regensburg), Christian Thiele (Universirty of Regensburg), Pratibha Dhumale (University of Münster), Lars Kullmann (University of Regensburg), Gudrun Mendl (University of Regensburg), Roland Houben (University Hospital Würzburg), Sebastian Haferkamp (University Hospital Regensburg), Andreas W. Püschel (University of Münster), Michael P. Krahn (University of Regensburg); dogliotti.giada@gmail.com

Abstract:
Mutations in the human Liver kinase B1 gene are the cause of the Peutz-Jeghers syndrome, an autosomal dominant disorder. The serine/threonine kinase LKB1 regulates various cellular processes such as cell proliferation, energy homeostasis and cell polarity and is frequently downregulated in various tumours and little is known about the upstream regulatory mechanisms that activate LKB1.

In Vitro and In vivo assays performed in mammalian cell culture and Drosophila M. were performed in our study.

Here we show that targeting of the kinase to the membrane by a direct binding of LKB1 to phosphatidic acid is essential to fully activate its kinase activity. Consequently, LKB1 mutants that are deficient of membrane binding are not capable to fully activate the downstream target AMPK in mammalian cells to control mTOR-signaling and to protect cells from apoptosis under energetic stress. Strikingly, we found LKB1 to be downregulated in malignant melanoma which exhibit aberrant activation of Akt and overexpress Phospholipase D, thus increasing cellular levels of phosphatidic acid.

These results provide evidence for a new mechanism of LKB1 activation and its implication in vivo and during carcinogenesis. We identified a new crosstalk between PLD-signaling and the LKB1-AMPK pathway which converge in balanced mTOR activation and thus control cell proliferation and cell growth. We identified a new mechanism of LKB1 controlling replication by H2B phosphorylation which recruits MCM proteins to stabilize origins of replications. In Downregulation or inactivation of LKB1 results in defective replication and impaired DNA repair mechanism.

Vortrag 9:

Titel: The transcription factor dach1 is essential for podocyte differentiation and function

Autoren/Adressen: Nicole Endlich (University Medicine Greifswald), Antje Blumenthal (University Medicine Greifswald), Katharina Schmidt (University Medicine Greifswald), Frances Kindt (University Medicine Greifswald), Pierre-Louis Tharaux (Paris Cardiovascular Centre), Nadine Artelt (University Medicine Greifswald), Maja Lindenmeyer (University of Munich), Clemens D. Cohen (University of Munich), Franziska Döring (University Medicine Greifswald), Regina Maciejewski (University Medicine Greifswald), Andreas W. Kuss (University Medicine Greifswald), Kerstin Amann (University Hospital Erlangen), Nazanin Kabgani (RWTH Aachen University Hospital), Marcus J. Moeller (RWTH Aachen University Hospital), Felix Kliewe (University Medicine Greifswald), Karlhans Endlich (University Medicine Greifswald); antje.blumenthal@uni-greifswald.de

Abstract:
Chronic kidney diseases are often caused by dedifferentiation of podocytes. Genome wide association studies have shown that a SNP in the cell fate determination factor Dach1 locus is associated with the glomerular filtration rate. Therefore, we have investigated the role of Dach1 for the differentiation of parietal epithelial cells (PEC) into podocyte-like cells.

PECs were transfected with a plasmid encoding for Dach1-GFP and were analyzed by immunocytochemistry, RT-PCR and Western blot. The expression of Dach1 and synaptopodin were studied in kidneys of mice and humans by immunohistochemistry. Knockdown of the zebrafish ortholog dachd was generated by morpholinos.

Podocytes express high levels of Dach1 in mice and humans in vivo and to lower extent in vitro. PECs express Dach1 only at very low levels. Transfection of PECs with a plasmid encoding for Dach1 induced the expression of synaptopodin, a podocyte-specific protein. Furthermore, we found that synaptopodin is located along actin fibers in punctate patterns in Dach1-expressing PECs comparable with differentiated podocytes. Moreover, dedifferentiating podocytes of isolated glomeruli showed a significant reduction of Dach1 expression together with synaptopodin after 9 days. The knockdown in zebrafish larvae resulted in morphological changes of the glomerulus accompanied by down-regulation of the nephrin expression and a leakage of the filtration barrier.
Further, Dach1 and synaptopodin were significantly reduced in isolated glomeruli from NTS-treated mice and in biopsies from patients suffering from diabetic nephropathy in contrast to biopsies from healthy individuals.

Taken together, Dach1 is a transcription factor that is essential for podocyte differentiation and proper glomerular function.

Vortrag 10:

Titel: Cytoskeletal properties as a marker for invasiveness in glioblastoma?

Autoren/Adressen: Tim Hohmann (Martin Luther Universität Halle Wittenberg), Kerstin Feese (Martin Luther Universität Halle Wittenberg), Urszula Grabiec (Martin Luther Universität Halle Wittenberg), Chalid Ghadban (Martin Luther Universität Halle Wittenberg), Faramarz Dehghani (Martin Luther Universität Halle Wittenberg); tim.hohmann@medizin.uni-halle.de

Abstract:
Cannabinoids are known to have an anti-tumorous effect, but the underlying mechanisms are only sparsely understood. Mechanical characteristics of tumor cells represent a promising marker to distinguish between tumor cells and the healthy tissue. We tested the hypothesis whether cannabinoids influence the cytoskeletal organization of tumor cells in the form of cell specific mechanical properties and if these factors are a prognostic marker for the invasiveness of tumor cells

3 different glioblastoma cell lines were treated with cannabinoids and to address alterations of cytoskeletal properties changes of mechanical properties of single cells and unspecific, short term adhesions were measured using atomic force microscopy. The invasiveness of cell lines was determined using a co-culture model with organotypic hippocampal slice cultures.

One key finding was that cannabinoids were able to reduce the short term adhesion energies for all cell types, correlating with the activation of focal adhesion kinases. We also found that cannabinoids are capable of influencing mechanical properties in a cell line specific manner. A network analysis revealed a correlation between a “generalized stiffness” and the invasiveness for all tumor cell lines and tumor invasion time points.

Consequently we could show that a “generalized stiffness” is a profound marker for the invasiveness of a tumor cell population in our model and thus might be of high clinical relevance for drug testing.

Vortrag 11:

Titel: Super resolution microscopy of peroxisomes

Autoren/Adressen: Srikanth Karnati (Justus Liebig University), Kathrin M Scherer (University College London; London), Adrian Pilatz (Justus Liebig University), Eveline Baumgart-Vogt (Justus Liebig University); srikanth.karnati@anatomie.med.uni-giessen.de

Abstract:
Introduction: Peroxisomes are the dynamic and essential organelles of the eukaryotic cells. Peroxisomes adjust their size, length and shape according to the necessity and metabolic status of the cell. Peroxisomal biogenesis disorders are caused by the defects in the peroxisomal matrix protein import, facilitated by peroxisome biogenesis proteins 13 and 14 (PEX13p and PEX14p) forming a docking complex on the peroxisomal membrane. So far, no structural information and distribution patterns are available on these membrane proteins of a peroxisome, which are challenging objects for microscopy. Moreover, the diameter of peroxisomes is close to the spatial resolution limit of conventional light microscopy, hence super resolution microscopy for imaging subperoxisomal protein distribution is necessary. Aims: 1) To quantify the number of PEX13p and PEX14 proteins on the peroxisomal membrane, and 2) whether length, size and shape of the peroxisomes correlate the structural distribution patterns of PEX13p and Pex14p?.

Innovation: This is the first report of structural organization and distribution of PEX13p and PEX14p on the membrane of lung peroxisomes using a combination of laser-scanning confocal microscopy (CLSM), super-resolution Structured Illumination Microscopy (SIM), and STimulated Emission Depletion microscopy (STED) to obtain a better insight into the structure of peroxisomes in C22 and T7 cells.

Deconvolved CLSM, STED and SR-SIM images were analyzed using a segmentation workflow using the Icy Bioimage software.

Conclusion: We achieved up to ~ 60 nm resolution in the focal plane and our findings suggest that PEX13p and PEX14p are unevenly distributed with different labeling intensities on the peroxisomal membrane.

Vortrag 12:

Titel: Caveolae promote urinary concentration via vascular and epithelial mechanisms

Autoren/Adressen: Yan Willière (Charité-Universitätsmedizin Berlin), Aljona Borschewski (Charité-Universitätsmedizin Berlin), Andreas Patzak (Charité-Universitätsmedizin Berlin), Tatiana Nikitina (Charité-Universitätsmedizin Berlin), Mauricio Sendeski (Charité-Universitätsmedizin Berlin), Carsten Dittmayer (Charité-Universitätsmedizin Berlin), Sebastian Bachmann (Charité-Universitätsmedizin Berlin), Kerim Mutig (Charité-Universitätsmedizin Berlin); kerim.mutig@charite.de

Abstract:
Caveolin-1 (Cav1) is essential for caveolae biogenesis. These cholesterol-rich membrane microdomains are involved in signal-transduction, vesicular trafficking, and functional modulation of plasma membrane proteins. Little is known about the role of the caveolae in the kidney. In this study we tested the hypothesis that caveolae interfere with renal NaCl- and water reabsorption thus affecting urinary concentration.

Cav1-deficient (Cav1-/-) mice were analyzed for their physiological kidney performance, kidney morphology, expression and activity of renal salt and water transport proteins, and renal vascular contractility.

In wildtype (WT) mouse kidneys, Cav1 was strongly expressed in vascular smooth muscle and endothelial cells and moderately expressed in epithelial cells of the late distal convoluted tubule (DCT2) and collecting duct. Cav1 expression and caveolae were not detectable in Cav1-/- mice. Physiological evaluation of WT and Cav1-/- mice revealed twofold higher urinary excretion of water and sodium upon Cav1 disruption. Immunoblotting showed reduced abundance of phosphorylated Na-Cl cotransporter in Cav1-deficient kidneys suggesting impaired salt reabsorption along the DCT2 upon Cav1 disruption. Functional analysis of renal interlobar arteries showed significantly decreased contractile response of Cav1-deficient vessels to phenylephrine. Concomitant inhibition of NO production by L-NAME abolished the differences between WT and Cav1-/- indicating that Cav1 disruption may stimulate NO synthases. Indeed, abundance of the endothelial NO synthase (eNOS) was increased by twofold in Cav1-/- kidneys compared to WT controls.

Our data suggest that Cav1 promotes urinary concentration via suppression of vascular NO production and stimulation of salt reabsorption along DCT2.

Vortrag 13:

Titel: Donor photoreceptor cell behaviour post transplantation in the mouse eye

Autoren/Adressen: Jasmin Balmer (Universität Oxford), Alun Barnard (Universität Oxford), Mandeep Singh (Johns Hopkins University Baltimore), Daniela Moralli (University of Oxford), Catherine Green (University of Oxford), Robert MacLaren (Universität Oxford); Jasmin.Balmer@vetsuisse.unibe.ch

Abstract:
Photoreceptor transplantation shows great promise for the treatment of blindness resulting from retinal degenerative diseases. However, if residual photoreceptors persist in the recipient, the question whether transplanted donor cells integrate in the remaining host photoreceptor layer has not been investigated yet. Another possible mechanism could be a transient fusion between transplanted donor cells and host photoreceptors, enabling an exchange of cytoplasmic content (green fluorescent protein (GFP)).

1. GFP+ photoreceptor precursors were transplanted into the subretinal space of adult host mice ubiquitously expressing DsRed. Two weeks post transplantation, we investigated whether those GFP+ cells were double positive for DsRed (host colour) and GFP (donor colour).
2. In a sex-mismatched experiment, GFP photoreceptors precursors from female pups were injected subretinally into eyes of male wild type recipient mice. Using FISH analysis, sex chromosomes were detected in the GFP positive cells two weeks post transplantation.

1. Two weeks post transplantation, 94 ± 4.1% of morphologically normal GFP+ cells in the host photoreceptor layer also co-localized DsRed.

2. In the sex-mismatched experiment using X- and Y-specific probes, subretinally transplanted female donor cells were carrying two X chromosomes. On the other hand, cytoplasmic GFP and Y-positive nuclei were found within the host outer nuclear layer. Thus, photoreceptor cells in the male host contain donor-derived GFP.

GFP+ cells in the host photoreceptor layer were not integrated donor cells but host cells that obtained GFP from the donors. The most likely mechanism of intercellular exchange might be cell fusion, which could be utilized as future therapeutic strategy.

Vortrag 14:

Titel: Reduced brain volume and astrocyte density in activity-based anorexia rats

Autoren/Adressen: Linda Frintrop (RWTH Aachen University), Johanna Liesbrock (RWTH Aachen University), Lisa Paulukat (RWTH Aachen University), Sonja Johann (RWTH Aachen University), Martien J. Kas (University Medical Center Utrecht), Rene Tolba (RWTH Aachen University), Nicole Heussen (Department of Medical Statistics), Joseph Neulen (Department of Gynecological Endocrinology and Reproductive Medicine), Kerstin Konrad (Department of Child and Adolescent Psychiatry, Psychosomatics and Psychotherapy), Beate Herpertz-Dahlmann (RWTH Aachen University), Cordian Beyer (RWTH Aachen University), Jochen Seitz (RWTH Aachen University); lfrintrop@ukaachen.de

Abstract:
Severe gray and white matter volume reductions were found in patients with anorexia nervosa (AN) that were linked to neuropsychological deficits and a poor clinical prognosis. However, their origin and underlying pathophysiology remain unclear. For the first time, we systematically analyzed brain volume changes and their cellular basis in an activity-based anorexia (ABA) model.

Adolescent female Wistar rats had 24 h/day running wheel access and received 40% of their baseline daily food intake until a 25% weight reduction was reached. The reduced body weight was maintained for two weeks. The volumes of the cerebral cortex and corpus callosum were determined. The number and total area of neurons and astrocytes and corresponding mRNA expression of cellular markers were analyzed and compared with normally fed controls.

In ABA rats, the volumes of the cerebral cortex and corpus callosum were significantly reduced compared to controls. Notably, the number and immunoreactive area of GFAP-positive astrocytes in the cerebral cortex and corpus callosum of ABA animals decreased, whereas no changes were observed in neurons. These findings were complemented by at least a 50% reduction in GFAP mRNA expression in both brain areas.

Volumetric brain alterations in ABA animals mirror those in human AN patients. Thus, a decisive underlying cellular mechanism appears to lower the cell count of GFAP-positive cells. Future studies should clarify the functional consequences of these astrocyte alterations. The role of astrocytes in AN could constitute a new target for research on the underlying pathophysiology and potential treatment strategies.

Vortrag 15:

Titel: Ultrastructural aspects of thalamus damages in a mouse model of osmotic demyelination syndrome: demyelination associated with oligodendrocyte injuries.

Autoren/Adressen: Jacques Gilloteaux (Universite de Namur), Joanna Bouchat (Laboratory Neurodegeneration and Regeneration, URPhyM - NARILIS), Bruno Couturier (Research Unit for the Study of Hydromineral Metabolism, General Internal Medicine, Erasme Hospital, Faculty of Medicine, Université Libre de Bruxelles, Bruxelles and Laboratory of Histology, Histopathology and Neuroanatomy, Faculty of Medicine, University); jacques.gilloteaux@unamur.be

Abstract:
Disproportionate correction of a chronic hyponatremia can be accompanied clinically by osmotic demyelination syndrome (ODS) as a non-inflammatory disorder of the CNS. The physiopathology remains unclear while hypothetical mechanisms include some sort of blood-borne, myelinotoxic factors caused by focal, osmotic fluctuations in both white and mixed-rich grey matter-regions.

A murine model of ODS was generated to investigate the progress of cerebral defects.

After 12-, 24- and 48-hours post-osmotic corrections, spatial and temporal changes of several molecular markers linked with neuroglial damages associate the demyelinating lesions along with other functional defects were found in the thalamus, mesencephalon, pons and subcortical regions. The lesions were associated with a significant decrease of APC+ and Cx47+ oligodendrocytes, loss of astrocyte markers (ALDH1L1, AQP4, S100β) along with blood-brain barrier disruption. Preliminary electron microscope investigations of the thalamic lesions revealed that amongst the neuroglial cells, oligodendrocytes displayed higher contrast than other cell types and worse injures than those noted of other cell type due to nuclear degradations, vacuoles, swelling of organelles and accumulation of lysosomes. The defectuous myelin displayed highest contrast, inter-lamellar splits and foamy-like damages in which neurite appeared intact. Astrocyte and endothelial cells surprisingly showed minimal or no evident damage contrarily to what was reported in another rodent model of ODS.

In conclusion, this ODS murine model is able to reproduce demyelinating lesions observed in human neuropathology and raises new questions about the early role played by astrocytes in demyelination.

Vortrag 16:

Titel: The chondroitin sulfate code hypothesis for signaling in the neural stem cell niche

Autoren/Adressen: Denise Harrach (Heidelberg University), Alexander von Holst (JGU Mainz); vonholst@uni-mainz.de

Abstract:
Chondroitin sulfate proteoglycans and their specific sulfation pattern by chondroitinsulfotransferases (Chsts) appear to play a crucial role for the behaviour of neural stem cells (NSCs) in the embryonic neural stem cell niche during mouse forebrain development. Here, we wanted to test the chondroitin sulfate code hypothesis by overexpression of one specific Chst UST in cortical neural stem cells.

We performed in utero electroporation experiments in E14.5 embryos to analyse the impact of a modified sulfation pattern by Ust overexpression on cortical neural stem cell fate in vivo. For this purpose we analyzed the proliferation and cell cycle progression of radial glia cells after EdU injections. Also, the number of newborn neurons and glia cells was determined.

At E16.5, two days after Ust overexpression, we observed a change in the thickness of the cortical plate (CP), but no obvious morphological alterations of the ventricular zone (VZ). Ust overexpression increased the ratio of precursor cells to neurons without increased proliferation rates suggesting an effect on cell cycle length and/or progression.

Our findings are in line with the interpretation of an enhanced FGF-signalling due to an efficient and functional Ust overexpression that leads to a defined modification of the sulfation pattern of defined chondroitin sulfate units in the neural stem cell niche of the developing cortex.

Vortrag 17:

Titel: Neurodegeneration in the anterior thalamus of SOD1(G93A) ALS mice

Autoren/Adressen: Sonja Johann (Institute of Neuroanatomy, RWTH Aachen), Berthold Debye (Institute of Neuroanatomy, RWTH Aachen), Cordian Beyer (Institute of Neuroanatomy, RWTH Aachen); sjohann@ukaachen.de

Abstract:
Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder and the most common motor neuron disease in adults. To date, ALS is considered a multisystem disorder, characterized by degeneration of motor neurons as well as neuropathological changes in non-motor regions. Therefore we aimed to address whether the thalamus region is affected in the widely used SOD1(G93A) mouse model for ALS.

In the present study, we investigated neurodegenerative changes including neuronal loss and glia pathology in the anterodorsal thalamic nucleus (AD) of SOD1(G93A) mice. To monitor developing AD pathology, we have analyzed pre-symptomatic and early symptomatic mice. Since activation of inflammasomes seem to play a crucial role in ALS, we examined protein expression of Nod-like receptor protein 3 (NLRP3), apoptosis-associated speck-like protein containing a caspase-1 recruitment domain (ASC) and interleukin 1 beta (IL1 beta in AD glial cells and neurons.

We detected a profound vacuolation of the neuropil, strong accumulation of mutant hSOD1 and neuronal loss in the AD. Neurodegeneration was accompanied by mild gliosis with unchanged oligodendrocyte cell numbers NLRP3 and ASC were significantly up-regulated in the AD of SOD1(G93A) mice. Finally, co-localization studies revealed elevated expression of NLRP3, ASC and IL1 beta in neurons.

Our study yielded two main findings: i) neurodegenerative changes in the AD occur early in disease progression and ii) NLRP3 activation may contribute to neuronal cell death. In conclusion, the SOD1(G93A) model may represent a model to study non-motor related thalamus pathology in ALS.

Vortrag 18:

Titel: In vitro generation of olfactory receptor neurons (orns)

Autoren/Adressen: Moritz Klingenstein (University Tübingen), Stefanie Raab (University Tübingen), Stefan Liebau (University Tübingen); moritz.klingenstein@uni-tuebingen.de

Abstract:
The inner cell mass of embryos can be isolated as embryonic stem cells (ESCs) and have the potential to proliferate and to differentiate into all three germ layers. The overexpression of specific factors leads to a reprogramming to induced pluripotent stem cells (iPSCs) which are like the ESCs pluripotent. With the iPSC model it is not only possible to create patient specific cell lines to investigate diseases, it is also feasible to differentiate specific cell types which are not easily to be obtained like olfactory receptor neurons (ORNs).

For reprogramming we use keratinocytes from plucked human hair as somatic cell source. This method is a well-established non-invasive possibility to gain cell samples for reprogramming. After approximately four weeks after infection with a lentivirus, iPSC colonies can be characterized for pluripotency. Our new established protocol for ORN differentiation includes several adherent and suspension steps with addition of various factors at different time points, mimicking the development of the ORNs. The ORNs were characterized via diverse methods like qRT-PCR and immunofluorescence for specific markers like OMP and functional assays like odorant depending calcium-imaging.

We could show an increased number of ORNs with our protocol via qPCR and immunofluorescence staining and we could also show functional responses of the ORNs to odorants.

We reprogrammed keratinocytes from plucked human hair to iPSCs and differentiated those to a neuronal culture with an increased amount of ORNs and proved their function with calcium responses after odorant stimulation.

Vortrag 19:

Titel: Pathologic alterations in the sensory system of the wobbler mouse, an animal model for the sporadic form of amyotrophic lateral sclerosis.

Autoren/Adressen: Bastian Ott (Ruhr-University Bochum), Carolin Dahlke (Ruhr-University Bochum), Darius Saberi (Ruhr-University Bochum), Beate Brand-Saberi (Ruhr-University Bochum), Thomas Schmitt-John (University of Aarhus), Carsten Theiss (Ruhr-University Bochum); ott.bastian@gmx.de

Abstract:
Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease that is well described to particularly affect α-motor neurons. Different animal models are well established to investigate the underlying morphological changes of the main three forms; the most relevant form sporadic ALS (sALS) (app. 90%), the familial ALS (fALS) with 5-10% and the very rare juvenile ALS (jALS). The wobbler mouse is the approved model for sALS. Symptoms of ALS are rapid progressive weakness, muscle atrophy, dysarthria, dysphagia and dyspnea, but normally no sensory failures. Since there are many different variations described for human ALS, including studies that revealed pathological alterations in the sensory system of some patients, we set our focus on the sensory system of the wobbler mouse.

We screened dorsal root ganglia (DRG) and peripheral nerves with immunohistochemistry analyzed with laserscanning microscopy. For ultrastructural analysis we performed high resolution electron microscopy and deep-etch freeze-fracture replica.

In our study we demonstrate similar neurodegenerative effects taking place in neurons of wobbler mouse DRG as well as in motor neruons. A highly impaired distribution of neurofilaments and especially an accumulation of heavy phosphorylated neurofilaments (pNfH) in DRG of wobbler mouse were observed.

Sensory neurons must be taken into account to understand all mechanisms leading to ALS despite the missing symptoms of patients. As neurofilament disorders are a common sign of neurodegeneration, here the accumulation of pNfH in DRG is a promissing link to studies promoting high levels of pNfH in the cerebrospinal fluid as an early hallmark for ALS in humans.

Vortrag 20:

Titel: Neuronal protection of ethyl pyruvate in excitotoxicity is microglia independent

Autoren/Adressen: Philipp Pieroh (Martin Luther University Halle-Wittenberg), Daniel-Christoph Wagner (Fraunhofer Institute for Cell Therapy and Immunology), Urszula Grabiec (Martin Luther University Halle-Wittenberg), Johannes Boltze (Fraunhofer Institute for Cell Therapy and Immunology), Angela Ehrlich (University of Leipzig), Chalid Ghadban (Martin Luther University Halle-Wittenberg), Constance Hobusch (University of Leipzig), Gerd Birkenmeier (University of Leipzig), Faramarz Dehghani (Martin Luther University Halle-Wittenberg); Philipp.Pieroh@medizin.uni-halle.de

Abstract:
Although Ethyl Pyruvate (EP) presented robust neuroprotective effects due to it´s antiinflammatory and antioxidative properties the distinct cellular and molecular targets for these effects are not revealed. In addition, the required concentration within the brain to evoke EP neuroprotection is still not determined.

Organotypic hippocampal slice cultures were left unlesioned or excitotoxically lesioned with 50 µM N-methyl-D-aspartate (NMDA, for 4 hours). The role of microglia was examined in an additional OHSC set treated with clodronate from day of preparation until excitotoxic insult. Thereafter, culture medium containing EP (0.84µM, 8.4µM, 42µM, 84µM, 168µM) was added until fixation. OHSC were stained with propidium iodide for degenerating neurons and with Isolectin IB4-FITC for microglia. Quantitative analyses were performed using confocal laser scanning microscopy. The effects of ethyl pyruvate (EP; 0.84µM, 8.4µM, 84µM) on astrocytes (0 hours to 48 hours) were investigated in the scratch-wound model.

EP ameliorated significantly neuronal survival following excitotoxicity (0.84µM, 8.4µM, 42µM, 84µM). Except for 84µM the microglia content was not alternated. The neuroprotective effects were not completely annulled by microglia depletion. EP abolished gap closure at 48h in the scratch wound model without affecting previous analysed time points.

EP mediated neuroprotection in excitotoxicitiy seems microglia independent. The impairment of wound healing suggests an astrocytic involvement. Furthermore, the necessary concentration of EP ameliorating neuronal survival is lower than previously described.

Vortrag 21:

Titel: Cajal-Retzius cells in the developing and mature hippocampus

Autoren/Adressen: Max Anstötz (University Medical Center Hamburg-Eppendorf), Sun Kyong Lee (Northwestern University, Feinberg School of Medicine), Tamra Neblett (Northwestern University, Feinberg School of Medicine), Gianmaria Maccaferri (Northwestern University, Feinberg School of Medicine), Gabriele Rune (University Medical Center Hamburg-Eppendorf); m.anstoetz@uke.de

Abstract:
Cajal-Retzius Cells (CR cells) are early-born neurons of the mammalian brain, which play essential roles during cortical development. It is commonly believed that they are transient cells, and that the numbers of CR cells progressively decrease after birth because of developmentally-regulated programmed cell death.

In order to study the ontogeny of CR cells of the hippocampal formation, we took advantage of three reporter mouse lines (CXCR4-EGFP, Wnt3a-tdTomato, PDE1c-tdTomato) that allow for an unambiguous identification of these neurons.

Unexpectedly, our data reveal a lifelong persistence of CR cells in the hippocampus, while neocortical CR cells seem to vanish soon and are hardly detectable after the first two postnatal weeks. Moreover, mice held in an enriched environment, which was shown to stimulate the hippocampal network, display a significant increase in CR cell numbers presumably due to enhanced survivability. Our results obtained from patch-clamp recordings and whole-cell reconstructions further suggest a hippocampal-specific intense connectivity, which is maintained during adulthood. Finally, optogenetic stimulation of CR cells reveals various types of as yet unknown target neurons.

In conclusion, our results indicate that CR cells remain plastically integrated in the mature hippocampal circuit and suggest that they play additional, non developmental, roles that may be regulated by specific types of environmental experience.

Vortrag 22:

Titel: Signature of neurodegeneration in an animal model of multiple sclerosis

Autoren/Adressen: Tanja Hochstrasser (Ludwig-Maximilians-University of Munich), Robert Haag (Ludwig-Maximilians-University of Munich), Caroline Roggenkamp (Ludwig-Maximilians-University of Munich), Franziska Kramer (Ludwig-Maximilians-University of Munich), Markus Kipp (Ludwig-Maximilians-University of Munich); tanja.hochstrasser@med.uni-muenchen.de

Abstract:
There is a broad consensus that Multiple sclerosis (MS) represents more than an inflammatory disease: it harbors several characteristic aspects of a classical neurodegenerative disorder, i.e. damage to axons, synapses and nerve cell bodies. While several treatment options exist to dampen inflammatory-driven tissue damage, modalities to prevent neurodegeneration are still in their infancy. A better understanding of the neurodegenerative signature in MS patients and respective animal models is therefore urgently needed.

Three distinct gold-standard methods to investigate the magnitude and morphological appearance of neurodegeneration in the cuprizone model were used. Loss of neuronal cell bodies was addressed by design-based stereology. Dendritic trees, spines and synapses were reconstructed in a three-dimensional (3D) environment using Neurolucida 360 to obtain reliable data about the size, shape, and complexity of neurons. Serial block-face scanning electron microscopy was used to detect different morphological features of axonal damage.

First analyses show that neuronal elements are subjected to degenerative processes in both, the grey and white matter. 3D-reconstruction of callosal fibers on the ultrastructural level reveals different types of axonal injury among (1) focal swellings of axon with intact myelin sheaths, (2) splitting of the innermost myelin lamella from the axon with focal swelling, or (3) complete axonal transection. Furthermore, morphological changes of synapses were detected in the cortex.

This study shows the complex nature of neurodegeneration in a progressive, non-immune cell driven, MS animal model. A better understanding of how such morphological changes occur will pave the way for the development of novel neuroprotective strategies.

Vortrag 23:

Titel: Distinct molecular requirements of synaptic vesicle and large dense-core vesicle docking

Autoren/Adressen: Cordelia Imig (Max Planck Institute of Experimental Medicine), Kwun-nok M. Man (Max Planck Institute of Experimental Medicine), JeongSeop Rhee (Max Planck Institute of Experimental Medicine), Nils Brose (Max Planck Institute of Experimental Medicine), Sonja M. Wojcik (Max Planck Institute of Experimental Medicine), Benjamin H. Cooper (Max Planck Institute of Experimental Medicine); imig@em.mpg.de

Abstract:
Secretory vesicle docking, priming, and fusion are orchestrated by a complex molecular machinery. The accurate assessment of vesicle docking requires electron microscopy to resolve intermembrane distances in the nm range, but information on proteins involved in this process has been partly inconclusive. Important reasons for experimental inconsistencies are that the assessment of vesicle docking is technically challenging and that diverse preparations, cell types, sample fixation methods, imaging approaches, and docking definitions have been employed.

To study the mechanisms of synaptic vesicle (SV) and large dense-core vesicle (LDCV) docking in a comparable experimental setting, with high precision, and in a near-native state, we analyzed hippocampal organotypic slice cultures and acute adrenal gland slices from mice lacking key proteins of the fusion machinery, using rapid cryofixation, and three-dimensional electron tomography.

We dissected previously indistinguishable, sequential steps in vesicle recruitment (tethering) and membrane attachment (docking) and found that SV docking requires Munc13/CAPS family priming proteins and all three neuronal SNAREs. However, in contrast to synapses, where most, if not all docked SVs are part of the readily releasable pool (RRP), our data show that the majority of docked LDCVs in chromaffin cells are not fusion competent and that the functional RRP cannot be distinguished from other docked LDCVs by current ultrastructural methods.

Our results therefore indicate that the molecular requirements of SV and LDCV docking are distinct.

Vortrag 24:

Titel: Short- and long-term plasticity of the axon initial segment in the mouse barrel cortex

Autoren/Adressen: Nora Jamann (Medical Faculty Mannheim, Heidelberg University), Corinna Corcelli (Medical Faculty Mannheim, Heidelberg University), Robin Wagener (University of Geneva), Jochen Staiger (University Medical Center,Georg-August-University Göttingen), Christian Schultz (Medical Faculty Mannheim, Heidelberg University), Maren Engelhardt (Medical Faculty Mannheim, Heidelberg University); Jamann@stud.uni-heidelberg.de

Abstract:
The axon initial segment (AIS), the site for action potential initiation, can undergo significant structural plasticity to regulate neuronal excitability. It elongates after sensory deprivation and shortens after elevated activity, indicating a homeostatic function. We previously showed that during development of the visual cortex, after onset of sensory input (eye opening), AIS shortening is observed. However, it is unknown if this experience-dependent plasticity is a common principle in other sensory cortices. We therefore investigated AIS plasticity during development of the mouse barrel cortex.

Using immunofluorescence and confocal analysis, we quantified changes in AIS length from E20.5 to P180. Furthermore, we performed deprivation experiments via daily bilateral whisker trimming, followed by periods of whisker regrowth for rescue conditions to determine if sensory activity is a driving factor for AIS plasticity.

Under normal conditions, AIS increase in length in cortical layers II/III and V until P15. After establishment of intracortical connections and onset of active whisking, AIS lengths rapidly decrease to reach mature levels in adult mice. Deprivation from P0-P15 causes an increase in AIS length at P15 and substantially diminishes shortening especially in layer II/III. We observed a rescue effect after restoration of sensory input until P45. To investigate whether AIS plasticity can also occur more rapidly, we exposed mice to enriched environments for 3 hours. Strikingly, a significant AIS shortening after this short period of increased activity was observed.

In summary, our findings indicate that AIS plasticity regulates excitability as a response to short- and long-term changes of sensory information.

Vortrag 25:

Titel: Extracellular matrix ensures temporally precise high frequency synaptic transmission

Autoren/Adressen: Christoph Körber (Heidelberg University), Denise Harrach (Heidelberg University), Thomas Kuner (Heidelberg University); koerber@ana.uni-heidelberg.de

Abstract:
In the mammalian brain, a small fraction of the neurons is surrounded by a special form of extracellular matrix, the so called perineuronal nets (PNNs). PNNs are a complex meshwork mainly composed of hyaluronan as a basic element, tenascin-R and chondroitin sulfate proteoglycans (CSPGs) that bind to hyaluronan and consist of glycosaminoglycan side chains (GAGs) bound to a core protein. Most of the neurons described so far that bear PNNs were also found to express parvalbumin and the Kv3.1 potassium channel subunit, suggesting they are fast-spiking interneurons. However, the physiological function of PNNs in synaptic transmission remains elusive.

In the medial nucleus of the trapezoid body (MNTB) of the auditory brainstem, all principal neurons are surrounded by PNNs. MNTB principal neurons receive their main excitatory input from a single calyx of Held synapse, a giant axo-somatic synapse that has evolved as a model system for synaptic transmission in recent years. We removed PNNs from MNTB neurons by chon-droitinase (ChABC) treatment and examined the effects on synaptic transmission.

ChABC treatment led to faster synaptic short-term depression (STD). However, this effect was prevented by addition of cyclothiazide and kynurenic acid to the extracellular solution. These results are suggestive of a role for PNNs in the effective clearing of glutamate from the synaptic cleft in order to prevent postsynaptic glutamate receptors from desensitization and/or saturation.

We thus propose that PNNs are necessary for ensuring fast glutamate clearance during high frequency firing thereby maintaining high fidelity signal transmission.

Vortrag 26:

Titel: Neuroligins and bdnf: trassynaptic teamwork

Autoren/Adressen: Andoniya Petkova (Universitätsmedizin Göttingen), Nina Gödecke (TU Braunschweig), Martin Korte (TU Braunschweig), Thomas Dresbach (Universitätsmedizin Göttingen); andonia.petkova@gmail.com

Abstract:
Synaptic maturation is a process that allows synapses to acquire their full functionality, and failures in synaptic maturation are thought to contribute to psychiatric disorders such as autism. Neuroligins are postsynaptic cell-adhesion molecules essential for postsynaptic and presynaptic maturation. But what are the transsynaptic pathways by which Neuroligins act to regulate presynaptic maturation?

Here, we show that Neuroligins and brain-derived neurotrophic factor (BDNF) cooperate to mediate presynaptic maturation. Applying BDNF to neuronal cultures mimicked the maturation-promoting effect of overexpressing the Neuroligin isoforms NL1 and NL2.

Reducing the levels of BDNF by applying a BDNF scavenger (TrkB-Fc) or depleting BDNF by Cre-lentivirus transduction blocked the action of NL1 and NL2. In particular, inhibiting endogenous BDNF signaling reduced the positive effects of NL1 on presynaptic maturation and of NL2 on synapse formation. Applying BDNF to cultures from NL1-knockout mice rescued impaired presynaptic maturation both in early (DIV6) and late (DIV15) culture stages, indicating that BDNF acts downstream of NL1-mediated cell adhesion.

Our data introduce BDNF as a novel and necessary component in a transsynaptic pathway linking NL-mediated cell adhesion, neurotrophin action and presynaptic maturation.

Vortrag 27:

Titel: Super-resolution microscopy detects presynaptic localization of the als-associated rna-binding protein fus

Autoren/Adressen: Michael Schön (Ulm University), Jochen M. Reichel (Ulm University), Maria Demestre (Ulm University), Stefan Putz (), Michael J. Schmeißer (Ulm University), Tobias M. Böckers (Ulm University); michael.schoen@uni-ulm.de

Abstract:
Fused in Sarcoma (FUS) has recently been associated with frontotemporal dementia (FTD) and familial amyotrophic lateral sclerosis (fALS). A common observation in ALS-FUS and FTD-FUS is a mis-localization of FUS from the nucleus into cytoplasmic aggregates. Our aim was to decipher the localization of FUS in the rodent central nervous system and the subsynaptic localization of the protein in hippocampal synapses with super-resolution microscopy.

In our study we applied several immunohistochemical approaches. For localization of FUS on a nanoscale level we used a single-molecule localization microscopy technique also referred to as direct stochastic optical reconstruction microscopy (dSTORM). Therefore, we labeled synapses with pre- and postsynaptic markers in combination with FUS.

We could confirm the abundant nuclear localization of FUS throughout the rat central nervous system. Moreover, we detected a considerable amount of FUS localizing to synapses with a developmental dynamic.
Super-resolution microscopy in hippocampal synapses of cultured rat neurons revealed a distribution of FUS in the presynaptic compartment of glutamatergic synapses. Within the bouton FUS is localized more proximal than the active zone protein Bassoon and overlaps almost completely with the synaptic vesicle marker Synaptophysin.

Our results show an abundant nuclear and also synaptic localization of the ALS-associated RNA-binding protein FUS. In hippocampal synapses FUS is localized in presynaptic boutons in close proximity to synaptic vesicles. We could add another regulator of local translation to the presynaptic compartment. Future studies have to reveal its function at synapses under normal and disease conditions

Vortrag 28:

Titel: Subthalamic nucleus volumes are highly consistent but decrease age-dependently – a combined magnetic resonance imaging and stereology approach in humans

Autoren/Adressen: Johann Zwirner (University of Leipzig), Dustin Möbius (University of Leipzig), Ingo Bechmann (University of Leipzig), Thomas Arendt (University of Leipzig), Karl-Titus Hoffmann (University Clinic of Leipzig), Carsten Jäger (University of Leipzig), Donald Lobsien (University Clinic of Leipzig), Robert Möbius (University of Leipzig), Uwe Planitzer (University Clinic of Leipzig), Dirk Winkler (University Clinic of Leipzig), Markus Morawski (University of Leipzig), Niels Hammer (University of Otago); medijo@gmx.de

Abstract:
The subthalamic nucleus (STN) is a main target structure of deep brain stimulation (DBS) in idiopathic Parkinson's disease. Nevertheless, there is an ongoing discussion regarding human STN volumes and neuron count, potentially having an impact on STN-DBS. Moreover, a suspected functional subdivision forms the basis of the tripartite hypothesis, which has not been morphologically substantiated. In this study, we aimed to investigate the human STN by means of combined magnetic resonance imaging (MRI) and stereology.

STN volumes were obtained from 14 individuals (ranging from 65-96 years, 25 hemispheres) in 3 T MRI and in Luxol-stained histology slices. Neuron number and related cell densities were investigated stereologically over the entire STN and in pre-defined subregions in anti-human neuronal protein HuC/D-stained slices.

STN volumes measured with MRI were smaller than in stereology but appeared to be highly consistent, measuring on average 99 ± 6 mm3 (MRI) and 132 ± 20 mm3 (stereology). The neuron count was 430,000 on average. STN volumes and cell count decreased age-dependently. Neuron density was different for the ventral, medial and dorsal subregions with significantly higher values ventrally than dorsally.

Small variations in STN volumes in both MRI and stereology contradict previous findings of large variations in STN size. Age-dependent decreases in STN volumes and neuron numbers might influence the efficacy of STN-DBS in a geriatric population. Though the study is limited in sample size, site-dependent differences for the STN subregions form a morphological basis for the tripartite theory.

Vortrag 29:

Titel: Flow-cytometric identification and small molecule-based modulation of neuroblastoma subpopulations in vitro

Autoren/Adressen: Fraua Ferlemann (Emmy Noether-Group for Stem Cell Biology), Vishal Menon (Emmy Noether-Group for Stem Cell Biology), Larisa Condurat (Emmy Noether-Group for Stem Cell Biology), Jan Pruszak (Emmy Noether-Group for Stem Cell Biology); fraua.ferlemann@neptun.uni-freiburg.de

Abstract:
Neuroblastoma (NB) is the most common extra-cranial solid tumor in children, representing up to 10% of childhood cancer. A developmental disorder of neural crest origin, its heterogeneous histology results in a broad spectrum of clinical outcomes. Beyond the known genetic risk factors (e.g., 1p deletion or MYCN amplification), there is a need to identify markers to further resolve cellular heterogeneity.

To investigate specific subsets of NB cell populations and their associated signaling networks, we subjected several human NB cell lines (SH-SY5Y, SH-EP, BE(2)-M17, Kelly) to a detailed surface molecular characterization by multiparametric flow cytometry.

Applying an established clustering algorithm (spanning tree progression of density normalized events; SPADE), neuroblastoma cell subpopulations were visualized and could be defined by combinatorial cytometric profiles. Employing multiwell-based screening platforms paired with cytometric readout, we identified chemical modulators of cellular phenotype and function. The combinatorial detection of glycoprotein epitopes (CD15, CD24, CD44, CD57, CD171), integrins (CD29, CD49c, CD49e) or the chemokine receptor CXCR4 (CD184) enabled the quantitative identification of cellular subsets differentially responding to small molecule modulators, most notably of bone morphogenetic protein (BMP) and retinoid signaling pathways.

Our approach may provide tools for an improved prognostic analysis of NB as well as for pharmacological screens towards developing novel avenues of NB diagnosis and treatment.

Vortrag 30:

Titel: Aflibercept counteracts pathological neovascularization, modulates inflammation and triggers a tip cell-driven regeneration of the retinal vasculature after hypoxic damage in mice

Autoren/Adressen: József Jászai (TU Dresden, Medizinische Fakultät), Jesus Eduardo Rojo Arias (TU Dresden, Medizinische Fakultät), Hector Montecino Garrido (TU Dresden, Medizinische Fakultät), Matina Economopoulou (TU Dresden, Uniklinikum), Henning Morawietz (TU Dresden, Uniklinikum, Med. Klinik III.), Richard H.W. Funk (TU Dresden, Medizinische Fakultät); jozsef.jaszai@tu-dresden.de

Abstract:
The primary objective of our study was to investigate the efficacy of VEGF-Trap (Aflibercept), a recombinant decoy VEGF-receptor-body, in inhibiting pathological retinal neo-vascularization and promoting microvascular regeneration (ordered revascularization) in the murine oxygen-induced ischemic retinopathy model (OIR). This model is a particularly well-studied paradigm of retinal hypoxic stress mimicking morphological features of pathologic retinal neo-vascularization observed in retinopathy of prematurity (ROP), a developmental pathology affecting pre-term individuals, and those of late severe forms of proliferative diabetic retinopathy (PDR). The OIR model offers an excellent system for testing the efficacy of anti-angiogenic substances and studying mechanistic aspects of microvascular regeneration.

To induce pathologic neovascularization (NV) in the retina, consecutive hyper- and normoxia (relative hypoxia) were applied to post-natal mice. Retinal microvasculature from mice subjected to OIR (control) and such after receiving VEGF-Trap were compared by morphological and biochemical methods.

The OIR protocol induces regression of the central retinal microvasculature and an uncoordinated proliferation of endothelial cells leading to the formation of pathological epiretinal glomeruloid tufts. Aflibercept application significantly inhibits aberrant vaso-proliferation and triggers a tip cell-driven mechanism responsible for an accelerated revascularization of avascular areas. The substance also modulates the inflammatory response associated with the hypoxic damage by decreasing vascular permeability and affects the activation state of phagocytic cells, increasing the proportion of cells with a ramified morphology.

Our results indicate that most of the pathological vascular changes could be reliably reduced by Aflibercept in OIR mice, resulting in faster vascular regeneration without significant side effects on vascular architecture.

Vortrag 31:

Titel: Knockdown of apol1 induces autophagy in the zebrafish pronephros

Autoren/Adressen: Ahmed Kotb (University Medicine Greifswald,,,, Assiut University Egypt), Frank Dombrowski (University Medicine Greifswald, Greifswald), Kerstin Amann (University Hospital Erlangen, Erlangen), Uwe Zimmermann (University Medicine Greifswald, Greifswald), Johanna Chluba (University of Burgundy Dijon), Tobias Huber (University Medical Center Freiburg), Karhans Endlich (University Medicine Greifswald), Nicole Endlich (University Medicine Greifswald); kotba@uni-greifswald.de

Abstract:
APOL1 which encodes for a secreted high density lipoprotein is expressed in podocytes of the human kidney. It was already shown that APOL1 genetic variants are associated with kidney diseases like focal segmental glomerulosclerosis (FSGS) in African Americans. Since APOL1 is not expressed in mice, the zebrafish is an ideal model to study the function of zApoL1. The aim of our study was to find out which role ApoL1 plays for autophagy in vitro and in vivo.

Transgenic GFP-Lc3 zebrafish strain (Tg(CMV:EGFP-map1Lc3b)) and human podocytes that were transfected with a plasmid encoding GFP-LC3 were used to study autophagy. Knockdown (KD) of zApoL1 in zebrafish larvae was generated by morpholino injection. APOL1 of human podocytes was knocked down by siRNA. The efficiency of the KD was confirmed by RT-PCR and Western blots. APOL1 and LC3 expression were studied in human kidney sections of patients suffering from FSGS by immunohistochemistry.

We found that APOL1 KD in cultured human podocytes induces an increase of LC3. The KD of the zebrafish ortholog zApoL1 resulted in pericardial edema, a hallmark of kidney disease. Zebrafish cryosections revealed that the Lc3 expression is markedly increased in podocytes after zApoL1 KD. Moreover, kidney biopsies of patients suffering from FSGS showed a significant decrease of the APOL1 expression combined with an increase of LC3 spots, specifically in podocytes.

APOL1 is important for proper kidney function. Loss of APOL1 induces autophagy as shown by the expression of LC3 in vitro and in vivo.

Vortrag 32:

Titel: Cardiac looping and the early topogenesis of the atrioventricular canal (avc) in the embryonic chick heart

Autoren/Adressen: Thomas Reichel (UMG, Georg-August-University Göttingen), Jörg Männer (UMG, Georg-August-University Göttingen); jmaenne@gwdg.de

Abstract:
The early embryonic heart is a tubular structure that forms by merging of left and right heart fields (HF) along the midline of the dorsal pericardial wall. During HF merging, the initially linear heart tube elongates and becomes deformed into a helical tube. This deformation is named cardiac looping. It is the first manifestation of visceral asymmetries and is controlled by molecular signals conferring left or right (LR) identities to the two body halves. Defects in LR-specification (isomerisms) frequently lead to AVC septation defects indicating that LR-specification plays an important role in AVC development. Fate-mapping has shown that, in the post-looping heart, the ventral wall of the AVC, which harbors the superior AV-cushion, derives from the left HF, while the dorsal wall of the AVC, which harbors the inferior AV-cushion, derives from the right HF. The process leading to this situation is unknown.

Positional changes of the AVC were documented in the looping embryonic chick heart (HH-stages 11-24) by 3D-OCT + SEM.

The original left and right halves of the AVC retain their initial LR-orientation during the first phase of cardiac looping (C-looping, HH-stages 11-13), which is characterized by ventral bending and rightward torsion of the future ventricles. Concomitantly with the subsequent caudal displacement of the ventricles (early S-looping, HH-stages 14-18), the AVC undergoes a torsion, which brings its original left and right halves into their final ventral and dorsal positions, respectively.

C-looping defines the direction of future AVC torsion, while AVC torsion itself is caused by early S-looping.

Vortrag 33:

Titel: Microglia functions and phenotypes during postnatal development of the nigrostriatal system

Autoren/Adressen: Abdelraheim Attaai (University of Freiburg), Ralf Gilsbach (University of Freiburg), Kerstin Krieglstein (University of Freiburg), Björn Spittau (University of Freiburg); bjoern.spittau@anat.uni-freiburg.de

Abstract:
Microglia are the resident innate immune cells of the central nervous system (CNS) and play important roles during pathological neuroinflammation and neurodegeneration. However, recent studies demonstrated essential physiological functions of microglia during CNS development and postnatal maturation of neuronal circuits and functional systems. Here, we describe microglia functions, activation phenotypes and gene expression patterns during postnatal development of the nigrostriatal system between postnatal day 0 (P0) and day 28 (P28).

TH-fiber density measurements and detection of dopamine using HPLC-ED was used to assess the maturation of the nigrostriatal system within the first 28 postnatal days in C57BL/6 mice. Immunoelectron microscopy was employed to detect microglial synaptic contacts. Immunohistochemistry for microglia quantifications and microglia phagocytosis as well as flow cytometry and gene expression arrays were used.

We demonstrate that microglia engulf apoptotic oligodendrocytes within the first two postnatal weeks in the basal ganglia as well as in the substantia nigra. Further, synpatic connections in the maturing nigrostriatal system are frequently contacted by microglial processes and microglia actively phagocytose synapses. Microglia shift from M2-like to M1-like activation states from P0 to P28 and further adopt their unique microglia-specific gene expression signature within these first four postnatal weeks. Interestingly, microglia maturation correlated with increased activation of microglial TGF-beta signalling, further underlining the importance of TGF-beta for microglia maturation and maintenance.

Together, our data indicate that microglia are active players in shaping the developing nigrostriatal system and further suggest an important role of TGF-beta during postnatal induction of a microglia-specific gene expression signature.

Vortrag 34:

Titel: Effects of trpa1 agonists on murine airways

Autoren/Adressen: Georg Haider (Justus-Liebig-University Giessen), Silke Wiegand (Justus-Liebig-University Giessen), Emma Spies (Fraunhofer Institute of Toxicology and Experimental Medicine), Armin Braun (Fraunhofer Institute of Toxicology and Experimental Medicine), Wolfgang Kummer (Justus-Liebig-University Giessen), Christina Nassenstein (Justus-Liebig-University Giessen); christina.nassenstein@anatomie.med.uni-giessen.de

Abstract:
TRPA1 is a cation channel of the transient receptor potential-channel family, predominantly expressed in sensory C-fibers and activated by a wide variety of environmental irritants and endogenous inflammatory mediators. Activation increases [Ca2+]i, induces inward currents, elicits action potential discharge in bronchopulmonary C-fibers and increases central reflex activity.

To examine our hypothesis that TRPA1 activation in C-fibers induces bronchoconstriction, we performed lung function analysis during inhalation of increasing cinnamaldehyde concentrations (CA, TRPA1 agonist).

Unexpectedly, CA induced a dose-dependent bronchodilation. To elucidate the nature of bronchodilation, organ bath experiments were performed on explanted, preconstricted tracheal rings. The CA effect was independent from central reflexes and mimicked by many electrophilic and non-electrophilic TRPA1 agonists, including arcrolein, AITC, 2-APB, thymol and carvacrol. Surprisingly, the bronchodilatory effect of CA was increased by pre-treatment with TRPA1-antagonists as well as in tracheas of TRPA1-KO mice, indicating that CA causes a TRPA1-mediated bronchoconstriction which is superimposed by a TRPA1-independent bronchodilation. Since a 5-day organotypic culture of the tracheas or pretreatment by RP-67580, a neurokinin 1 receptor (NK1R) antagonist, respectively, was associated with an increased bronchodilation, we conclude that the TRPA1-dependent bronchoconstriction involves sensory neurons and is dependent on NK1R signaling. However, the nature of the TRPA1-independent bronchodilation remains unclear since iberiotoxin, indomethacin, tetrodotoxin, propranolol and Nω-nitro-L-arginine could not prevent the bronchodilation.

We conclude that activation of TRPA1 in C-fibers induces a NK1R-dependent bronchoconstriction superimposed by a bronchodilatory effect of unknown etiology.

Vortrag 35:

Titel: The role of microglia-derived signaling molecules as a brain-intrinsic trigger for blood-brain barrier breakdown in multiple sclerosis

Autoren/Adressen: Miriam Scheld (Institute of Neuroanatomy), Markus Kipp (Department of Anatomy II), Bernd Rüther (Institute of Neuroanatomy), Bernd Denecke (Interdisciplinary Center for Clinical Research), Kim Ohl (Department of Pediatrics), Oliver Pabst (Institute of Molecular Medicine), Cordian Beyer (Institute of Neuroanatomy), Tim Clarner (Institute of Neuroanatomy); mscheld@ukaachen.de

Abstract:
Multiple sclerosis (MS) is characterized by widespread demyelinating lesions within the CNS. The general view of lesion pathogenesis is an initial immune dysregulation causing the immune system to attack CNS structures. Otherwise, there exists evidence that brain-intrinsic degenerative cascades might be initially involved in lesion development.

To further investigate this hypothesis and elucidate mechanisms of lesion formation, we combined two common murine MS models, the demyelinating cuprizone model and the autoimmune EAE model. In this new combinatory model, we identified massive and widespread immune cell recruitment into the forebrain. The histological appearance of these lesions reflected MS pathology in many aspects. Since microglia is known to play important roles in CNS inflammatory processes, we used the combinatory model to investigate the role of reactive microglia for the disruption of the blood-brain barrier (BBB).

Therefore, genome-wide gene expression profiles of ex vivo isolated microglia from EAE, cuprizone and the combinatory model were analyzed. Disease-related biochemical pathways are currently tested for their significance for BBB disruption and role in lymphocyte invasion. The inclusion of microglia-specific knock-down strains in our studies will help to pave the way for understanding how microglia interacts with the BBB and peripheral immune cells during MS pathogenesis.

Our results clearly illustrate the significance of brain-intrinsic degenerative cascades for the formation of new MS lesions and highlight the importance of microglia for BBB breakdown and peripheral cell invasion into the CNS.

Vortrag 36:

Titel: Abrogation of microglial tgf-beta signalling results in distinct microglia pheontypes in vitro and in vivo

Autoren/Adressen: Tanja Zöller (University Freiburg), Artur Schneider (University Freiburg), Christian Kleimeyer (University Freiburg), Kerstin Krieglstein (University Freiburg), Björn Spittau (University Freiburg); bjoern.spittau@anat.uni-freiburg.de

Abstract:
Transforming growth factor beta 1 (TGF-beta1) has been shown to be an endogenous factor to regulate microglia functions and activation states in vitro and in vivo by promoting anti-inflammatory effects leading to resolution of microglia-driven neuroinflammation. Here, we describe the phenotypes after microglia-specific deletion of the TGFb receptor type II (Tgfbr2) and the intracellular downstream mediator Smad4.

Tgfbr2flox/flox/Cx3cr1CreERT and Smad4flox/flox/Cx3cr1CreERT mice were used to specifically target TGF-beta signalling in microglia. Validation of recombination was performed using the ROSA26-EYFP reporter mouse line as well as qPCR and functional analysis of TGF-beta signalling. Flow cytometry, cytokine/chemokine arrays and PathScan intracellular signalling arrays were employed to analyse microglia phenotypes and activation states. Immunohistochemistry was further used to address microglia numbers and morphological changes in vivo.

We demonstrate that Tgfbr2-deficient microglia are insensitive to TGF-beta1 and display increased expression of surface activation markers CD36, CD86 and CD206. Moreover, increased phosphorylation of TAK1 in mutant microglia was accompanied by augmented secretion of Ccl2 and Cxcl10. Tgfbr2flox/flox/Cx3cr1CreERT mice showed no gross abnormalities but presented considerable morphological changes of microglia and moderate neuroinflammation. Interestingly, these phenotypic changes were not observed in Smad4flox/flox/Cx3cr1CreERT mice, which presented only marginal microglia activation.

Our data demonstrate the importance of TGF-beta signalling for microglia quiescence under physiological conditions and further indicate that Smad4 might be dispensable for TGF-beta-mediated regulation of microglia activation.

Vortrag 37:

Titel: Peroxisomes are essential for germ cell development

Autoren/Adressen: Ann-Kristin Brauns (UKE Hamburg), Eveline Baumgart-Vogt (Justus Liebig University Giessen), Georg H. Lüers (UKE Hamburg); a.brauns@uke.de

Abstract:
Peroxisomes are cell organelles with important functions in various metabolic processes such as lipid synthesis, β-oxidation of very long chain fatty acids and degradation of reactive oxygen species. Peroxisomal disorders lead to severe neurological diseases as a result from changes in cellular architecture of the brain as well as disturbed myelination of axons. Patients with a peroxisomal biogenesis disorder also show testicular pathologies and are infertile. Infertility affects 6% of male population. However, 25% of the exact causes are still unknown.

We have established a mouse model with a conditional knockout for Pex13 in pre-meiotic germ cells under the control of a Stra8-cre promoter. The peroxisomal membrane protein Pex13 is part of the translocation machinery required for import of peroxisomal matrix proteins into the organelle.

The inactivation of Pex13 leads to a biogenesis defect of peroxisomes with loss of all metabolic functions. In addition to reduced testis volume, histological analysis of knockout mice also revealed an interruption in germ cell differentiation with loss of mature spermatozoa and the generation of multinucleated giant cells. Gas chromatography revealed alterations in plasmalogen and triglyceride composition in testis of KO mice. FADS2 expression is increased, suggesting a regulatory effect on β-oxidation. Moreover there is also evidence for a disturbance of the blood-testis barrier leading to impermeability.

These results indicate an important effect of peroxisomes on germ cell development.

Vortrag 38:

Titel: Bdnf: mrna expression in urine cells of patients with chronic kidney disease and its role in kidney function

Autoren/Adressen: Nicole Endlich (University Medicine Greifswald), Tim Lange (University Medicine Greifswald), Jana Kuhn (University Medicine Greifswald), Paul Klemm (University Medicine Greifswald), Ahmed Kotb (University Medicine Greifswald), Florian Siegerist (University Medicine Greifswald), Maja T. Lindenmeyer (University of Munich), Clemens D. Cohen (University of Munich), Rainer Rettig (University of Greifswald), Uwe Lendeckel (University of Greifswald), Uwe Zimmermann (University Medicine Greifswald), Kerstin Amann (University Hospital Erlangen), Beate Fiene (University Medicine Greifswald), Sylvia Stracke (University Medicine Greifswald), Karlhans Endlich (University Medicine Greifswald); tim.lange@uni-greifswald.de

Abstract:
Podocyte injury and loss are major causes of chronic kidney disease (CKD). Since the number of patients suffering from CKD is continuously increasing during the last decades, it is important to find biomarkers predicting the individual risk.

RNA was isolated from urinary cells of 120 CKD patients and the mRNA level of the podocyte-specific proteins nephrin, podocin, podocalyxin and potential biomarkers like BDNF, KIM-1 and cathepsin L were determined by qRT-PCR. Kidneys were co-stained for synaptopodin and BDNF. BDNF was knocked down in zebrafish larvae by injection of morpholinos. 2-Photon microscopy (2PM) was used for in vivo studies.

We found a strong positive correlation between BDNF and the kidney injury marker KIM 1 which were also significantly correlated with nephrin. A significant difference in BDNF and KIM-1 expression was observed between diabetics and non-diabetics. Co-staining of synaptopodin and BDNF showed that BDNF is localized in the cell body and major processes of healthy podocytes. In contrast, only a few podocytes were stained in DN patients for BDNF.
The knockout of bdnf in zebrafish larvae resulted in pericardial edema, a hallmark for kidney injury. Histological studies revealed an increased Bowman`s space, enlarged capillaries, podocyte loss and impaired glomerular filtration that was confirmed by 2-PM.

Expression of BDNF, which we show to be highly correlated with KIM-1 in urine cells of CKD patients, could be an alternative biomarker for CKD. Moreover, we found that BDNF plays an important role for proper podocyte morphology and function.

Vortrag 39:

Titel: E-selectin ligand binding affinity to determine anti-metastatic efficacy of proteasome inhibition.

Autoren/Adressen: Tobias Lange (University Medical Center Hamburg-Eppendorf), Ursula Valentiner (University Medical Center Hamburg-Eppendorf), Daniel Wicklein (University Medical Center Hamburg-Eppendorf), Hanna Maar (University Medical Center Hamburg-Eppendorf), Vera Labitzky (University Medical Center Hamburg-Eppendorf), Ann-Kristin Brauns (University Medical Center Hamburg-Eppendorf), Kristoffer Riecken (University Medical Center Hamburg-Eppendorf), Christian Boernchen (University Medical Center Hamburg-Eppendorf), Rainer Kiefmann (University Medical Center Hamburg-Eppendorf), Valsamma Abraham (University of Pennsylvania), Horace DeLisser (University of Pennsylvania), Timo Gemoll (University Medical Center Schleswig-Holstein), Jens Habermann (University Medical Center Schleswig-Holstein), Guido Sauter (University Medical Center Hamburg-Eppendorf), Benjamin Otto (University Medical Center Hamburg-Eppendorf), Thomas Streichert (University Medical Center Hamburg-Eppendorf), Gerrit Wolters-Eisfeld (University Medical Center Hamburg-Eppendorf), Udo Schumacher (University Medical Center Hamburg-Eppendorf); to.lange@uke.de

Abstract:
E-selectin-mediated adhesion of circulating tumor cells (CTCs) to vascular endothelial cells (ECs) initiates CTC extravasation at a distant site and is hence a critical step of metastatis formation. This study aimed to inhibit CTC extravasation in order to reduce metastasis formation.

tumor cell adhesion in situ and in vitro, spontaneous metastasis formation in vivo, glycosyltransferase expression and activity, inhibition of glycosylation steps, RNA-interference, immunoprecipitation, western blot, clinical studies

We show that the approved compound bortezomib (BZM) counteracts cytokine-mediated up-regulation of E-selectin on ECs. This can lead to impaired adhesion of tumor cells in vitro and in situ and reduced spontaneous metastasis in vivo. However, the efficacy of BZM crucially depends on the nature of the tumor cell's E-selectin ligands. BZM-'sensitive' tumors (neural crest-derived tumors) are characterized by a newly specified, non-canonical, low affinity E-selectin ligand, namely poly-N-acetyllactosamine. BZM-'resistant' tumors (gastrointestinal adenocarcinomas) are characterized by the canonical, high affinity E-selectin ligand sialyl Lewis A (CA 19-9). For both classes of ligands, we exemplarily demonstrate MGAT5B and C2GNT2 as key glycosyltransferases and CD44 and CD24 as important glycoprotein carriers on neuroblastoma and colorectal cancer cells, respectively. The identified glycoconjugates are shown to be frequently co-expressed in corresponding clinical samples and experimental metastases.

Our study demonstrates anti-metastatic efficacy of BZM in a subset of solid human tumors. This novel option might be useful to prevent metastasis during transient periods of increased CTC release (biopsy and surgery). Serum sLeA (CA 19-9 tumor marker) levels might predict therapy response.

Vortrag 40:

Titel: Par-6 functions as a tumor suppressor regulating cell polarity and hippo signaling by preventing degradation of pals1

Autoren/Adressen: Olga Panichkina (University of Regensburg), Ludwig Lagleder (University of Regensburg), Rui Sun (University of Regensburg), Michael Krahn (University of Regensburg); Olga.Panichkina@vkl.uni-regensburg.de

Abstract:
Epithelial cell polarity is of crucial importance for many tissues. Disruption of epithelial cell polarity leads to disassembly of cell-cell and cell-matrix contacts and tissue disorganisation and finally results in epithelial-to-mesenchymal transition (EMT), which is implicated in carcinogenesis and metastasis. Several studies report a cross-talk between the apical complexes, in particular a link between PAR-6 and the Crumbs-complex. Therefore we elucidated in this study how PAR-6 regulates the positioning of the Crumbs complex.

MDCK and HCT116 cancer cell lines were maintained in DMEM medium. shRNA against Pals1 were constructed into the pSuperior vector. Immunohistochemical and immunofluorescent stainings, immunoblotting and RT-PCR were performed according to standard protocols.

In PAR-6-mutant cells, Pals1 is strongly diminished, resulting in an intracellular mislocalization of Crumbs and subsequent disturbance of apical-basal polarity. Besides degradation of Pals1, Par-6-mutant cells show decreased phosphorylation and enhanced nuclear translocation of Yap, indicating a dysregulation of the Hippo-signalling pathway. Furthermore, downregulation of Pals1 caused an aberrant cell morphology, a reduction of E-cadherin and increased EMT-markers, resulting in enhanced cell motility. Finally, the expression of PAR-6 is reduced in invasive colon cancer samples, whereas Rpn13 is overexpressed. Consequently, Pals1 is degraded, whereas Pals1 mRNA is not affected, resulting in disruption of apical-basal polarity and cell-cell contacts of tumor cells.

Taken together, PAR-6 functions as a tumor suppressor by preventing the degradation of Pals1 to ensure apical-basal cell polarity, cell-cell-adhesion and controlled cell proliferation, thus preventing EMT and cancer progression.

Vortrag 41:

Titel: Hensen's node of the chick bears matrix filled microcavities prior to morphological node asymmetry

Autoren/Adressen: Tobias Karl Pieper (University of Goettingen), Meriam Carpaij (University of Goettingen), Helen Sang (University of Edinburgh), Christoph Viebahn (University of Goettingen), Nikoloz Tsikolia (University of Goettingen); tobias.pieper@stud.uni-goettingen.de

Abstract:
Asymmetrical morphogenesis of the primitive node is the first sign of left-right symmetry breaking in the chick. However, the mechanisms leading to this asymmetry are unknown and a causal link between asymmetrical morphogenesis and molecular patterning has to be elucidated.

Focusing on node asymmetry we studied the morphology and gene expression in the node area between stages 4- and 5+ by means of transmission electron microscopy (TEM), in situ hybridization, immunohistochemistry and live imaging of GFP-transgenic chicken embryos.

Both morphological analysis and live imaging revealed an increasing tilt of the node during early node regression stages (HH 4+ to 5+). The right node shoulder is continuous with the notochord whereas, as the result of node tilting, the left part of the node is shifted posteriorly. At stage 5+, together with the emerging left-sided expression of the gene nodal, both domains of the node are separated by a less dense region. Localization of N-Cadherin at stage 5 in the node follows this morphology with a particularly strong reaction in the primitive streak, the right node shoulder and the emerging notochord. Interestingly, detailed analysis of semithin sections revealed intercellular matrix filled spaces, which are specifically localized in the Hensen's node from stages 4- until 8. TEM of these microcavities shows short cellular processes and a filamentous structure within the matrix.

We demonstrate an asymmetrical morphogenesis of axial mesoderm during node tilting and regression, and we hypothesize that microcavities in the node play a role in axial mesoderm formation.

Vortrag 42:

Titel: Plakoglobin is Required for Positive Adhesiotropy in Cardiomyocytes

Autoren/Adressen: Camilla Schinner (Ludwig-Maximilians-Universität), Angela Schlipp (Ludwig-Maximilians-Universität), Vera Rötzer (Ludwig-Maximilians-Universität), Ahmed Messoudi (Ludwig-Maximilians-Universität), Anja Horn (Ludwig-Maximilians-Universität), Franzsika Vielmuth (Ludwig-Maximilians-Universität), Volker Spindler (Ludwig-Maximilians-Universität), Jens Waschke (Ludwig-Maximilians-Universität); camilla.schinner@med.uni-muenchen.de

Abstract:
Desmosomes, as part of the intercalated disc (ICD), are essential for coordinated contraction and structural integrity of the heart. Recently, we reported sympathetic signaling to regulate cardiomyocyte cohesion. Adrenergic stimulation strengthened desmoglein 2 (Dsg2)-mediated adhesion paralleled with reorganization of the ICD. Here we demonstrate this effect to be strictly dependent on the plaque protein plakoglobin (Pg).

We applied immunostaining, dissociation assay and atomic force microscopy (AFM) to HL-1 cardiomyocytes, established a cardiac specific Pg knock-out (Pg KO) mouse model and performed working heart experiments with Pg KO hearts.

Similar to Dsg2, Pg staining was enhanced along intercellular junctions following adrenergic stimulation, while distribution of other desmosomal proteins was not altered. Revealed by AFM, silencing Pg abrogated cAMP-dependent enhancement of Dsg2-mediated binding events at cell junctions. Deleting Pg blocked the positive effect of adrenergic stimulation on cardiomyocyte cohesion and prevented the positive inotropic and chronotropic impact of sympathetic signaling ex vivo. Furthermore, ICDs of Pg KO hearts challenged by adrenergic stimulation were ruptured compared to challenged wild type hearts. Interestingly, in Pg KO hearts all other ICD components were present whereas Dsg2 was selectively reduced before onset of dilatation and fibrosis.

These data indicate that strengthening of cardiomyocyte cohesion via Pg and Dsg2 is required for a sufficient response to adrenergic stimulation and maintenance of ICD integrity. Because reduction of Pg at the ICD has been reported to be a feature of arrythmogenic cardiomyopathy, this mechanism may be of high medical relevance.

Vortrag 43:

Titel: Regeneration of critical size bone defects via the implantation of novel ß tricalcium phosphate scaffolds in transgenic mice

Autoren/Adressen: Mersedeh Tohidnezhad (ukaachen), Tobias Heigl (ukaachen), Nazanin Barahmand Pour (ukaachen), Christian Bergmann (ukaachen), Michaela Bienert (ukaachen), Hans-Christoph Pape (ukaachen), Sabine Neuss-Stein (ukaachen), Horst Fischer (ukaachen), Philipp Lichte (ukaachen), Thomas Pufe (ukaachen);mtohidnezhad@ukaachen.de

Abstract:
Vascular endothelial growth factor (VEGF) and VEGFreceptor (VEGFR) are important in generating a microenvironment that facilitates bone growth.
Aim of the present study was the investigation of the fracture healing process in VEGFR2-luc and NFκB-luc mice using novel ß tricalcium phosphate (ß-TCP) scaffold (with and without strontium (Sr))

A critical size fracture was performed and stabilized using external fixation . The ß-TCP and ß-TCP+Sr scaffolds were implanted in the bone defect The expression levels of VEGFR2 and NFκB could be longitudinal monitored in a Xenogen imaging system. After two months, animals were euthanized and the fracture sites were histologically examined.

We observed the highest peaks of luciferase activity in the ß-TCP+Sr group at the 15th day. Additionally, Sr reduced inflammation by means of NFκB activity in the early phase of healing (15th days), but it was increased again in the late healing stage.
The histological analysis showed that much more osseous tissue has been formed in ß-TCP+Sr when compared to ß-TCP. In both, ß-TCP and ß TCP+Sr, the connection of newly formed tissue within the scaffold area to the fracture ends was clearly visible.

This study for the first time gives an overview of VEGFR2 and NFκB expression profiles during fracture healing. A new tissue was observed in the centerof the scaffolds. Addition of Strontium in scaffolds influence the inflammation in different stages of the healing and leads to increase of ossification. This effect might influence the healing process.

Vortrag 44:

Titel: Desmoglein 3 binding properties depend on keratin filaments

Autoren/Adressen: Franziska Vielmuth (Ludwig-Maximilians-Universität München), Fanny Loschke (University of Leipzig), Thomas M. Magin (University of Leipzig), Jens Waschke (Ludwig-Maximilians-Universität München), Volker Spindler (Ludwig-Maximilians-Universität München); Franziska.Vielmuth@med.uni-muenchen.de

Abstract:
Desmosomes are supramolecular protein complexes required for strong intercellular adhesion. The interaction between the adjacent cells is provided by desmosomal cadherins, such as Desmoglein (Dsg) 3, which are intracellularly linked with the intermediate filament cytoskeleton by several adapter proteins. We recently showed that Dsg3 single molecule binding forces are not uniformly distributed but are higher at cell borders compared to cell surface areas. In view of the compromised adhesion resulting from intermediate filament loss, we here tested the relevance of keratins for the binding properties of Dsg3.

Atomic force microscopy (AFM), keratin-deficient keratinocytes, immunostaining

In human keratinocytes, Dsg3, the adapter molecule desmoplakin and keratin filaments assemble on the cell surface. Murine keratinocytes lacking all keratin filaments (KtyII k.o.) displayed reduced intercellular adhesion compared to controls (KtyII wt). Surprisingly, the protein levels and membrane localization of Dsg3 was increased in KtyII k.o. cells. In line with this, by using AFM, a higher frequency of Dsg3-mediated binding events was observed in Ktyll k.o. cells. Interestingly, loss of keratins do not abrogate the different binding forces at distinct cell areas, but the binding forces of individual binding events were significantly reduced compared to KtyII wt cells at both cell areas.

Our data for the first time show that keratins regulate binding properties of desmosomal cadherins and therefore provide new insights into the mechanisms of desmosomal adhesion.

Vortrag 45:

Titel: Neurovascular Egfl7 regulates subventricular neural stem cells, neurogenesis and olfactory perception

Autoren/Adressen: Frank Bicker (Johannes Gutenberg University Mainz, School of Medicine), Mirko HH Schmidt (Johannes Gutenberg University Mainz, School of Medicine); mirko.schmidt@unimedizin-mainz.de

Abstract:
Adult neural stem cells reside in a specialized niche in the subventricular zone (SVZ)and give rise to adult-born neurons in the olfactory bulb (OB), thus contributing to neural plasticity and pattern discrimination. Here we analyzed the molecular mechanisms applied by blood vessels to govern neural stem cells (NSCs) and neurogenesis.

We used various mouse in vivo models such as conventional and conditional knock-out mice as well as cerebroventricular injections of adenoviruses followed by molecular and cellular analyses in vivo, ex vivo assays, electrophysiology or mouse behavior paradigms.

The neurovascular protein EGFL7 was secreted by endothelial cells of the SVZ to shape the vascular stem cell niche. Loss of EGFL7 caused an accumulation of activated NSCs while ectopic EGFL7 pushed activated NSCs towards quiescence and neuronal progeny towards differentiation. EGFL7's effects were achieved by promoting Dll4-induced Notch signaling at the blood vessel-stem cell interface. Less inhibitory neurons formed in the OB of EGFL7 knock-out mice, which increased the absolute signal conducted from the mitral cell layer of the OB but decreased neuronal network synchronicity. Consequently, EGFL7 knock-out mice displayed physiological defects in olfactory behavior and perception.

EGFL7 is a protein secreted by blood vessels to govern neural stem cells and neurogenesis in the SVZ, and altered the neural circuitry in the OB and affected mouse behavior. Further, the larger pool of activated NSCs increased neurogenesis in old mice suggesting the manipulation of EGFL7 levels as a tool to attenuate cognitive decline happening during aging.

Vortrag 46:

Titel: The orientation and organization of the presynaptic active zone protein bassoon: from the golgi to the synapse.

Autoren/Adressen: Tina Ghelani (University Medical Center, Göttingen), Fabian GÖTTFERT (Max Planck Institue for Biophysical Chemistry), Rene EBRECHT (University Medical Center, Göttingen), Fred WOUTERS (University Medical Center, Göttingen), Nina Wittenmayer (University Medical Center, Göttingen), Thomas DRESBACH (University Medical Center, Göttingen); tinaghelani@gmail.com

Abstract:
Bassoon is one of the largest scaffolding proteins found in the cytomatrix of the active zone (CAZ) of a neuron's presynaptic terminal. The CAZ is a specialized sub-compartment assembled in close proximity to the neurotransmitter release site, or active zone, and is comprised of interconnected active zone proteins. The CAZ and its proteins have been shown to promote short-term plasticity and long-term plasticity by enabling priming and docking of synaptic vesicles and binding to Ca2+ channels. Despite its integral role in presynaptic transmission, the mechanisms of mammalian CAZ formation are still poorly understood.

To visualize the steps involved in the biogenesis of the CAZ, we generated single and double tagged full-length Bassoon constructs optimized for visualizing the orientation and organization of Bassoon molecules with super-resolution imaging techniques. Using specific nanobodies against the fluorescent tags and stimulated emission depletion (STED) nanoscopy, we are able to resolve and characterize the orientation of the N- and C-termini of the protein, and using FLIM imaging the intermolecular organization of neighbouring Bassoon molecules in maturing cultured neurons.

This study describes, the orientation and organization of Bassoon at different sub-cellular localizations, during different stages of CAZ biogenesis, of neurons.

Understanding the changes in conformation of Bassoon, during neuronal development, enhances our knowledge of AZ assembly and synapse maturation, and serves as a ruler to compare orientation of other CAZ and synaptic proteins at the mammalian synapse.

Vortrag 47:

Titel: Heterogeneity of the axon initial segment in interneuron and pyramidal cells of rat visual cortex

Autoren/Adressen: Felix Höfflin (Medical Faculty Mannheim, Heidelberg University), Alexander Jack (Ruhr-University Bochum), Corinna Corcelli (Medical Faculty Mannheim, Heidelberg University), Julia Bucher (Medical Faculty Mannheim, Heidelberg University), Christian Schultz (Medical Faculty Mannheim, Heidelberg University), Petra Wahle (Ruhr-University Bochum), Maren Engelhardt (Medical Faculty Mannheim, Heidelberg University); maren.engelhardt@medma.uni-heidelberg.de

Abstract:
In most cortical neurons, the site of action potential initiation, the axon initial segment (AIS), is usually located at the proximal axon. Morphological studies have shown that in various cortical regions (somatosensory, visual or cingulate cortex), AIS thus appear as a relatively homogeneous domain. However, in the hippocampal CA1 region, for example, up to 50% of AIS emerge from distal dendritic branches. In these particular cells, AIS are separated from the soma by a visible gap. Another structural parameter that can vary is overall AIS length. A direct correlation of AIS length and position with cellular excitability has been shown. We set out to investigate AIS morphology in greater detail, hypothesizing that the initial observation of seemingly homogeneous AIS in a given cortical region is inadequate and depends on which neuronal class is investigated.

Neurons in visual cortex-derived organotypic cultures were labeled with biolistic gene transfer and classified using established morphological criteria. Utilizing AIS immunolabeling, confocal microscopy and morphometric analysis, we analyzed AIS position and length.

Interestingly, we find a substantial AIS heterogeneity in visual cortex: pyramidal neurons have significantly longer and more distal AIS than interneurons. Noncanonical AIS phenotypes are observed, from multiple branch points to AIS “extra domains”. The latter show spot-like immunoreaction to AIS markers, often appearing at a remarkable distance to the distal end of the actual AIS.

Our data contribute to the emerging understanding that AIS morphology and also plasticity can have a significant impact on neuron function and thus, network formation and maintenance.

Vortrag 48:

Titel: Identification of the neuroanatomical circuits related to autistic-like behavior using cell type specific shank mutant mice

Autoren/Adressen: Michael Schmeisser (Ulm University), Sasa Peter (Erasmus Medical Center, Rotterdam), Claudia Reinelt (Ulm University), Sonja Halbedl (Ulm University), Dominik Reim (Ulm University), Chris de Zeeuw (Erasmus Medical Center, Rotterdam), Tobias Boeckers (Ulm University); michael.schmeisser@uni-ulm.de

Abstract:
The SHANKS are scaffold proteins contributing to the morphological and functional integrity of excitatory synapses. Importantly, SHANK mutations have been linked to a significant number of individuals with autism spectrum disorder (ASD). We have previously shown that both Shank2 and Shank3 mutant mice exhibit autistic-like behavior. However, the neuroanatomical circuits involved remained unknown.

Having created several cell type specific Shank mutants using the Cre-lox system, we now provide data on the neuroanatomical origin of these phenotypes

One of our most striking results is that deletion of Shank2 in cerebellar Purkinje cells alters synaptic transmission at the parallel fiber-Purkinje cell synapse and is sufficient to cause core autistic-like features in mice.

Taken together, our cell type specific Shank mutants are magnificent tools to dissect the neuroanatomical circuits related to autistic-like behavior.

Vortrag 49:

Titel: NMDA-receptor inhibition restores alterations in homeostatic synaptic plasticity of dentate granule cells in slice cultures prepared from amyloid-precursor-protein (APP)-deficient mice

Autoren/Adressen: Andreas Vlachos (Heinrich-Heine-University, Düsseldorf), Christos Galanis (Goethe-University, Frankfurt), Maximilian Lenz (Heinrich-Heine-University, Düsseldorf), Denise Becker (Goethe-University, Frankfurt), Meike Hick (Goethe-University, Frankfurt), Ulrike Müller (Ruprecht-Karls University, Heidelberg), Thomas Deller (Goethe-University, Frankfurt); Andreas.Vlachos@med.uni-duesseldorf.de

Abstract:
The physiological role of the Amyloid-Precursor-Protein (APP) remains not well understood. Recent work has implicated APP in the regulation of neural plasticity: changes in dendritic arborisation, alterations in spine density, and defects in LTP have been reported in APP-deficient mice. These studies established a link between APP and its cleavage products in experience-dependent circuit formation and plasticity. However, it is not clear whether APP-deficiency also affects other forms of plasticity. We here studied the role of APP in homeostatic synaptic plasticity, which plays a fundamental role in maintaining functional stability of neuronal networks.

Single- and paired-recordings, local viral injections and pharmacological treatments were employed in entorhino-hippocampal slice cultures of APP-deficient mice and their age- and time-matched wild type litter mates.

We demonstrate that homeostatic plasticity of excitatory neurotransmission onto dentate granule cells requires the presence of APP. Interestingly, inhibition of NMDA-receptors rescues the ability of APP-deficient granule cells to express homeostatic synaptic plasticity.

Hence, we propose that alterations in APP or APP processing may affect the functional stability of neuronal networks and that clinically used NMDA-receptor inhibitors may protect neurons from alterations in homeostatic synaptic plasticity.
(supported by Deutsche Forschungsgemeinschaft; FOR1332).

Vortrag 50:

Titel: Quantitative analysis of cortical structural plasticity probed with large-scale in-vivo two-photon imaging

Autoren/Adressen: Hongwei Zheng (Institute of Anatomy and Cell Biology), Sanjeev Kumar Kaushalya (Institute of Pharmacology), Johannes Knabbe (Institute of Anatomy and Cell Biology), Björn Ommer (Interdisciplinary Center for Scientific Computing), Rohini Kuner (Institute of Pharmacology), Thomas Kuner (Institute of Anatomy and Cell Biology); zheng@ana.uni-heidelberg.de

Abstract:
In vivo two-photon imaging has established structural alterations of dendrites and spines as a key mechanism of neuronal plasticity. We investigate structural plasticity of mid cingulate cortex (MCC) dendrites and thalamic axons as a cellular mechanism potentially underlying chronic pain. To address this, we use a longitudinal in vivo imaging and behavior approach allowing us to correlate behavioral readouts with changes in dendritic and axonal structure. Furthermore, we aim at identifying cell types and cellular compartments engaging in structural plasticity to gain insights into the organization of MCC circuits.

We acquired high-resolution two-photon image stacks containing the full morphology of entire neurons at weekly intervals before and after spared nerve injury (SNI), a model of chronic neuropathic pain. Neuronal morphology was identified by using an automated computing and analysis workflow consisting of several steps: preprocessing, registration, tracing of structures and quantitative analysis of dendritic tree geometry.

To improve the tracing fidelity and to measure efficiency of structural changes from entire neurons to local dendritic segments and spines, a novel Bayesian incremental learning and 3D tracing algorithm has been developed to compute large-scale 3D datasets in a weighted global to local optimization manner. Blood vessels and neighboring cells can be identified without influencing the tracing of target neurons. Furthermore, exact 3D tracing and vectorization support accurate 4D correspondence.

Our preliminary results reveal a segmental organization of changes in spine density and changes in the density of presynaptic thalamic boutons in response to SNI.

Vortrag 51:

Titel: Analysis of vasopressin v1a vs. v2 receptor distribution in the mammalian kidney

Autoren/Adressen: Torsten Giesecke (Charité Universitätsmedizin Berlin), Taka-aki Koshimizu (Jichi Medical University, Shimotsuke-shi, Tochigi-ken), Katsumasa Kawahara (Kitasato University School of Medicine, Kitasato), Maurice Manning (University of Toledo, College of Medicine and Life Science), Alexander Paliege (Charité Universitätsmedizin Berlin), Sebastian Bachmann (Charité Universitätsmedizin Berlin), Kerim Mutig (Charité Universitätsmedizin Berlin); torsten.giesecke@charite.de

Abstract:
Vasopressin facilitates urinary concentration via the V2 receptor (V2R). Activation of the V1a receptor (V1aR) may modulate renal acid-base handling and stimulate the renin-angiotensin-aldosterone axis. Data on the renal distribution of these receptor subtypes is still scarce owing to the lack of specific antisera.

We have generated specific antibodies and compared segmental and cellular distribution of V1aR and V2R along mouse, rat, and human kidney tubules. Segmental and cellular distribution of the receptor subtypes was analyzed by immunofluorescence. Functional studies were performed in AVP-deficient Brattleboro rats using the agonists AO-4-67 for V1aR and desmopressin for V2R.

The V2R was localized basolaterally in thick ascending limb (TAL), distal convoluted tubule (DCT), and principal cells of connecting tubule (CNT) and collecting duct (CD), whereas macula densa (MD) and intercalated cells (IC) were negative. The V1aR antibody produced signal exclusively in MD cells and IC of CNT/CD. At the cellular level, MD cells and type A IC (A-IC) showed basolateral V1aR distribution, whereas type B IC (B-IC) showed no basolateral but rather the diffuse apical receptor distribution. Administration of desmopressin in Brattleboro rats induced apical trafficking of aquaporin 2 in the CNT/CD principal cells, whereas application of AO-4-67 resulted in luminal trafficking of V-ATPase in A-IC of CNT/CD.

Our data extend information on renal distribution of vasopressin receptors at protein level. The divergent renal distribution patterns of V1aR and V2R provide morphological support for the epithelial effects of vasopressin mediated by these receptors.

Vortrag 52:

Titel: Us-guided perineural injection at the ulnar tunnel

Autoren/Adressen: Ulrike Hamscha (Medical University Vienna), Ines Tinhofer (Medical University Vienna), Lukas Reissig (Medical University Vienna), Wolfgang Grisold (KFJ Hospital, Vienna), Wolfgang J. Weninger (Medical University Vienna), Stefan Meng (Medical University Vienna); ulrike.hamscha@meduniwien.ac.at

Abstract:
The objective of this anatomical study was to investigate the feasibility of ultrasound-guided injection of fluids into the perineural sheath of the ulnar nerve (UN) inside the ulnar tunnel (UT).

Using a standard ultrasound machine, we performed ultrasound guided injections of 1 mL ink into the UT of a total of 21 upper limbs. Injections were targeted at the perineural sheath of the UN. The UT was then dissected, the UN exposed and the distribution of ink examined.

In all specimens, ink was solely distributed inside the perineural sheath of the ulnar nerve. No ink was found inside the epineurium of the nerve or in surrounding blood vessels and tendons. The superficial branch was stained in all cases (100%), on a length of 2.97 1.26 cm (mean SD). In 19 of the 21 specimens (90.48 %) also the deep branch was stained, on a length of 2.11 1.0 cm (mean SD).

Our results proof that targeted injection of fluids into the perineural sheath of the UN inisde the UT is feasible. Therefore our study might serve as proof of concept for the design of clinical trails aiming at testing whether patiens suffering from UT syndrome could profit from injections of cortison into the UT.

Vortrag 53:

Titel: Alveolar micromechanics in progressing bleomycin-induced lung injury

Autoren/Adressen: Lars Knudsen (Hannover Medical School), Elena Lopez-Rodriguez (Hannover Medical School), Lennart Berndt (Hannover Medical School), Caroline Boden (Hannover Medical School), Clemens Ruppert (Justus-Liebig-University Giessen), Heinz-Gerd Hoymann (Fraunhofer Institute for Toxicology and Experimental Medicine), Jason H Bates (University of Vermont), Bradford Smith (University of Vermont), Matthias Ochs (Hannover Medical School); knudsen.lars@mh-hannover.de

Abstract:
Surfactant-dysfunction related alterations of alveolar micromechanics including intra-tidal alveolar derecruitment and recruitment (R/D) and heterogeneous ventilation contribute to acute lung injury. This study quantified alveolar micromechanics as function of positive end-expiratory pressure (PEEP) ventilation and disease progression in bleomycin-induced lung injury.

One (D1) and three (D3) days after bleomycin instillation lungs were evaluated by invasive pulmonary function tests at PEEP values between 1 and 10 cm H2O to determine organ-scale tissue elastance (H). Lungs were then perfusion fixed at corresponding end-expiratory airway opening pressures (Pao) and assessed with design-based stereology. These data were used to parameterize a physiologically-based computational multi-compartment model of alveolar micromechanics.

While in healthy controls the number of open alveoli remains stable with Pao=1-20 cmH2O, bleomycin challenged lungs demonstrate alveolar R/D with Pao1-10cmH2O and an increase of alveolar size variance at Pao=10cmH2O. These structural changes correlated with organ-scale tissue elastance. Moreover, with disease progression at D3, 40% of alveoli remained closed at high Pao=20cmH2O. Computational modelling showed that individual elastance at a given alveolar size of open alveoli decreased with injury and surfactant dysfunction.

Alveolar R/D and alveolar size variation dominate alveolar micromechanics at Pao 1-10cmH2O and correlate with organ-scale elastance at corresponding PEEP in bleomycin-injured lungs. Alveolar derecruitment and increased alveolar size heterogeneity are associated with increased H. The open alveoli and alveolar ducts are more compliant most likely due to outward tethering forces generated by adjacent collapsed alveoli.

Vortrag 54:

Titel: Teaching and assessing medical students' pattern recognition skills

Autoren/Adressen: Ralph Nawrotzki (University of Heidelberg), Falk Herrmann (University of Heidelberg), Joachim Lirsch (University of Heidelberg); nawrotzki@ana.uni-heidelberg.de

Abstract:
Pattern recognition is a fast and automatic process that requires little mental effort and leaves capacity to perform other tasks at the same time. In contrast to analytic reasoning, it is rarely taught at university. Here we analyzed i) whether effective pattern recognition can be taught to first-year medical students and ii) whether a twelve question multiple-choice test is suitable to assess students' pattern recognition skills.

Three groups of ten participants each (Group A – no ultrasound course, Group B – completed course, Group C – tutors) were asked to think aloud while answering text and image questions on the anatomy of the abdomen. Answers were recorded, transcribed and analyzed by content analysis. Concept maps were generated to visualize students' choice of cognitive strategy (fast or slow thinking).

The analysis of outcome and process variables revealed that students in groups B and C favored fast strategies in their answers (instant statements of recognized patterns) whereas group A members responded in analytic ways. Response times and average number of words per answer decreased from group A to group C (A: 68 to B: 31 and C: 14 seconds and A: 122, B: 84, C: 55 words).

Our data reveal that students' abilities to recognize images have a profound impact on their choice of cognitive strategy while retrieving knowledge. The simplicity of our MC test suggests that similar studies can be performed in other fields of medical expertise. Teaching and assessing medical students' pattern recognition skills will support their efforts to develop effective clinical reasoning skills.

Vortrag 55:

Titel: Cadaver-specific ct scans visualized at the dissection table combined with virtual dissection tables improve learning performance in general gross anatomy

Autoren/Adressen: Daniel Paech (German Cancer Research Center Heidelberg), Frederik Giesel (University Hospital Heidelberg), Roland Unterhinninghofen (Karlsruhe Institute of Technology), Kerstin Klopries (Heidelberg University), Heinz-Peter Schlemmer (German Cancer Research Center Heidelberg), Thomas Kuner (Heidelberg University), Sara Doll (Heidelberg University); d.paech@dkfz.de

Abstract:
The purpose of this study was to quantify the benefit of the incorporation of radiologic anatomy (RA), in terms of first-year medical student training in RA-seminars, cadaver CT scans and life size virtual dissection tables on the learning success in general anatomy.

The intervention group (year 2015, n1=50) with training in radiologic image interpretation (RA-seminar) and additional access to cadaver CT scans was compared to a control group without any radiologic image interpretation training (2011, n3=98). All participants took a test of 40 highly discriminating questions, comprising of 10 questions each on head & neck, abdomen, thorax and extremities derived from the 10% most difficult questions of the National Board Examinations (2005-2010). Furthermore, the students' perception was assessed qualitatively through a survey.

The average test score of the intervention group (21.8 ± 5.0) was significantly higher compared to the control group (17.1 ± 4.7) ($p<0.001$). The intervention group showed highly significant improvements in the subcategories head & neck ($p<0.001$) and extremities ($p<0.001$). In the category of thorax small but significant differences ($p<0.05$) were detectable between the students of the intervention and the control group. 87.8% of the students agreed that teaching with radiologic imaging modalities was a good supplement to first year gross anatomy and a large majority of 85.4% stated that it was sensible to learn how to read CT scans at the beginning of their medical studies.

Medical imaging and virtual dissection should therefore be considered to be part of the standard curriculum of gross anatomy.

Vortrag 56:

Titel: Why the Anatomische Gesellschaft excluded six unwanted members in 1949

Autoren/Adressen: Andreas Winkelmann (Medizinische Hochschule Brandenburg); andreas.winkelmann@mhb-fontane.de

Abstract:
A letter in the Nachlass (estate) of Würzburg anatomist Curt Elze reveals that the first post-war board of the Anatomische Gesellschaft decided not to re-admit six former members after the official re-foundation of the Gesellschaft in 1949. This analysis intended to clarify the reasons for these exclusions.

Archival research.

The following anatomists were excluded: Robert Wetzel of Tübingen, Otto Popp and Andreas Pratje of Erlangen, Richard Wegner of Greifswald, Bernhard Lange formerly of Breslau, and Max Clara of Munich. With the exception Wegner, they were never re-admitted into the Gesellschaft. Four of them had been members of the NSDAP, but only three could be called "Nazi activists". Only Pratje and Wetzel had agitated to bring the traditional Anatomische Gesellschaft under Nazi influence. At least Clara, Popp and Pratje, however, were regarded as having used party contacts to boost their career. In one way or other, the scientific achievements of all six were seen critical, and some kind of dishonesty in their career paths was assumed.

As other active Nazis, like Eduard Pernkopf or Enno Freerksen, were re-admitted to the Anatomische Gesellschaft after 1949, "being a Nazi" in the past was not a criterion for exclusion in 1949. I suggest that the common ground for an exclusion of members at the time was their perceived breach of existing hierarchies and of an implicit code of entrance into the circle of established anatomists. Such a breach could consist of, e.g., basing a career on political rather than scientific merits.

Vortrag 57:

Titel: Sex-dependent regulation of aromatase-mediated synaptic plasticity in basolateral amygdala

Autoren/Adressen: Roland Bender (University of Hamburg), Lepu Zhou (University of Hamburg), Ricardo Vierk (University of Hamburg), Gabriele M. Rune (University of Hamburg); r.bender@uke.de

Abstract:
The basolateral amygdala (BLA) is pivotal for fear learning. For this purpose, it integrates sensory input from cortical and subcortical regions, a function that requires marked synaptic plasticity. Here we studied the contribution of aromatase (AROM), the enzyme that converts testosterone to 17-BETA-estradiol (E2), to this plasticity.

1) Adult mice were systemically injected (i. p.) for 7 days with the AROM-inhibitor letrozole. 2) Amygdalar organotypic cultures of neonatal rats were incubated with letrozole for 7 days. 3) Synaptic densities were determined in BLA using electron microscopy. 4) LTP was recorded in BLA of letrozole-treated acute slices from juvenile rats.

A significant reduction of synaptic density in BLA was observed in adult mice (in vivo) and in organotypic cultures (in vitro) only if the tissue originated from females, but not from males. Similarly, LTP in BLA was virtually abolished by letrozole in acute slices from female, but not male, juvenile rats.

The contribution of endogenous (AROM-derived) E2 to synaptic plasticity in BLA is sex-dependent, suggesting that sex-specific regulatory mechanisms are involved. This may have implications for the interpretation of certain sex-biased mood disorders and side effects of AROM-inhibitor-based cancer therapies.

Vortrag 58:

Titel: Hippocampal mossy fiber synapses represent individual computational units

Autoren/Adressen: Alexander Drakew (Zentrum für Molekulare Neurobiologie Hamburg), Urban Maier (Zentrum für Molekulare Neurobiologie Hamburg), Michael Frotscher (Zentrum für Molekulare Neurobiologie Hamburg);
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Abstract:
Hippocampal mossy fiber (MF) synapses display different forms of synaptic plasticity, which are expressed on the presynaptic as well as on the postsynaptic side at different time scales and stimulation intensities. These properties point to heterogeneity of MF synapses. Moreover, in light of the structural heterogeneity of MF synapses it is tempting to speculate that individual MF synapses reside in different functional states, which is difficult to assess experimentally. In order to analyze the structure and function of single MF synapses, we combined single-bouton stimulation and two-photon imaging of spines postsynaptic to the stimulated mossy fiber bouton.

We labeled hilar mossy cells in organotypic entorhino-hippocampal slice cultures in the whole-cell patch clamp configuration using dye-filled pipettes. Alexa 594 was used to visualize the morphology of spines, whereas Fluo-4ff served to report calcium transients in single spines. Alexa 488 released from a second pipette transiently stained the extracellular space and allowed for targeted patching of unlabeled boutons presynaptic to labeled identified spines.

Stimulation of the bouton evoked very heterogeneous synaptic responses pointing to a varying contribution of different glutamate receptors. Moreover, plasticity of individual mossy fiber synapses in response to a defined stimulation pattern depended on the initially encountered synaptic state.

These results suggest that each mossy fiber synapse contributes individually to computation in the hippocampus.

Vortrag 59:

Titel: Order under the guise of chaos: functional neuroanatomy of the barrel cortex of the *reeler* mouse

Autoren: Julien Guy (1), Alexandra Sachkova (1), Martin Möck (1), Mirko Witte (1), Robin J. Wagener (2), Jochen F.Staiger (1)

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Abstract:
Rodents possess an array of facial whiskers on either side of their snout, which they use to collect tactile information about their surroundings. Layer IV of the somatosensory cortex contains the barrel field, a somatotopic representation of the whiskers. Barrels receive direct sensory input from the thalamus, which is then distributed to other layers of the cortex resulting in widespread cortical activation. In the *reeler* mouse, the loss of function of the reelin protein causes abnormal development of the cortex leading to the loss of cortical lamination. Neurons that ought to be grouped together in layers are found in ectopic positions across the cortical depth. Although evidence exists that barrel equivalent structures may form in *reeler*, the functional organization and connectivity of its somatosensory cortex deserves further investigation. Using *in vivo* intrinsic signal optical imaging, we show that sensory input reaches and activates the *reeler* barrel cortex normally, and that somatotopy persists in spite of laminar disorganization. Furthermore, using *in vitro* whole cell recordings and optogenetics, we demonstrate that thalamic input to layer IV equivalent neurons is direct in *reeler*. Thalamic input is weakened, while network activity increases with respect to normal cortex. These results reveal a paradox in the *reeler* brain, opposing a weakened thalamic input to a normal activation of cortical networks. We propose a model of cortical network changes that addresses this paradox, whereby a weakened thalamic input is rescued by an increase in the gain of its intracortical amplification and a shift in the excitation-inhibition balance.

Vortrag 60:

Titel: Structural and functional mechanisms of homo- and heterosynaptic plasticity at the entorhinal cortex-granule cell synapse in the dentate gyrus in vivo

Autoren/Adressen: Peter Jedlicka (Goethe-University Frankfurt), Tassilo Jungenitz (Goethe-University Frankfurt), Marcel Beining (Goethe-University Frankfurt), Hermann Cuntz (Ernst-Strüngmann Institute (ESI) for Neuroscience in Cooperation with Max-Planck Society), Schwarzacher W. Stephan (Goethe-University Frankfurt), Thomas Deller (Goethe-University Frankfurt); jedlicka@em.uni-frankfurt.de

Abstract:
LTP and LTD are critical synaptic mechanisms of learning and memory. High-frequency stimulation of main inputs to the dentate gyrus is known to induce homosynaptic LTP (hom-LTP) at tetanized synapses and simultaneous heterosynaptic LTD (het-LTD) at non-tetanized synapses. Hom-LTP and het-LTD might represent a homeostatic mechanism for keeping the total input strength of excitatory synapses within a physiological range. However, little is known about the structural and functional mechanisms mediating hom-LTP and het-LTD.

We employed high-frequency stimulation of entorhinal afferents to the dentate gyrus of anesthetized rats to elicit LTP of the tetanized medial perforant path input and LTD of the non-tetanized lateral perforant path input. Following high-frequency stimulation, we used 3D-reconstructions of dendrites and spines of stimulated granule cells to study the structural correlates of hom-LTP and het-LTD.

We demonstrate on the level of single neurons that whereas hom-LTP is accompanied by an expansion of spines, het-LTD is associated with a shrinkage of spines. In addition, using biophysically realistic models of granule cells we show that these changes can be sufficiently explained by computational rules of synaptic plasticity and metaplasticity.

In sum, our results provide evidence that plasticity-inducing mechanisms in the dentate gyrus of intact animals lead to an activity-dependent structural remodeling of spines underlying input-specific LTP of stimulated synapses and associated LTD in neighboring non-stimulated synapses. We suggest that homosynaptic and heterosynaptic structural plasticity of spines is a fundamental mechanism for maintaining the firing rate of granule cells in response to afferent stimulation within a homeostatically defined range.

Vortrag 61:

Titel: Pilocarpine-induced status epilepticus is associated with changes in synaptopodin in the mouse hippocampus

Autoren/Adressen: Maximilian Lenz (Heinrich-Heine-University, Düsseldorf), Marina Ben Shimon (The Chaim Sheba Medical Center, Tel HaShomer), Thomas Deller (Goethe-University, Frankfurt), Nicola Maggio (The Chaim Sheba Medical Center, Tel HaShomer), Andreas Vlachos (Heinrich-Heine-University, Düsseldorf); maximilian.lenz@med.uni-duesseldorf.de

Abstract:
Epilepsy is a complex neurological disorder severely affecting neural function. Some patients may experience a life-threatening state of ongoing seizure activity, a status epilepticus, which is associated with postictal cognitive dysfunction. However, the molecular mechanisms by which status epilepticus influences brain function beyond seizure activity remain not well understood. We here addressed the question of whether pilocarpine-induced status epilepticus affects the actin-modulating protein synaptopodin (SP), which plays an important role in regulating the ability of neurons to express synaptic plasticity. This makes SP an interesting marker for epilepsy-related alterations in neuronal plasticity.

Immunohistochemistry and quantitative PCR was used to assess pilocarpine-induced changes in SP-expression, while extracellular field potential recordings in acute hippocampal slices were used to assess synaptic plasticity.

Indeed, intraperitoneal pilocarpine-injection in three-month old male C57BL/6J mice led to a reduction of SP-cluster sizes and numbers in CA1 stratum radiatum (90 min after seizure-onset). In line with this observation a defect in the ability to induce long-term potentiation (LTP) of Schaffer collateral-CA1 synapses was observed.

Based on these findings we propose that status epilepticus may exert its after-effect on cognition at least in part by perturbing SP-dependent mechanisms of synaptic plasticity.

Vortrag 62:

Titel: Prg-1 regulates synaptic plasticity via intracellular pp2a/ß1-integrin signaling

Autoren/Adressen: Xinfeng Liu (University Medical Center Mainz), Jisen Huai (University Medical Center Mainz), Heiko Endle (University Medical Center Mainz), Leslie Schlüter (University Medical Center Mainz), Michael Schmeisser (Ulm University), Tobias Boeckers (Ulm University), Thomas Deller (Goethe University Frankfurt), Andreas Vlachos (Goethe University Frankfurt), Robert Nitsch (University Medical Center Mainz), Johannes Vogt (University Medical Center Mainz); johannes.vogt@unimedizin-mainz.de

Abstract:
Alterations in dendritic spine numbers are linked to deficits in learning and memory. While we previously revealed that postsynaptic plasticity-related gene 1 (PRG-1) controls lysophosphatidic acid (LPA) signaling at glutamatergic synapses via presynaptic LPA-receptors, we now analyze the role of PRG-1 in regulating spine formation and synaptic plasticity.

We used molecular, biochemical and cell biological techniques in combination with electrophysiological and behavioral analyzes.

Here, we show that PRG-1 affects spine density and synaptic plasticity in a cell-autonomous fashion via protein phosphatase 2A (PP2A) / ß1-integrin activation. PRG-1-deficiency reduces spine numbers and ß1-integrin activation, alters long-term potentiation (LTP), and impairs spatial memory. The intracellular PRG-1 C-terminus interacts in a LPA-dependent fashion with PP2A, thus modulating its phosphatase activity at the PSD. This results in recruitment of adhesome components src, paxillin and talin to lipid rafts and ultimately in activation of ß1-integrins. Consistent with these findings activation of PP2A with FTY720 rescues defects in spine density and LTP of PRG-1-deficient animals.

Together with the functional and behavioral data, our molecular and morphological studies provide evidence for the fact that PRG-1 drives a cell-autonomous signaling pathway involved in the regulation of spine density, and subsequently memory formation.

Vortrag 63:

Titel: Connectomics of the rat hippocampal formation

Autoren/Adressen: Oliver Schmitt (University Rostock), Ann-Christin Klüncker (University Rostock), Sebastian Schwanke (University Rostock); schmitt@med.uni-rostock.de

Abstract:
A complete and consistent connectome of the rat hippocampal formation of the left and right hemisphere which includes collateral data, pathway data as well as estimates of connectional densities was not available until now. Thus, connectional data were extracted exclusively from 5376 peer reviewed tract tracing publications. The aim of the study was
to analyse the intrinsic and extrinsic connectional architecture of the hippocampal formation.

Neuronal connections which are detected by applying classical anterograde, retrograde and bilateral tract tracing methods, as well as neurotrophic virus applications and intracellular fillings were collated. These data were described in 5376 publications. The connectional data were imported into a neuroVIISAS connectome database and analyzed with regard to global and local connectome features.

All known pathways of hippocampus (perforant tract, mossy fiber system, commissural system i.a.) are included in the hippocampal connectom data. We found significant alternative pathways which may bypass the characteristic connectivity of the hippocampus. The analysis at the level of cytoarchitectonic layers revealed a small world connectional architecture rather than the analysis of 32 subregions delineated within the stereotaxic atlas of Paxinos (2015). Certain network-motifs like partial and full reciprocal 3-node subgraphs show a stastically significant appearence within the hippocampal connectome.

A characteristic connectional architecture of the hippocampal connectome has been determined. It shows features of a small world network, specific contralalteral connectivity and alternative pathways with regard to known major intrinsic and extrinsic hippocampal connections.

Vortrag 64:

Titel: A genetically encoded system with high spatiotemporal resolution for modification of neuronal network activity patterns in vivo

Autoren/Adressen: Firat Terzi (University of Heidelberg), Niklas Schneider (University of Heidelberg), Sidney Cambridge (University of Heidelberg); cambridge@ana.uni-heidelberg.de

Abstract:
The patterns of neuronal network activity are fundamental to brain function. To correlate changes in network activity with genetic manipulation of neurons, we established a method that allows targeted transgene expression in a defined set of previously identified neurons. The goal is that these neurons can be analyzed before, during, and after induced gene expression to precisely correlate phenotypic changes to the genetic manipulation of neuronal excitability.

To achieve transgene expression with high spatio-temporal control in living mice, we developed an optimized version of the inducible Tetracycline (TetOn) system with substantially reduced background expression. For acute genetic manipulation of neuronal excitability, we chose the inward rectifying potassium channel Kir2.1 which cell-autonomously silences neurons.

In vitro, we found that Kir2.1 induction led to a significant reduction of neuronal activity within three hours. Two-photon microscopy in cortices of living mice indicated that injection of doxycycline and induction of Kir2.1 reduced neuronal activity within hours as visualized by a co-expressed GCamP6 calcium indicator.

The goal is to induce transgene expression of Kir2.1 in a subset of cells to investigate the consequences on neuronal network homeostasis of larger ensembles. By imaging of the network patterns and morphology before and after Kir2.1 expression, the phenotypic homeostatic network changes can be directly correlated to the changes in gene expression. In particular, with this flexible high-resolution approach, the dynamics of homeostasis can be captured in much detail including potentially very early events which was previously not possible.

Vortrag 65:

Titel: Mover: a synapse-specific regulator of plasticity

Autoren/Adressen: Julio S. Viotti (Universitätsmedizin Göttingen), Hermes Pofantis (Universitätsmedizin Göttingen), Asha Kiran Akula (Universitätsmedizin Göttingen), Thomas Dresbach (Universitätsmedizin Göttingen); julioviotti@yahoo.com.br

Abstract:
Rare are the proteins in the neurotransmitter release machinery that escape the evolutionary conservation that makes synapses among nematodes, flies, mice and rats quite similar. Mover is one of these rare exceptions. Mover is a vertebrate-specific, synaptic vesicle-bound phosphoprotein. It binds both the highly conserved Calmodulin and the vertebrate-specific Bassoon. Moreover, it is differentially expressed among synapses.
Knock-down of Mover at the calyx of Held has been shown to increase the rate of vesicle reloading after synaptic depression, as well as the calcium sensitivity and probability of release.
Shedding light onto the role of this new participant in the synaptic machinery would help us comprehend three fundamental questions: a) how is release probability regulated? b) how is synaptic heterogeneity accomplished? c) are there special signatures of vertebrate synapses?

In this study, we have used a Mover knockout mouse line in combination with imaging and electrophysiology to understand the role of this protein in synaptic transmission.

Knockout of Mover increases frequency facilitation, paired-pulse ratio and high-frequency facilitation in the Hippocampal Mossy Fibers but not in the Schaffer Collaterals.

These discoveries, together with the changes in the Calyx of Held, suggest that Mover is a synapse-specific regulator of synaptic plasticity. At the Mossy Fibres, activity-dependent regulation of Mover could increase the dynamic range for the induction of frequency facilitation and working memory.

Vortrag 66:

Titel: S-scam/magi2 is essential for synapse formation and maintenance

Autoren/Adressen: Nina Wittenmayer (University of Göttingen Medical School, Brandenburg Medical School), Julio Viotti (University of Göttingen Medical School), JeongSeop Rhee (Max-Planck Institute for Experimental Medicine), Sebastian Kügler (University of Göttingen Medical School), Thomas Dresbach (University of Göttingen Medical School); nina.wittenmayer@med.uni-goettingen.de

Abstract:
The synaptic scaffolding molecule (SSCAM) / membrane-associated guanylate kinase inverted-2 (MAGI-2) was originally characterized as a scaffold protein that interacts with N-methyl-D-aspartate (NMDA) receptors at excitatory synapses. SSCAM is essential for maintaining the surface pool of GluA2-containing AMPA receptors at synapses. However, whether SSCAM specifically affects GluA2-containing receptors, or has more wide-ranging effects on synapse formation and function, is unknown.

Using an RNAi based knockdown approach, we found that S-SCAM/MAGI-2 regulates synaptogenesis, spinogenesis and synapse maintenance in general.

Simultaneous knockdown of all three S-SCAM/MAGI-2 isoforms in cultured rat hippocampal neurons during early synaptogenesis leads to a dramatic reduction of the number of synapses: clustering
of pre- and postsynaptic proteins is nearly abolished, remaining clusters are misaligned, and both spontaneous and evoked synaptic transmission are dramatically reduced. All isoforms of S-SCAM/MAGI-2, α, β or γ, are competent to rescue the clustering of synaptic markers. Moreover, S-SCAM/MAGI-2 knock-down in rat cultures and in vivo resulted in a drastic reduction of spinogenesis. Acute knockdown in mature cultures likewise reduced the number of synapses.

These findings indicate that the postsynaptic scaffolding protein S-SCAM/MAGI-2 is crucial for multiple molecular mechanisms controlling synapse formation and maintenance.

Vortrag 67:

Titel: Pitx2, the hypoblast and axial inversion in pre-streak amniote embryos

Autoren/Adressen: Ruben Plöger (Georg - August - Universität), Claudio Stern (University College London), Christoph Viebahn (Georg - August - Universität Göttingen); ruben.ploeger@t-online.de

Abstract:
Formation of the anterior-posterior (AP) axis in the amniote embryo is controlled through inhibition of the hypoblast's own inhibitory role on primitive streak (PS) formation in the epiblast. Morphologically, the site of PS formation is ear-marked by Koller's sickle at the posterior margin of the chick embryo, while the mammalian embryonic disc (ED) has its first sign of the AP axis prior to PS formation at the anterior margin. To understand the functional relevance of this topographical inversion we analyse the expression pattern of Pitx2 – a positive regulator of PS formation in the chick – in the rabbit; the latter was chosen because of its flat ED, which is typical for mammals and easily compared amongst amniotes.

The cellular distribution of Pitx2 expression was determined by in situ hybridization and serial sectioning of 4.0- to 6.5-day rabbit blastocysts.

Prior to AP axis formation Pitx2 is expressed in single cells of extraembryonic tissues surrounding the ED, first, and later also in the hypoblast. Stronger expression is detected in both layers of the ED next to the anterior marginal crescent, which appears at stage 1, while at stage 2 expression is also in the hypoblast of the posterior gastrulation extension (later, the area of PS formation).

Simultaneous expression of Pitx2 in both anterior and posterior margins of the mammalian embryo appears to accompany the inversion of initial AP axis topography in amniotes. Pitx2 function in the mammalian ED may thus be subject to more complex regulation than in the avian embryo.

Vortrag 68:

Titel: Mechanism causing loss of keratinocyte cohesion dependent on different pemphigus autoantibody profiles

Autoren/Adressen: Elias Walter (Anatomische Anstalt LMU München), Franziska Vielmuth (Anatomische Anstalt LMU München), Lukas Rotkopf (Anatomische Anstalt LMU München), Volker Spindler (Anatomische Anstalt LMU München), Jens Waschke (Anatomische Anstalt LMU München); Elias.Walter@med.uni-muenchen.de

Abstract:
Pemphigus vulgaris is an autoimmune dermatosis, leading to mucosal and epidermal splitting. Usually, the different phenotypes of pemphigus are determined by the autoantibody profiles against the desmosomal cadherins desmoglein 1 (Dsg1) and Dsg3.
Here we characterized the mechanisms engaged by pemphigus autoantibody fractions (PV-IgG) from patients with different clinical phenotypes and autoantibody profiles.

Adhesion assays, Immunostaining, Western blotting, Ca2+ imaging, Atomic force microscopy (AFM), ELISA

All autoantibody fractions applied in this study caused loss of cell cohesion, induced retraction of keratin filaments and compromised binding of Dsg3 but not Dsg1 as revealed by AFM. This was accompanied by rapid Ca2+ influx and activation of p38MAPK and Src. Erk activation was induced by a PV-IgG fraction from a patient with mucocutaneous pemphigus (antibodies targeting Dsg1 and Dsg3) only. Interestingly, strong alterations in distribution and loss of Dsg3 were caused by both the mucocutaneous PV-IgG as well as fractions from an atypical case of pemphigus where skin blistering was associated with antibodies against Dsg3 exclusively. In contrast, AK23, a mouse monoclonal antibody against Dsg3, caused mild alterations of Dsg3 immunostaining without a strong depletion. Inhibition of p38MAPK by SB202190 blocked loss of keratinocyte cohesion and Dsg3 reorganization in response to all autoantibody fractions and the Erk inhibitor U0126 was protective against mucocutaneous IgG fractions.

These results indicate that loss of Dsg3 binding as well as p38MAPK-induced alterations of desmosome integrity contribute to blister formation in patients.

Vortrag 69:

Titel: Signaling mechanisms in acute inflammatory endothelial barrier disruption

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Abstract:
Endothelial cells provide a physical barrier between blood and the surrounding tissue. During inflammation, disruption of endothelial adherens junctions (AJ) and tight junctions leads to increased microvascular permeability. Especially since many studies focused on thrombin, which is not a typical inflammatory mediator, the signaling mechanisms underlying acute endothelial barrier breakdown are not entirely clear yet. In this study we used histamine in comparison to thrombin to study barrier disruption and the corresponding signaling pathways in primary human microvascular endothelial cells.

Immunostaining, Western blotting, fura-2 measurement, GTPase GLISA, transendothelial electrical resistance, Fluorescence-Resonance-Energy-Transfer (FRET)

Both histamine and thrombin induced a rapid and transient Ca2+ increase and barrier breakdown which was blocked by the intracellular Ca2+ chelator BAPTA-AM. The histamine response was blocked by the histamine receptor type 1 antagonist diphenhydramine but not by the H2R antagonist ranitidine. Interestingly, GLISA measurements revealed activation of RhoA but not inactivation Rac1 at the timepoint of barrier breakdown. Next, we established FRET measurements of RhoA and Rac1 activity in microvascular endothelial cells using CNF1 and CNFy which activate Rho family GTPases. For thrombin, our FRET experiments show activation of RhoA at intercellular junctions. At the same time course both thrombin and histamine increased tensions at endothelial AJs indicated by staining with the conformation-sensitive antibody targeting the α18-subunit of α-catenin.

This data suggests that the patterns of signaling mechanisms engaged in acute inflammatory barrier disruption differ to more delayed responses as seen in sepsis. Furthermore, Rho-mediated tension may be involved in acute endothelial barrier disruption.

Vortrag 70:

Titel: Adhesive and signaling properties of Desmoglein 2 in intestinal epithelial barrier regulation

Autoren/Adressen: Hanna Ungewiß (Anatomische Anstalt LMU München), Franziska Vielmuth (Anatomische Anstalt LMU München), Daniela Kugelmann (Anatomische Anstalt LMU München), Jens Waschke (Anatomische Anstalt LMU München); hanna.ungewiss@med.uni-muenchen.de

Abstract:
Intestinal epithelial barrier properties are maintained by a junctional complex consisting of tight junctions (TJ), adherens junctions (AJ) and desmosomes. Previously we have shown that Desmoglein 2 (Dsg2), an adhesion molecule of desmosomes, is required for epithelial barrier properties. Moreover, our data suggest that impaired Dsg2 binding may contribute to barrier defects in Crohn`s disease as Dsg2 was downregulated in patients' biopsies.

Western blot , Triton extraction, Immunostaining evaluation by Confocal microscopy and structured illumination microscopy (SIM), Transmission electron microscopy (TEM), Immuno-electron microscopy (Immuno-EM), Transepithelial electrical resistance (TER), dispase-based dissociation assay, Atomic force microscopy (AFM)

Here, for the first time we identified extradesmosomal Dsg2 on the surface of differentiated enterocytes displaying junctional complexes by Triton extraction, confocal microscopy, SIM and Immuno-EM. AFM revealed Dsg2-specific binding events on the surface of enterocytes with a mean unbinding force of around 34 pN which were more prominent close to cell borders. However, in contrast to an antibody targeting E-cadherin, which is the adhesion molecule of AJs, an inhibitory Dsg2 antibody did neither cause epithelial barrier breakdown nor impaired barrier reformation in a Ca2+ switch assay, under conditions where it significantly reduces cell cohesion in a dissociation assay and Dsg2-specific binding in AFM. Under the same conditions, the inhibitory Dsg2 antibody induced activation of p38MAPK, which is a key regulator of desmosomal adhesion.

Our data suggest that Dsg2, in addition to its adhesion function, may regulate desmosomal adhesion via cellular signaling pathways.

Vortrag 71:

Titel: Loss of desmoglein 2 promotes tumorigenic behavior in pancreatic cancer cells through erk activation and plakoglobin destabilization

Autoren/Adressen: Katharina Hütz (Ludwig-Maximilians-Universität München), Julian Zeiler (Ludwig-Maximilians-Universität München), Jens Waschke (Ludwig-Maximilians-Universität München), Volker Spindler (Ludwig-Maximilians-Universität München); volker.spindler@med.uni-muenchen.de

Abstract:
The ability to maintain cell-cell adhesion is crucial for tissue integrity and organization. Accordingly, loss of cohesiveness plays a critical role in cancer invasion and metastasis. Desmosomes are cell junctions providing strong intercellular adhesive strength and dysregulation of desmosomal constituents was shown to contribute to cancer progression through altered cell signaling pathways.
Here, we studied the role of the desmosomal adhesion molecules desmoglein 2 (Dsg2) and desmocollin 2 (Dsc2) for tumorigenic behavior of pancreatic cancer cells.

Migration assays, Invasion assays, Kinase arrays, Proliferation assays, Immunostaining

siRNA-mediated knock down of Dsg2 but not Dsc2 resulted in loss of cell cohesion and enhanced migration of pancreatic adenocarcinoma cells (AsPC-1). We established stable Dsg2 knockdown cell lines in which loss of Dsg2 caused an increase in cell migration and enhanced invasion through extracellular matrix without influencing cell proliferation. To identify potential pathways regulated by Dsg2, we screened major kinases and detected the activity of ERK to be significantly enhanced in Dsg2-deficient cells. Consequently, inhibition of ERK phosphorylation in Dsg2 knockdown cells led to normalized migration. Interestingly, loss of Dsg 2 was accompanied with reduced levels of the desmosomal adapter protein and transcriptional regulator plakoglobin, whereas other desmosomal molecules were not altered. Consistent with this observation, overexpression of plakoglobin rescued enhanced migration induced by silencing of Dsg2.

Loss of Dsg2 but not Dsc2 promotes tumorigenic behavior of pancreatic cancer cells by regulation of ERK activity and the levels of plakoglobin.

Vortrag 72:

Titel: The regulation of gap junctions by desmoglein 2-binding and camp in cardiomyocytes

Autoren/Adressen: Bernd Erber (Ludwig-Maximilians-Universität München), Camilla Schinner (Ludwig-Maximilians-Universität München), Angela Wölfel (Ludwig-Maximilians-Universität München), Jens Waschke (Ludwig-Maximilians-Universität München); bernd.erber1993@web.de

Abstract:
Arrhythmogenic cardiomyopathy (AC) is an inherited disease which causes cardiac arrhythmia and sudden cardiac death. Mutations mainly affect desmosomal proteins including the cadherin-type adhesion molecule desmoglein 2 (Dsg2) and the plaque protein plakoglobin (Pg) which are components of intercalated discs and known to be required for mechanical cohesion of cardiomyocytes. Previously we showed that loss of Dsg2-adhesion leads to reduced heart rate and decreased response to beta-adrenergic stimulation, whereas cAMP enhances desmosomal binding. In the present study we investigated the regulation of GJ by desmosomal binding and cAMP signaling.

Immunostaining, Western blotting, immunoprecipitation, fura-2 measurement, dispase-based dissociation assay, microelectrode array measurement (MEA)

We observed association of both Dsg2 and β1AR with the GJ component connexin 43 (Cx43) by immunoprecipitation. L-tryptophan (L-Tryp), which interferes with cadherin-mediated binding, as well as the SERCA pump blocker thapsigargin impaired cardiomyocyte cohesion in a dissociation assay and disturbed Cx43 distribution and GJ-mediated excitation conduction in cultured HL-1 cardiomyocytes plated on a microelectrode array as well as in fura-2 measurements. Similarly, siRNA-induced knock-down of Dsg2 or Pg disturbed GJ function. All effects were largely abolished by both beta-adrenergic stimulation and stabilization of desmosomal binding by a Dsg-specific tandem peptide. These data show that adrenergic signaling enhances cardiomyocyte cohesion and propagation of cardiomyocyte excitation.

Desmosomal adhesion is required for GJ integrity and function and therefore stabilization of desmosomal binding may be a suitable approach to treat arrhythmia in AC patients.

Vortrag 73:

Titel: The il-6 receptor is a new proteolytic substrate for meprin metalloproteases

Autoren/Adressen: Philipp Arnold (CAU Kiel), Michelle Rothaug (CAU Kiel), Neele Schumacher (CAU Kiel), Janna Schneppenheim (CAU Kiel), Juliane Lockau (CAU Kiel), Ute Pickhinke (CAU Kiel), Rielana Wichert (CAU Kiel), Björn Rabe (CAU Kiel), Christoph Gabers (CAU Kiel), Stefan Rose-John (CAU Kiel), Ralph Lucius (CAU Kiel), Christoph Becker-Pauly (CAU Kiel); p.arnold@anat.uni-kiel.de

Abstract:
Inflammatory processes are orchestrated by signaling molecules (cytokines) through binding to specific cytokine receptors at the cell surface. Interleukin 6 (IL-6) binds to its primary receptor the IL-6 receptor (IL-6R) and subsequently associates with the secondary receptor gp130. This complex then initiates an intracellular signaling cascade. The metalloproteases ADAM10 and 17 were previously described to shed the IL-6R from the cell surface and generate a soluble IL-6R (sIL-6R) that can be found in the serum. Here we describe the two metalloproteases meprin ALPHA and meprin BETA as new sheddases for the IL-6R.

In our approach we used a cell culture overexpresion system, western blot analysis, a peptide cleavage assay and a cell proliferation assy with a murine B lymphoma cell line (Ba/F3 cells).

We show that soluble meprin ALPHA and membrane bound meprin BETA cleave the IL-6R at the cell surface. Thereby a biological active sIL-6R is generated that induces trans-signaling on Ba/F3 cells (murine B lymphoma cell line).

Our findings render meprins as potential players in the onset and/or progression of inflammatory responses as for example Kawasaki and inflammatory bowel disease through the cleavage of cytokine receptors.

Vortrag 74:

Titel: New Insights into Glucoprotamin Fixation.

Autoren/Adressen: Bernhard Hirt (Eberhard-Karls-Universität Tübingen);
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Abstract:
Recently Glucoprotamin (GP), a C12-pyrrolidine derivate, was identified as a highly effective fixative, which easily penetrates the tissue, inhibits the autolysis of the tissue by the complete blockage of enzymes, provides good tissue qualities, is low in costs, nontoxic, easy to handle and has a high antimicrobial effect. It has been proposed as an alternative to formaldehyde in the field of gross anatomy as well as histology.

We will now introduce a composition of chemicals which contain GP as the active substance and describe the fixation effect on cadavers and histologic preparations.

Glucoprotamin was synthesized with a purity of more than 98%, revealed by mass spectrometry and gas chromatography. The effect on enzymatic activities was tested for Glucoprotamin, the single additives of the composition, the composition as a whole and for formaldehyde as a control sample by using FRET based techniques. The anti-microbial effect after fixation was tested on biopsies of fixed cadavers. Cadaver dissections and histological tissue sections were used for detailed morphological analysis.

Fixation with GP provides cadavers and tissue with realistic visual and haptic properties which are suitable for both macroscopic preparations and histological investigations. GP can be used as a sole fixating agent in the field of clinical anatomy as well as an additive in conventional fixative compositions to lower the toxic load of formaldehyde in dissection courses.