ANATOMISCHE GESELLSCHAFT

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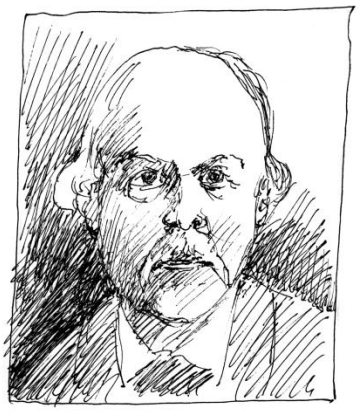
In memory of Koelliker's 200th birthday



Rudolf Albert von Koelliker

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der Universität Würzburg

1886

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Vortrag 1:

Titel: ACBD5 and VAPB mediate membrane contact zones between peroxisomes and the ER

Autoren/Adressen: Markus Islinger (Universität Heidelberg), Joseph L Costello (University of Exeter), Inés Castro (University of Exeter), Andreas Manner (Uiversität Heidelberg), Christian Hacker (University of Exeter), Tina A Schrader (University of Exeter), Jeremy Metz (University of Exeter), Dagmar Zeuschner (Max-Planck Institut für molekulare Biomedizin), Afsoon S Azadi (University of Exeter), Luis F Godinho (University of Exeter), Victor Costina (Universität Heidelberg), Peter Findeisen (Universität Heidelberg), Michael Schrader (University of Exeter); markus.islinger@medma.uni-heidelberg.de

Abstract:
Peroxisomes and the endoplasmic reticulum (ER), which cooperate in cellular lipid metabolism, form tight structural associations, which were first observed in ultrastructural studies decades ago. ER-peroxisomal contacts have been suggested to contribute to a number of cell biological processes such as lipid metabolism, phospholipid exchange, metabolite transport, antiviral signaling, peroxisome biogenesis, and pexophagy. Nevertheless, information about their molecular composition, structure, regulation and function remains scarce.

In order to identify proteins tethering peroxisomes to the ER, we applied a combination of biochemical (IP, mass spectrometry, gradient centrifugation) and microscopic techniques (Immuno-EM, IF, live cell imaging).

Here we show that the peroxisomal membrane protein, acyl-CoA binding protein 5 (ACBD5) and the resident ER protein vesicle-associated membrane protein-associated protein–B (VAPB) generate interactions across organelle boundaries. This ACBD5-VAPB complex tethers the membranes of peroxisomes and the ER in order to form specialized contact zones. Both proteins are anchored in the respective organelle membrane via a C-terminal membrane-spanning domain with their N-termini projecting into the cytoplasm. We were able to reveal that the N-terminal cytosolic parts of ACBD5 and VAPB directly interact with each other via a FFAT-like (two phenylalanines (FF) in an acidic tract) motif in ACBD5. Moreover, we demonstrate that disruption of the ACBD5-VAPB complex results in loss of peroxisome-ER association which perturbs peroxisomal membrane expansion and increases peroxisomal motility.

Our findings reveal the first molecular mechanism for establishing peroxisome-ER contact zones in mammalian cells and report a new function for ACBD5 in peroxisome-ER tethering.

Vortrag 2:

Titel: Super resolution microscopy and automatized digital image processing as a novel and rapid diagnostic tool for podocyte foot process effacement

Autoren/Adressen: Florian Siegerist (University Medicine Greifswald), Silvia Ribback (University Medicine Greifswald), Frank Dombrowski (University Medicine Greifswald), Kerstin Amann (University Medicine Erlangen-Nürnberg), Uwe Zimmermann (University Medicine Greifswald), Karlhans Endlich (University Medicine Greifswald), Nicole Endlich (University Medicine Greifswald); florian.siegerist@stud.uni-greifswald.de

Abstract:
Podocyte foot process morphology plays an essential role for proper glomerular filtration. In minimal change disease, foot processes are lost and replaced by flattened protrusions. Since foot processes of podocytes are around 200-300nm in width, analysis could only be performed by electron microscopy. Recently, super resolution techniques have been developed which allow lightmicroscopic resolution beyond Abbe's optical resolution limit of ~200nm. Using structured illumination microscopy (SIM) it is possible to double the resolution limit. We hypothesized that SIM would allow the analysis of podocyte morphology and to diagnose foot process effacement.

We used SIM of renal tissue of human, murine and rat origin stained with an antibody against the slit diaphragm protein nephrin. For automatic evaluation of the slit diaphragm length / glomerular capillary area, we programmed an ImageJ plugin.

In human samples, we measured a mean foot process width of 0.249±0.68µm in healthy and 0.675±0.246µm in diseased patients. By the use of custom made software we measured a slit diaphragm density of 3.099±0.268µm/µm2 in healthy seubjects compared to 1.825±0.493µm/µm2 in patients. Using both methods we found statistically significant differences between the patients in comparison to the healthy control subjects. Furthermore, we show that using SIM we can image foot processes in animal models.

Taken together, we have established a novel method which allows quick analysis of kidney sections for foot process effacement in an automated fashion. Including this technique into the diagnostic routine could effectively shorten the time until diagnosis of podocyte foot process effacement.

Vortrag 3:

Titel: Rise of urethral brush cells

Autoren/Adressen: Klaus Deckmann (Justus-Liebig-University), Chrissy Kandel (Justus-Liebig-University), Luisa Schulz (Justus-Liebig-University), Amir Rafiq (Justus-Liebig-University), Paul Scholz (Ruhr-University), Sabrina Baumgart (Ruhr-University), Wolfgang Kummer (Justus-Liebig-University); Klaus.Deckmann@anatomie.med.uni-giessen.de

Abstract:
We recently identified a novel cholinergic chemosensory cell in the urethra of 14 mammalian species including humans (urethral brush cell = UBC). UBC utilize the canonical bitter and umami taste transduction signaling cascade to detect putative harmful compounds (e.g. bacterial bitter substances) and initiate reflex detrusor contractions by cholinergic transmission to sensory nerve fibers. Here, we addressed their postnatal and their ontogenetic origin and identify potential novel markers for these cells.

We utilizing choline acetyltransferase (ChAT)-eGFP and Wnt1-tomato reporter mice, deep sequencing, and a mouse model (MyD88-knockout).

Postnatal appearance of UBC was assessed in urethral whole mounts of ChAT-eGFP reporter mice of both genders from day P0 to P100. UBC appear first at P4-P6 in male and at P13-P14 in female mice. After about 10 weeks, UBC numbers have reached the same level in both genders. We identify MyD88 as a key factor for UBC genesis and revealed doublecortin like kinase 1 (DCLK1) as strong UBC marker. Antibodies against this marker were applied to urethral sections of Wnt1-cre tomato mice, a reporter strain for cells of neural crest origin. Neither DCLK1-positive UBC nor serotonin-positive endocrine cells expressed Wnt1-driven tomato, and, hence, are not derived from the neural crest. On the other hand, UBC were immunolabeled with antibodies against the epithelial marker cytokeratin 18 in ChAT-eGFP reporter mice. These data were also supported by deep sequencing.

Taken together, UBC represent cholinergic epithelial cells not derived from the neural crest which develop postnatally in a MyD88-dependent manner.

Vortrag 4:

Titel: Deletion of endothelial TGF-β signaling causes choroidal neovascularization

Autoren/Adressen: Barbara Maria Braunger (University of Regensburg), Anja Schlecht (University of Regensburg), Sarah V Leimbeck (University of Regensburg); Barbara.Braunger@ur.de

Abstract:
To identify the role of TGF-β signaling for CNV formation, we generated mice with a conditional deletion of the TGF-β type II (TβRII) receptor which is essential for TGF-β signaling.

We generated a series of mutant mouse models with induced conditional deletion of TGF-β signaling in the entire eye, the retinal pigment epithelium (RPE) or the vascular endothelium. The successful deletion of TβRII was confirmed by real time RT-PCR, Western blotting and immunohistochemistry. Retinal/choroidal structure and function were studied by light and transmission electron microscopy (TEM), immunohistochemistry, FITC-dextran perfusions, fluorescence angiography, CLARITY imaging, real time RT-PCR, and electroretinography.

Deletion of TGF-β signaling in the entire eye of newborn mice resulted in a significant upregulation of retinal Vegf-a, Fgf-2, Angpt2 and Igf expression levels. At the age of 6 weeks, CNV was detected by CLARITY imaging of the eyes of isolectin B4 injected animals and on meridional sections of dextran perfused eyes. TEM analyses showed the thickening of the Bruch's membrane (BM) and fine fibrillar extracellular material between the basal lamina of the choriocapillaris and the elastic layer of BM. Quite intriguingly, the specific deletion of TGF-β signaling in vascular endothelial cells caused CNV and a phenotype quite similar to that observed after the deletion in the entire eye.

Impairment of TGF-β signaling in the vascular endothelium of the eye is sufficient to trigger CNV formation. Our findings indicate a fundamental role of TGF-β signaling in the pathogenesis of ocular diseases associated with CNV.

Vortrag 5:

Titel: PKA-mediated phosphorylation of plakoglobin at s665 is required for positive adhesiotropy in cardiomyocytes and may enhance gap junction function

Autoren/Adressen: Camilla Schinner (LMU Munich), Bernd Erber (LMU Munich), Thu Kim Co (LMU Munich), Mariya Y. Radeva (LMU Munich), Jens Waschke (LMU Munich); jens.waschke@med.uni-muenchen.de

Abstract:
In arrythmogenic cardiomyopathy (AC), a genetic disease causing arrhythmias and sudden cardiac death, mainly desmosomal proteins such as desmoglein-2 (Dsg2) or plakoglobin (Pg) are affected. Because dysregulation of gap junctions (GJ) may contribute to arrhythmia, here we investigated the mechanisms regulating cardiomyocyte cohesion and gap junction function.

We performed in vitro kinase assay, dissociation assay, atomic force microscopy, multi electrode array, PhosTag and western blot analysis with HL-1 cardiomyocytes, and performed Langendorff perfusions in an AC mouse model lacking Pg.

As shown recently, cardiomyocyte cohesion is enhanced by cAMP signaling. Here we demonstrate this effect to be directly mediated by phosphorylation of Pg at S665 through PKA. siRNA-mediated depletion of Dsg2 or the adherens junction protein N-cadherin (N-Cad) abolished positive adhesiotropy. Interestingly, reduction of N-Cad disrupted baseline cell cohesion more extensively than depletion of Dsg2. Stabilization of Dsg2 binding via a linking peptide designed to specifically crosslink Dsg2 molecules (Dsg2-LP) ameliorated arrhythmia in a Pg-deficient AC mouse model and enhanced propagation of excitation in culture cardiomyocytes. Both, cAMP signaling and Dsg2-LP increased connexin-43 phosphorylation at S368. Inhibition of PKC abolished propagation of excitation and phosphorylation of connexin-43.

Our data indicate that PKA-mediated phosphorylation of Pg is required for positive adhesiotropy for which expression of other intercalated disc components are essential. Increased intercellular cohesion may regulation GJ function via phosphorylation of connexin-43.

Vortrag 6:

Titel: Morphogenesis of the optic fissure margins is setting the stage for consecutive fusion, pioneered by a distinct subset of margin cells using a hyaloid vessel as scaffold

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

Abstract:
The optic fissure is a transient gap in the developing optic cup of vertebrates. Persisting optic fissures, termed coloboma, are a frequent reason for blindness in children. A plethora of genes have been linked to coloboma, however, it has remained unclear how the two optic fissure margins, framed by two conjoined epithelia, are fusing to form a continuous neuroretina and retinal pigmented epithelium (RPE) respectively. Furthermore, highly variable morphologies of coloboma phenotypes strongly argue for a diverse set of underlying pathomechanisms.

Here we investigated the contribution of the individual cell types with 4D in vivo time-lapse analyses using zebrafish (Danio rerio).

We show that optic fissure closure is initiated already during fissure morphogenesis, by a bilateral tissue flow. On the temporal side this is a continuation of the dynamic optic cup morphogenesis, on the nasal side, however, the cells are derived from the optic stalk. Subsequently a distinct cell population is translocated into the fissure margins from the optic stalk. The fusion of the margins is eventually triggered by an epithelial disassembly, driven by bi-potential pioneer cells, which establish the contact in between the margins and take the fate of both, neuroretina and RPE. Thus, the epithelial margins, framed by two conjoined epithelia, are transformed into the inner neuroretina and the outer RPE.

The process described here represents a fundamental mechanism of a seamless connection of aligned epithelial margins. Our findings can likely be applied to similar fusion processes, like palatal shelf fusion with key relevance for development and growth.

Vortrag 7:

Titel: Highly variable penetrance of abnormal phenotypes in embryonic lethal knock out mice

Autoren/Adressen: Robert Wilson (The Francis Crick Institute), 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), Emily Hardman (The Francis Crick Institute), Fabrice Prin (The Francis Crick Institute), Christina McGuire (The Francis Crick Institute), Ramiro Ramirez-Solis (Wellcome Trust Sanger Institute), Jacqui White (Wellcome Trust Sanger Institute), Antonella Galli (Wellcome Trust Sanger Institute), Catherine Tudor (Wellcome Trust Sanger Institute), Elizabeth Tuck (Wellcome Trust Sanger Institute), Cecilia Mazzeo (Wellcome Trust Sanger Institute), James Smith (The Francis Crick Institute), Elizabeth Robertson (University of Oxford), David Adams (Wellcome Trust Sanger Institute), Timothy Mohun (The Francis Crick Institute), Wolfgang Weninger (Medical University of Vienna);

Abstract:
The Deciphering the Mechanisms of Developmental Disorders (DMDD) programme (https://dmdd.org.uk/) characterises morphological defects in mouse embryos of embryonic lethal and subviable knockout lines with the aim of uncovering the role of the targeted gene in normal embryonic development and establishing correlates with human congenital abnormalities.

Homozygous mutant and wild type E14.5 embryos of the C57BL/6 strain were imaged in the DMDD project by using High resolution episcopic microscopy (HREM) and screened for morphological defects.

Virtually all mutant embryos we have analysed show multiple abnormal phenotypes, and amongst the 42 lines we have analysed the defects observed affect most organ systems. Within each mutant line, the phenotypes of individual embryos form distinct but overlapping sets. Prevalent phenotypes include subcutaneous edema, malformations of the heart or great vessels, abnormalities in forebrain morphology and the musculature of the eyes, as well as loss or abnormal size of the hypoglossal nerve. Strikingly, no matter how profound the malformation, almost no phenotypes are fully penetrant, and within a single line different phenotypes can vary considerably in penetrance because of the overlapping but distinct spectra of abnormalities observed between individual embryos.

These findings have challenging implications for efforts to identify human disease correlates, although we present some potential mouse models of known human diseases, which appear to replicate the symptoms of the human disease.

Vortrag 8:

Titel: Generation of a human vascularized in vitro 3D tumor model using induced pluripotent stem cells

Autoren/Adressen: Philipp Wörsdörfer (University of Würzburg), Nahide Dalda (University of Würzburg), Katharina Günther (University of Würzburg), Frank Edenhofer (University of Würzburg), Erik Henke (University of Würzburg), Süleyman Ergün (University of Würzburg);

Abstract:
In vitro tumor models including stromal components are important tools for research and can serve as screening platforms for the development of new drugs. The aim of our study is to develop a novel vascularized all human in vitro tumor model system.

We used human induced pluripotent stem cells (iPSCs) to generate mesodermal progenitors with splanchnopleuric identity. For that purpose we induced mesodermal fate by Wnt-activation and provided further patterning cues by applying additional cytokines e.g. BMP4. We hypothesized that such cells should be able to give rise to vascular as well as hematopoetic cell types, both important components of the tumor stroma.The induced mesodermal progenitor cells were mixed with GFP-labeled tumor cells (e.g. the breast cancer line MDA-MB 435s) and grown as three-dimensional sphere cultures with a diameter of approximately 500 µm.

Capillary structures developed within the generated tumor spheres as demonstrated by immunofluorescence analyses using specific antibodies targeted against CD34, CD31 as well as VE-Cadherin. Moreover, a capsule formed by smooth muscle actin positive cells covering the surface of the sphere was observed. Furthermore, Collagen I deposition could be detected.

We present a novel strategy for the development of an all human in vitro tumor model system which could be used e.g. for preliminary screening of anticancer drugs in cell culture.

Vortrag 9:

Titel: Meprin BETA regulates the phagocytic potential of macrophages via TREM2 proteolysis

Autoren/Adressen: Philipp Arnold (Kiel University), Dennis Berner (Kiel University), Janna Schneppenheim (Kiel University), Christian Haass (Deutsches Zentrum für Neurodegenerative Erkrankungen), Ralph Lucius (Kiel University), Christoph Becker-Pauly (Kiel University); p.arnold@anat.uni-kiel.de

Abstract:
With this project we aim to better understand the proteolytic role of the metalloproteinase meprin BETA during inflammatory processes. Here, we focus on the cellular response after meprin BETA-mediated cleavage of the phagocytosis associated receptor TREM2, which was previously identified as an ADAM10 substrate.

In this project we use different cell culture and biochemical methods. These include protein overexpression, Western blot, FACS analysis and phagocytosis assays.

We show that meprin BETA can remove the TREM2 receptor from the cell surface. Inhibition of the GAMMA-secretase complex with DAPT revealed an increase in meprin β-mediated C-terminal fragments of TREM2. However, unlike ADAM10, meprin BETA completely degrades the ectodomain of the receptor, which significantly impairs phagocytosis of the macrophage cell line THP-1.

In recent years we showed that meprin BETA cleaves many substrates that play important roles during inflammatory processes. For instance, meprin BETA influences cellular transmigration through cleavage of CD99 and regulates the level of the cytokine receptor IL-6 receptor on human granulocytes. Here, we show that the proteolytic activity of meprin BETA lead to decreased phagocytosis potential in macrophages through cleavage of the TREM2 receptor. As reduced levels of TREM2 are associated with elevated transcriptional levels of cytokines such as IL-6, this could lead to an increased recruitment of immune cells, as IL-6 dependent signaling in endothelial cells induces immune-cell diapedesis to the site of inflammation.

Vortrag 10:

Titel: The tumor microenvironment: a physical barrier protecting tumor cells

Autoren/Adressen: Rajender Nandigama (Universitaet Wuerzburg), Leonie Rossow (Universitaet Wuerzburg), Süleyman Ergün (Universitaet Wuerzburg), Erik Henke (Universitaet Wuerzburg); erik.henke@uni-wuerzburg.de

Abstract:
In addition to malignant tumor cells solid tumors are formed by a variety of non-transformed cellular and structural components. These components, the tumor vasculature, its stroma, infiltrating immune cells and the extracellular matrix (ECM) are commonly referred to as the tumor microenvironment (TME). The TME differs substantially from other organs and several of these altered aspects result in a strongly reduced efficacy of therapeutic agents and contributes to drug resistance.

Using systematic expression profiling approaches we identified targetable regulators of the TME correlated with intrinsic resistance to therapy. Results of the profiling approaches were validated in engineered 3D tumor models and in wide set of different in vivo cancer models.

We developed novel ECM- and vasculature-targeted approaches that are suitable to increase intratumoral drug delivery and therapeutic response. In various tumor models inhibition of collagen stabilization and cross-linking improved tumor supply and oxygenation. As a result response to chemotherapy was significantly enhanced even in metastatic disease. Moreover, our research indicated a strong interdependence of vascular and matrix properties, cooperating to protect tumor cells from exposure to therapeutic agents. Consequently, combining ECM-destabilization with therapies resulting in improved vascular functionality further increased efficacy of subsequently administered chemotherapy.

We demonstrate that both increased deposition of extracellular components and the dysfunctionality of the tumor vasculature provide a physical barrier that protects tumor cells from exposure to therapeutic agents. Re-engineering the TME is promising way to reduce side effects of cancer therapy, increase efficacy and to circumvent intrinsic resistance.

Vortrag 11:

Titel: The actin-binding protein adducin regulates Dsg3-dependent adhesive functions and trafficking

Autoren/Adressen: Matthias Hiermaier (Ludwig-Maximilians-Universität München), Franziska Vielmuth (Ludwig-Maximilians-Universität München), Jens Waschke (Ludwig-Maximilians-Universität München), Volker Spindler (Ludwig-Maximilians-Universität München); volker.spindler@med.uni-muenchen.de

Abstract:
Desmosomes are cell-cell junctions which provide strong intercellular adhesion and couple the intermediate filament cytoskeleton to sites of cell-cell contact. We have previously shown that the actin-binding protein adducin is required for desmosome function. The aim of this project was to gain new insights into the role of adducin for desmosomal molecule turnover, trafficking to the cell membrane and incorporation into desmosomes.

Experiments were conducted in human keratinocytes with siRNA-mediated knock-down of adducin and in knock-out cell lines obtained with the CRISPR/CAS9 system. Atomic force microscopy (AFM) was used on living keratinocytes to investigate the binding properties of the desmosmomal adhesion molecule desmoglein(Dsg)3 and trafficking was evaluated by life cell imaging of GFP-tagged Dsg3. Intercellular cohesion was quantified by Dispase-based dissociation assays and correlated with immunostainings, Western blots and biotinylation assays.

Adducin-deficient keratinocytes showed a lower frequency of Dsg3-mediated binding events in AFM measurements but surprisingly the binding forces of individual binding events were increased compared to control cells. Increased amounts of membrane tethers in adducin-deficient cells indicate a less rigid membrane structure and are in line with the role of adducin in organizing the cortical actin cytoskeleton. Dispase-based dissociation assays confirmed an overall reduced intercellular binding strength in cells lacking adducin although the desmosomal molecules Dsg2 and Dsg3 were upregulated in the membrane. Life cell imaging experiments revealed a less directed transport of Dsg3-containing vesicles to the cell membrane.

Our data indicate that adducin contributes to intercellular adhesion by regulating Dsg3-dependent adhesive functions and Dsg3 trafficking.

Vortrag 12:

Titel: Adaptor proteins of the crumbs complex in health and disease

Autoren/Adressen: Rui Sun (University of Regensburg), Olga Panichkina (University of Regensburg), Pavel Nedvetsky (University of Münster), Daniela Sparrer (University of Regensburg), Michael P. Krahn (University of Münster and University of Regensburg); Michael.Krahn@ukmuenster.de

Abstract:
The Crumbs complex has been intensively analyzed regarding its function during the establishment of apical-basal polarity and tight junction formation in epithelia. However, recent data suggest that this complex, in particular its adaptor proteins Pals1 and PATJ, are not only essential for cell polarization but also play a crucial role in different pathways, namely the Hippo- and TGFbeta-signaling cascades. Thus, the Crumbs complex serves a signaling hub, integrating different stimuli and regulates not only cell polarization but cell proliferation, contact inhibition and cell migration, too.

Using cell culture models, conditional knockout mice and xenograph transplantation models, we aim to elucidate the mechanisms of the Crumbs complex regulating different cellular processes in health and disease.

Currently we investigate the role of the Crumbs complex proteins during the pathogenesis of colorectal cancer, in which Pals1 but not Crumbs is frequently downregulated. Pals1-deficient cells exhibit an increased migration capacity in vitro and produce significantly more metastases in a mouse xenograph implantation model in vivo. On the other hand, Pals1 as well as PATJ are involved in the regulation of tubular structures and an impaired function of these proteins results in the formation of kidney cysts. Finally, we identified PATJ as a critical mechanosensor in endothelial cells, which is important for the regulation of blood pressure.

Taken together, the Crumbs complex, in particular its adaptor proteins emerge as mediators of critical cellular pathways, regulating several cellular functions and organ morphogenesis, far beyond the simple formation of the paracellular diffusion barrier.

Vortrag 13:

Titel: CEACAMs as novel factors that modulate the risk of helicobacter pylori caused gastritis and gastric cancer

Autoren/Adressen: Bernhard B. Singer (University Duisburg-Essen), Marc Reschke (University Duisburg-Essen), Verena Schmitt (University Duisburg-Essen), Anahita Javaheri (Technische Universität München), Kristof Moonens (Vrije Universiteit Brussel), Raquel Mejias-Luque (Technische Universität München), Steffen Backert (Friedrich Alexander University Erlangen), Han Remaut (Vrije Universiteit Brussel), Gunther Wennemuth (University Duisburg-Essen), Alexej Schmidt (Umeå University), Markus Gerhard (Technische Universität München); bernhard.singer@uk-essen.de

Abstract:
Gastric cancer is the second most common cause of cancer-related deaths and far more than half of all cases of stomach cancer are linked to Helicobacter pylori infection. This bacterium establishes a life-long infection in humans and is estimated to inhabit the stomach lining of more than half the world's population. Although H. pylori infection does not cause illness in most cases, it remains a major risk factor.

Utilizing a broad range of collaborations and methods e.g. flow cytometry, western blot, sequencing and crystallography we tested if members of the human CEA-related cell adhesion molecule (CEACAM) family interact with H. pylori. CEACAMs were already described as pathogen receptors for e.g. Moraxella and Neisseria.

We identified various human CEACAMs as novel receptors of H. pylori and identified HopQ as the adhesin that specifically binds CEACAM1, CEACAM3, CEACAM5 and CEACAM6. The HopQ - CEACAM binding is glycan- and pH-independent and targeted to the N-domain of CEACAMs. H. pylori binding induces CEACAM1 mediated signaling, and the HopQ-CEACAM1 interaction enables translocation of the virulence factor CagA into host cells. Based on the crystal structure of HopQ, we found that a β-hairpin insertion (HopQ-ID) in HopQ's extracellular 3+4 helix bundle domain is important for CEACAM binding. Utilizing our HopQ derived mAb we found that different H. pylori strains carry different amounts of HopQ and thus one could speculate that highly HopQ positive H. pylori are more pathogenic.

Taken together, our data suggest the HopQ-CEACAM1 interaction as promising novel therapeutic target to combat H. pylori-associated diseases.

Vortrag 14:

Titel: Local vs. systemic effects of calcineurin inhibition on salt reabsorption along the distal nephron of the kidney

Autoren/Adressen: Katharina Ilse Blankenstein (Charité-Universitätsmedizin Berlin), Aljona Borschewski (Charité-Universitätsmedizin Berlin), Sebastian Bachmann (Charité-Universitätsmedizin Berlin), Kerim Mutig (Charité-Universitätsmedizin Berlin); kerim.mutig@charite.de

Abstract:
Calcineurin dephosphorylates nuclear factor of activated T cells transcription factors, thereby stimulating T cell-mediated immune responses. Calcineurin inhibitors are instrumental for immunosuppression after organ transplantation but may cause side effects, including hypertension and electrolyte disorders. Kidneys were recently shown to display activation of the furosemide-sensitive Na-K-2Cl cotransporter (NKCC2) in the thick ascending limb (TAL) and the thiazide-sensitive Na-Cl cotransporter (NCC) in the distal convoluted tubule (DCT) upon calcineurin inhibition using cyclosporin A (CsA). A role of major hormones like arginine vasopressin (AVP) or renin-angiotensin-aldosterone system has been proposed. This study addresses local vs. systemic mechanisms of CsA-induced NKCC2 and NCC activation.

Wistar rats, vasopressin (AVP)-deficient Brattleboro rats, and cultured rat TAL and DCT cells were treated with CsA at short (30 mg/kg for 1-4h) or long term (30 mg/kg for 14 days).

Acute administration of CsA to Wistar rats significantly increased abundance of phosphorylated NKCC2, NCC, and their activating kinase SPAK suggesting intraepithelial activating effects. Chronic CsA administration led to salt retention and hypertension, along with stimulation of renin and suppression of renal cyclooxygenase 2, indicating contribution of endocrine and paracrine mechanisms at long term. In Brattleboro rats, CsA induced activation of NCC, but not NKCC2, and similar effects were obtained in cultured cells in the absence of AVP. Stimulation of cultured TAL cells with AVP agonist restored their responsiveness to CsA.

Our results suggest that the CsA-induced activation of NCC can be achieved solely by direct epithelial action of calcineurin inhibition, whereas its effect on NKCC2 requires concomitant stimulation by AVP.

Vortrag 15:

Titel: Conditional deletion of vegfr2 mimicks essential characteristics of neovascularization glaucoma in mice

Autoren/Adressen: Anita Grundl (University of Regensburg), Herbert Jägle (University Clinic Regensburg), Ernst R Tamm (University of Regensburg), Barbara M Braunger (University of Regensburg); Barbara.Braunger@ur.de

Abstract:
Neovascular glaucoma develops as complication of ocular hypoxia as seen in patients suffering from diabetic retinopathy, central vein oclusion or high blood pressure. Here we conditionally deleted vascular endothelial growth factor receptor 2 (VEGFR2) in the entire eye and generated an animal model that mimicks essential characteristics of neovascularization glaucoma.

Floxed VEGFR2 mice were crossed with CAG-Cre mice, expressing the Cre recombinase under control of a tamoxifen-responsive chicken actin promoter. The successful deletion of VEGFR2 was confirmed. Retinal structure and function were studied by light and electron microscopy, immunohistochemistry, real time RT-PCR and western blot analyses.

Deletion of VEGFR2 resulted in an avascular retina, concomittant with elevated HIF-1α stabilization and elevated expression levels of VEGF-a and Angiopoetin2. Consequently, the proliferation of the vessels of the iris occured. These capillaries exibited a fenestrated endothelium, resulting in their leakyness and hemorrhages in the anterior chamber of the eye.

These data support a novel and flexible model of neovascularisation glaucoma in mice. Furthermore, they highlight the importance of VEGFR2 for retinal angiogenesis.

Vortrag 16:

Titel: Modulation of formyl peptide receptors activity reduced aβ1-42-induced glial cell activation in Alzheimer's disease

Autoren/Adressen: Nicole Schröder (RWTH Aachen University), Anja Schaffrath (RWTH Aachen University), Josua Welter (RWTH Aachen University), Angelika Griep (University of Bonn), Michael Heneka (University of Bonn), Thomas Pufe (RWTH Aachen University), Lars-Ove Brandenburg (RWTH Aachen University); nischroeder@ukaachen.de

Abstract:
The chemotactic G-protein coupled formyl peptide receptor (FPR), a receptor linked with the signal transduction and uptake of Aβ1-42 (Aβ) in glial cells, seems to be linked with the massive increase of gliosis in the brain due to the chronic inflammation of Alzheimer's disease (AD). Our work is focused on mice FPRs, mFPR1 and mFPR2, which are orthologous to human FPR1 and FPRL1. We assume that their activity is critically involved in neuroinflammation and neurodegeneration in the Aβ-affected brain.

At first, we investigated the direct influence on inflammatory processes by stereotactic injection of fibrillary or oligomeric Aβ in the hippocampus of wildtype, mFPR1- or mFPR2-deficient mice. Moreover we used an AD mouse model to inject the pro-inflammatory FPR agonist fMLF, FPR1/2 antagonist Boc-FLFLFL (Boc) or anti-inflammatory FPR2 agonist Ac2-26 intraperitoneally twice a week about 20 weeks. After a Morris water maze (MWM) glial cell density and mRNA expression of Aβ-degrading enzymes were analyzed.

Whereas mFPR2-deficient mice showed a significant decreased astrocytes cell density, FPR1 deficiency resulted in a higher microglial cell density after one week of Aβ injection. Long-term injection of fMLF and Boc reduced significantly the glial cell density in the hippocampus, Boc appears to improve behavior in the MWM and the mRNA expression of Aβ-degrading enzymes where significantly increased after Boc treatment in AD mice.

Altogether, our results suggest that FPRs have an impact on inflammation and glial cell activation in the course of AD. Especially inhibition with Boc seems to be a promising approach.

Vortrag 17:

Titel: The taste of bacteria - a bacterial signal peptide increases mucociliary clearance in explanted mouse trachea by stimulating cholinergic chemosensory cells

Autoren/Adressen: Alexander Perniss (Justus-Liebig-University Giessen), Bernd Bufe (University of Saarland), Frank Zufall (University of Saarland), Gabriela Krasteva-Christ (University of Saarland), Wolfgang Kummer (Justus-Liebig-University Giessen); Alexander.Perniss@anatomie.med.uni-giessen.de

Abstract:
Bacterial signal peptides trigger innate immune responses via formyl peptide receptors (FPRs), present in immune cells. We investigated their effects on mucociliary clearance, which is a major defense mechanism against invasive bacteria in the trachea.

The trachea of C57Bl6, TRPM5- (transient receptor potential channel 5), PLCβ2-deficient mice (phospholipase Cβ2) (two crucial components of the canonical taste transduction cascade) and FVB/NCrl mice was explanted and particle transport speed (PTS) was visualized by tracking directed transport of dynabeads. The transcriptome of single tracheal ciliated and brush cells, a cholinergic chemosensory epithelial cell type, was analyzed by single cell deep sequencing.

Deep sequencing of single epithelial cells showed FPR expression in both ciliated and brush cells. The N-formylated bacterial signal peptide FL185 (10 µM), a product of pathogenic bacteria (e.g. E. coli), increased PTS from 45±2 to 73±3 µm/s (mean±SEM). Specific FPR1 and FPR2 inhibitors [cyclosporine H (1 µM) and t-BOC2 (10 µM)] did not reduce the effect, it was also conserved in FVB/NCrl mice lacking a functional FPR3. In contrast, FL185 was ineffective to increase PTS in TRPM5- and PLCβ2-deficient mice. Atropine (1 µM), a muscarinic antagonist, diminished the effect of FL185.

A bacterial signal peptide of pathogenic origin stimulates cilia-driven mucociliary clearance. Rather than being mediated by FPR, this mechanism depends on classical taste transduction cascade components which are uniquely coexpressed in the trachea by brush cells, and subsequent cholinergic signaling to ciliated cells. We interpret this as a novel defense mechanism against bacteria.

Vortrag 18:

Titel: Identification of an essential brain centre for vocalisation

Autoren/Adressen: Luis R. Hernandez-Miranda (Max-Delbrück-Centrum), Piere-Louis Ruffault (Max-Delbrück-Centrum), Gilles Fortin (CNRS), Carmen Birchmeier (Max-Delbrück-Centrum); luis.hernandes@mdc-berlin.de

Abstract:
Neuronal circuits controlling vocalisation rely on motor neurons that innervate laryngeal and expiratory muscles. How these motor neurons are coordinated to produce calls is not fully understood. Furthermore, functionally important premotor neurons in vocalisation are little known. Here, we set out to identify premotor neurons essential for vocalisation.

We analysed call production in various mouse mutant strains that affect development of distinct but overlapping hindbrain neural populations. In doing so, we identify the nucleus of the solitary tract (NTS, located in the hindbrain) to be essential for vocalizations. Genetically engineered mice to exclusively lack this nucleus were mute, i.e. unable to produce calls. Plethysmographic recordings illustrated that these mice are incapable of generating the expiratory pressure required for vocalisation. Connectivity studies showed that NTS neurons form functional connection with both laryngeal and expiratory motor neurons. Behavioral studies revealed that mothers neglect vocally affected pups.

Our recent findings show that NTS neurons directly connect to and entrain the activity of expiratory and laryngeal motor neurons at birth. Mice that specifically lack the NTS are mute and do not produce a call. This is caused because in the absence of the NTS mice are not able to regulate the laryngeal tension and expiratory pressure need in sound production. Furthermore, mothers neglect vocally impaired newborns.

Altogether, our data show that the NTS is essential for vocalisation in mice. Moreover, our work demonstrates that the NTS is an obligatory component of the neuronal circuitry that transforms breaths into calls.

Vortrag 19:

Titel: Getting smarter without EGFL7

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

Abstract:
In the adult hippocampus neural stem cells reside in the subgranular zone of the dentate gyrus where they give rise to adult-born neurons throughout life. Here we analyzed the molecular mechanisms applied by hippocampal neurons to govern neural stem cells and adult neurogenesis in the hippocampus. In particular, we studied the non-canonical Notch ligand EGFL7, which we have previously shown to regulate neural stem cells (Nat Cell Biol, 2009) in the subventricular zone (Nat Commun, 2017).

We applied conventional and tissue-specific conditional mouse models, advanced imaging techniques, molecular and cellular analyses in and ex vivo plus mouse behavior paradigms.

In the dentate gyrus EGFL7 was secreted by mature neurons, neural stem cells and blood vessels. The general loss-of-EGFL7 increased the amount of active neural stem cells and mature neurons. Conditional knock-out studies revealed that the tissue-specific loss-of-EGFL7 caused unique phenotypes that in combination reflected the general knock-out. RNASeq analyses suggest that the loss-of-EGFL7 led to a deregulation of epigenetic factors that caused the changes observed in adult hippocampal neurogenesis. Interestingly, EGFL7 knock-out animals displayed enhanced intelligence in the Morris water maze, touchscreen or Intellicage assay

In conclusion, EGFL7 is a protein secreted in the dentate gyrus by mature neurons and applied as a feedback mechanism to govern neural stem cells in order to contain excessive, unproductive neurogenesis. Loss-of-EGFL7 stimulated adult neurogenesis and neuronal survival, which increased intelligence in several mouse behavior paradigms. Further studies will reveal whether EGFL7 may serve as a tool to attenuate cognitive decline happening during aging.

Vortrag 20:

Titel: Synaptic bioactive phospholipid signaling: a new target for treating psychiatric disorders

Autoren/Adressen: Carine Thalman (University Medical Center Mainz), Guilherme Horta (University Medical Center Mainz), Irmgard Tegeder (Goethe University Frankfurt), Torfi Sigrudsson (Goethe University Frankfurt), Konstantin Radyuschkin (University Medical Center Mainz), Robert Nitsch (University Medical Center Mainz), Johannes Vogt (University Medical Center Mainz); johannes.vogt@unimedizin-mainz.de

Abstract:
Recent data suggest that synaptic lysophosphatidic acid (LPA) regulates cortical excitation-inhibition (E/I) balance controlling cortical sensory information processing in mouse and man. However, the source of synaptic LPA as well as its dynamic regulation by glutamatergic transmission, and the effects of LPA-synthesis inhibition on synaptic function are still not clear.

Immunofluorescence and electron microscopy were performed to localize the expression of autotaxin (ATX), the main LPA-synthesis enzyme, at the synapse. Astrocytic cell cultures and mass spectrometry were used to determine the dynamic regulation of ATX-function, while in-vitro and in-vivo electrophysiology, and behavioral assessment were used to assess the effect of ATX-inhibition.

We detected ATX in perisynaptic lamellae of astrocytes and found that ATX-sorting towards fine astrocytic processes and its enzymatic activity exerted in the extracellular space was dynamically regulated via astrocytic glutamate receptors. Pharmacological and genetical ATX-inhibition, both, rescued hyperexcitability syndromes due to altered bioactive lipid signaling in a mouse model of a human loss-of-function single nucleotide polymorphism (SNP) or in a ketamine model of schizophrenia. Interestingly, only altered behaviors observed in these syndromes were recovered by ATX-inhibition, while normal animals were not affected. Paired recordings of long-range cortical connections in freely moving animals revealed that increased gamma coherence, which relied on cortical hyperexcitability and is an endophenotype associated with schizophrenia, was reverted to control levels by ATX-inhibition.

Our data suggest that targeting ATX might be a versatile strategy for a novel drug therapy of psychiatric disorders.

Vortrag 21:

Titel: Synaptic activity regulates neuronal energy metabolism - evidence for a neuronal warburg effect

Autoren/Adressen: Carlos Bas Orth (Ruprecht-Karls-University Heidelberg), Yan-Wei Tan (Ruprecht-Karls-University Heidelberg), David Lau (Ruprecht-Karls-University Heidelberg), Hilmar Bading (Ruprecht-Karls-University Heidelberg); Bas-Orth@nbio.uni-heidelberg.de

Abstract:
Synaptic activity drives changes in gene expression to promote adaptations of neuronal structure and function. One example is the build-up of acquired neuroprotection, a synaptic activity- and gene transcription-mediated increase in the resistance of neurons against harmful conditions. A hallmark of acquired neuroprotection is the stabilization of mitochondrial structure and function. To better understand the basis of this mitoprotection, we here analyzed activity-mediated changes of the molecular composition of mitochondria and asked how these changes affect mitochondrial function.

Activity-regulated genes with known roles in mitochondrial function were identified by in-silico analysis of previously published transcriptome data and were confirmed by qRT-PCR analysis in mouse and rat hippocampal cultures and mouse and rat hippocampus in vivo. We used FACS sorting to show that, in mixed neuron glia cocultures, activity-dependent regulation of the identified genes occurred in neurons. Functional consequences of activity-induced gene expression changes were analyzed by Immunoblotting, biochemical quantification of L-lactate, and fluorescence based respirometry.

Synaptic activity caused an increased expression of glycolytic genes and a decreased expression of genes related to oxidative phosphorylation, mitochondrial biogenesis, and maintenance. This coordinated up- and down-regulation of functionally related genes caused a decrease in oxidative phosphorylation and a concomitant increase in aerobic glycolysis, also known as Warburg effect.

We identified a neuronal Warburg effect that represents a novel form of activity-dependent neuroadaptation. We suggest that the shift towards aerobic glycolysis reduces oxidative phosphorylation-related generation of reactive oxygen species and maintains spare respiratory capacity, and thus contributes to the build-up of acquired neuroprotection.

Vortrag 22:

Titel: When were you born where? A dynamic brain-wide atlas of VZ-born neurons

Autoren/Adressen: Robin J. Wagener (University of Geneva), Andrea V.S. Lopes (University of Geneva), Natalia Baumann (University of Geneva), Denis Jabaudon (University of Geneva);
JanRobin.Wagener@unige.ch

Abstract:
During development of the mammalian central nervous system, neurons are born from two distinct types of germinal zones: (1) ventricular zones (VZ), which line the walls of the ventricular system, or (2) sub-ventricular zones (SVZ), located away from the ventricular cavities. Understanding the dynamic relationships between VZ and SVZ-born neurons across brain regions during development may be important to understand brain structure and circuit function, yet classical methods of neuronal birthdating using thymidine analogues to label DNA does not distinguish between VZ- and SVZ-born neurons.

Here, we take advantage of the FlashTag birthdating approach developed in our laboratory (Telley, Govindan et al., Science 2016) to label VZ-born neurons as they are being born in contact with the ventricular wall.

By combining FlashTag labeling with chronic BrdU administration, we are generating a dynamic spatio-temporal map of VZ-born neurons between embryonic days (E)10.5 and E17.5 in the mouse brain.

The atlas will be provided as an online resource to systematically investigate the relationship between neuronal place and date of birth, and specific connectivity features. This could help in understanding the principles underlying brain architecture and wiring patterns across the central nervous system.

Vortrag 23:

Titel: Synaptic maintenance in the absence of synaptic activity in the auditory brainstem

Autoren/Adressen: Christoph Körber (Heidelberg University), Marc Willaredt (Carl von Ossietzky University Oldenburg), Hans Gerd Nothwang (Carl von Ossietzky University Oldenburg);koerber@ana.uni-heidelberg.de

Abstract:
The maintenance and integrity of synapses are thought to rely on the presence of neuronal activity. This includes the release of synaptic vesicles (SVs) at presynaptic active zones (AZs), either in response to an action potential or spontaneously. SV release is inhibited by bacterial neurotoxins which cleave neuronal SNARE proteins thereby preventing the assembly of the critical SNARE complex. One of these neurotoxins is tetanus toxin (TeNT) which cleaves the SNARE protein synaptobrevin/VAMP2.

We expressed TeNT in the bushy cells of the ventral cochlear nucleus (VCN) in the auditory brainstem using a specific Cre-driver mouse line (Math5). The globular bushy cells of the VCN give rise to the calyx of Held in the contralateral medial nucleus of the trapezoid body (MNTB), a giant axo-somatic synapse that comprises 300-700 individual AZs.

The expression of TeNT at this specific synapse led to a gradual decrease of SV release with the virtual absence of neurotransmission by P15. However, we did not observe any alterations in the MNTB, neither on the number and size of the MNTB principal cells, nor on the morphology of calyx of Held synapse. Moreover, TeNT expression did not lead to a reduction in AZ number or a loss of SVs from AZs, albeit the number of "docked" SVs close to the plasma membrane was strongly reduced

We therefore conclude that synaptic activity is not necessary for the maintenance of synapses but rather contributes to the remodeling of synapses in order to meet the current requirements of the neuronal circuit.

Vortrag 24:

Titel: CPI-17 as a new oligodendrocyte marker and its influence on remyelination

Autoren/Adressen: Stella Nyamoya (Faculty of Medicine, RWTH Aachen University), Julian Lanvermann (Faculty of Medicine, RWTH Aachen University), Carsten Dornblut (Fritz Lipmann Institute), Helen Morrison (Fritz Lipmann Institute), Markus Kipp (Ludwig-Maximilians-University of Munich); snyamoya@ukaachen.de

Abstract:
In the central nervous system, oligodendrocytes synthesize a specialized membrane, the myelin membrane, which enwraps the axons in a multilamellar fashion to provide fast action potential conduction and to ensure axonal integrity. The process of axonal wrapping is complex and poorly understood, but requires a tight regulation of sequential events, among dynamic changes of the cytoskeleton. In this project, we investigate the role of CPI-17 (C-kinase-activated protein phosphatase-1 (PP1) inhibitor, 17kDa), an endogenous myosin phosphatase inhibitor known to regulate smooth muscle motility.

Genome-wide array analyses were performed to screen for the relation of oligodendrocyte dysfunction and expressional changes of proteins involved actin-myosin dynamics. Different de- and remyelination in vivo models as well as optic nerve preparations were used to address the expression of CPI-17 along the oligodendrocyte lineage. CPI-17 deficient mice were included to perform functional studies. Oligodendrocyte cultures were used to investigate membrane protrusion and motility.

Gene-array analyses revealed a dramatic reduction of CPI-17 expression in a model of primary oligodendrocyte apoptosis. Loss of CPI-17 was confirmed in inflammatory and non-inflammatory demyelination models as well as human multiple sclerosis lesions. Immunoflourescence double labelling experiments revealed expression of CPI-17 in mature oligodendrocytes but not oligodendrocyte progenitor cells. Developmental myelination as well as remyelination was impaired in CPI-17-/- mice. Finally, in vitro studies revealed disturbed membrane dynamics in cultured CPI17-/- oligodendrocytes.

In this study, we identified a novel regulator of myelination and remyelination. We speculate that CPI17-induced membrane blebbing generates protrusion forces and/or rapid membrane extension during myelination and remyelination.

Vortrag 25:

Titel: VEGF – a stimulus for nerve regeneration

Autoren/Adressen: Verena Theis (Institute of Anatomy, Ruhr-University Bochum), Lukas Pieczora (Institute of Anatomy, Ruhr-University Bochum), Carsten Theiss (Institute of Anatomy, Ruhr-University Bochum); verena.theis@rub.de

Abstract:
The vascular endothelial growth factor (VEGF) represents an important factor for angiogenesis and vascularization. Additionally VEGF also affects glial and neuronal cells within the CNS and PNS. Our group has shown that VEGF increases somato- and dendritogenesis in neonatal but not in mature CNS neurons and leads to axonal growth cone guidance in the PNS. All these effects are mediated by VEGF receptor 2 (VEGFR2). We now assume that microRNAs regulate VEGFR2 expression on post transcriptional- and translational level.

By means of miRNA profiling we identified miRNAs that are closely connected to VEGFR2 expression. Using in situ hybridization these miRNAs were located within different nerval structures, the amount of VEGFR2 mRNA and miRNAs was quantified by qPCR. To perform functional studies we cultivated dissociated neurons of the CNS and PNS and transfected these cells with so-called mimics and inhibitors.

In the CNS these microRNAs seem to block VEGFR2 mRNA during postnatal maturation of Purkinje cells and motoneurons, so that functionality of the receptor might be minimized and sensitivity for VEGF is decreased. In contrast, in sensory dorsal root ganglia the expression of VEGFR2 first increases during development and then descends slowly. Here, other microRNAs are co-expressed with VEGFR2. The transfection with inhibitors results in an increased amount of VEGFR2.

It is conceivable that these mechanisms lead to different capacities for neuroregeneration within the CNS as well as the sensory compared to the motoric system which are probably due to different sensitivities for VEGF.

Vortrag 26:

Titel: Molecular mechanisms of synapse formation

Autoren/Adressen: Tina Ghelani(1), Julio Viotti(1), Thomas Dresbach(1), Nina Wittenmayer(2), (1)Institute for Anatomy, University of Goettingen Medical School Goettingen, Germany (2)Brandenburg Medical School Theodor Fontane, Neuruppin, Germany; Nina.wittenmayer@mhb-fontane.de

Abstract:
Accompanying synaptogenesis, the transport of new synthesized synaptic proteins to synaptic sites involves association or processing of the proteins through the Golgi apparatus, loading on precursor transport organelles and transport along dendrites or axons to synapses.

By using high-resolution microscopy techniques, we can show the distribution of synaptic proteins at the Golgi apparatus and on their transport organelles in cultured hippocampal neurons. Using an RNAi based knockdown approach, we characterized the synaptogenic function of SSCAM /MAGI-2.

The analysis with STED microscopy reveals a punctate distribution of endogenous Munc13-1, Bassoon and Piccolo at specific Golgi subcompartments, which indicates their assembly on specific transport organelles. By using diaminobenzidine (DAB)-photoconversion and electron microscopy, we can demonstrate that recombinant CFP-Bassoon is loaded on 50nm clear core vesicles in the somatic compartment of neurons. Further, knockdown of all three S-SCAM/MAGI-2 isoforms in rat hippocampal neurons during early synaptogenesis leads to a dramatic reduction of synapses on these cells. Electrophysiological data show that synaptic transmission is severely reduced and spinogenesis is impaired in vitro and in vivo.

The analysis of synaptic proteins at the golgi apparatus and in the somatic region of neurons with high-resolution microscopy techniques indicates their early assembly on specific transport organelles at distinct golgi lamellae. Additionally, we found the postynaptic scaffolding molecule SSCAM /MAGI-2 as a regulator of synapse formation and maintenance in general.

Vortrag 27:

Titel: Keratin-dependent p38MAPK regulation is important for Dsg3 binding properties and required for loss of intercellular adhesion in pemphigus

Autoren/Adressen: Franziska Vielmuth(LMU Munich), Elias Walter (LMU Munich), Vera Rötzer (LMU Munich), Fanny Loschke (Leipzig University), Thomas M. Magin (Leipzig University), Volker Spindler (LMU Munich), Jens Waschke (LMU Munich); Franziska.Vielmuth@med.uni-muenchen.de

Abstract:
Alterations of the keratin filament cytoskeleton correlate with loss of intercellular adhesion in pemphigus vulgaris (PV), a blistering skin disease caused by autoantibodies against desmogleins(Dsg) 1 and 3 (PV-IgG). However, the contribution of keratins to altered adhesion in PV is largely unknown.

Atomic force microscopy(AFM) was combined with FRAP and biochemical approaches such as immunoprecipitation and Western blot analyses.

Keratin-deficient keratinocytes(KtyII k.o.) displayed weaker Dsg3-mediated binding events compared to wildtype cell line(KtyII wt) whereas Dsg1-mediated binding events showed an altered distribution pattern at cell borders, indicating that pemphigus antigens are differentially regulated by keratins. AFM and FRAP experiments revealed higher mobility of Dsg3 molecules in KtyII k.o. cells and demonstrated that depletion of Dsg3 in response to PV-IgG occurred faster. In contrast, incubation with PV-IgG had only a minor additional effect on impaired cell adhesion in keratin-deficient cells. This could be due to the robust p38MAPK activation in KtyII k.o. cells in the steady state. In support, inhibition of p38MAPK reinforced intercellular adhesion, increased binding strength of Dsg3 in steady state conditions and blocked PV-IgG-induced loss of cell cohesion in KtyII k.o. cells. The observation that anisomycin-mediated p38MAPK activation reduced cell cohesion indicates that keratin-independent mechanisms regulating p38MAPK activity also contribute to keratinocyte adhesion. In support, we identified a complex consisting of Dsg3, p38MAPK and the keratin adaptor RACK1 in keratinocytes.

These results indicate that keratins regulate p38MAPK signaling thus are important for impaired cell cohesion in pemphigus.

Vortrag 28:

Titel: Fiber heterogeneity in the sagittal stratum – decoding its fine structure at ultra-high resolution

Autoren/Adressen: Svenja Caspers (Heinrich-Heine-University Düsseldorf), Markus Axer (Research Centre Jülich), David Grässel (Research Centre Jülich), Katrin Amunts (Heinrich-Heine-University Düsseldorf), Karl Zilles (Research Centre Jülich); svenja.caspers@hhu.de

Abstract:
The sagittal stratum (SagStrat) of the human brain is a major white matter tract containing connections between posterior cortical regions and subcortical nuclei. With only limited knowledge about their specific origin (monkey tracer studies), the present study provides the first comprehensive analysis of the SagStrat fiber architecture in vervet monkey and human brains using ultra-high resolution 3D polarized light imaging (3D-PLI) and identifies relevant features for informing tractography and in-vivo diffusion magnetic resonance imaging (dMRI).

Coroanl cryo-microtome sections through posterior parts of a vervet monkey brain and a human hemisphere were used for 3D-PLI analysis (in-plane resolution: 1 µm), providing 3D high-resolution fiber orientation maps (FOMs). In-vivo dMRI data from an adult human were obtained at 3T, reconstructed for voxel-wise fiber orientations.

3D-PLI shows that SagStrat is a bipartite structure, consisting of an outer and inner stripe of largely sagittally running fibers. Small fiber bundles enter and depart almost perpendicular to the main sagittal direction. This is also visible in in-vivo dMRI data. Fibers entering and leaving SagStrat are topographically arranged. In its dorsal to lateral parts, parietal intermingle with transcallosal fibers, while in the middle and ventral parts, temporo-occipital fibers are found.

With 3D-PLI showing all fibers at ultra-high spatial resolution, we demonstrated a dorso-ventral topographical arrangement of different intermingling fiber populations, beyond selective staining of single fiber bundles obtained in tracer studies. 3D-PLI thus provides information on fiber architecture both in monkey and human, which can be used for interpreting in-vivo dMRI in human basic and clinical neuroscience.

Vortrag 29:

Titel: Ex vivo microangioct: new dimensions in vascular imaging

Autoren/Adressen: Ruslan Hlushchuk (University of Bern), Sebastièn Barré (University of Bern), Laura Schaad (University of Bern), David Haberthür (University of Bern), Valentin Djonov (University of Bern); ruslan.hlushchuk@ana.unibe.ch

Abstract:
In angiogenesis research there are a few modalities available for the visualization of the vasculature. But none of them provides 3D-visualization of the vasculature down to the capillary level with the possibility for quantitative analysis.

Therefore, we aimed to develop a contrast agent appropriate for ex vivo microangioCT with superior perfusion features in order to visualize microvasculature, including capillaries. Moreover, it should be suitable for the successive morphological analysis using light or transmission electron microscopy.

After washing blood out the mice or rats were perfused with the novel contrast agent developed in cooperation with Fumedica AG (Switzerland). The organs of interest (kidney, skeletal muscles, brain, eye, heart etc.) were then harvested and fixated in PFA-solution. The samples were scanned (SkyScan 1172 or 1272) at different resolution levels: overview scans followed by detailed scan of the organ/site of interest. The volumes of interest were, if needed, excised, and processed for paraffin/epon embedding.

Besides the developed contrast agent we have developed an ex vivo microangioCT approach that swiftly provides 3D-microCT datasets of superior quality, which are sufficient for qualitative and quantitative analysis of the vasculature down to the capillary level. For example, in kidneys the glomeruli could be visualized and software-detected allowing their exhaustive counting in the whole organ within 24 hours.

The developed ex vivo microangioCT-approach provides microvascular imaging at unprecendented level and enables corrrelative microscopy. The aforementioned features and high reproducibility make it a reliable partner in a broad spectrum of basic research studies and clinically-oriented research where the microvasculature are of significant importance.

Vortrag 30:

Titel: 4D-high speed digital-holographic-microscopy (DHM) reveals new insights in sperm flagellar waveform

Autoren/Adressen: Michael Muschol (University of Duisburg Essen), Caroline Wenders (University of Duisburg Essen), Donner Babcock (University of Washington), Gunther Wennemuth (University of Duisburg Essen); gunther.wennemuth@uk-essen.de

Abstract:
2D projections of flagellar waveform of sperm on a XY plane are used in most studies to describe sperm swimming behavior. Such conventional microscopic imaging is used in andrology diagnostic tools such as computer-assisted sperm analysis (CASA). With conventional microscopic imaging no attention is paid to the Z-plane excursion of the sperm waveform. With this study we analyze, for the first time, the impact of movement in all 4 dimensions on sperm swimming pattern.

High speed digital-holographic-microscopy was applied on sperm to analyze flagellar waveforms and sperm swimming paths in four dimensions (X, Y, Z, and t).

During each beat cycle, excursions into the Z-plane travel as a wave down the sperm flagellum. These Z-plane excursions have nearly the same amplitude as the envelope of the flagellar waveform projected onto the XY-plane. Tracking of the heads of free swimming mouse sperm shows alternating changes between left-face- and right-face down-most configurations. At the maximum positive Z-plane excursion sperm roll from right face to left face and then roll back to their right face at the subsequent maximum negative Z-plane excursion. Surprisingly, there is a constant alternating chirality of rolling which is always counter clockwise for the first roll and clockwise for the counter-roll.

Sperm roll from one face to the other and back with a specific chirality and a chiral memory of the tail. We propose that this memory resides in a hypothetical elastic linkage built into the flagellar machinery. For the first we were able to visualize the waveform of free swimming sperm in four dimensions.

Vortrag 31:

Titel: Characterization of the transcription factor Math6 as an essential regulator of placental development

Autoren/Adressen: Marion Böing (Ruhr-Universität Bochum), Markus Napirei (Ruhr-Universität Bochum), Beate Brand-Saberi (Ruhr-Universität Bochum); marion.boeing@rub.de

Abstract:
The basic helix-loop-helix transcription factor Math6 (mouse atonal homolog 6) is important in numerous differentiation processes. The current work aims at the characterization of the recently developed Math6 knockout mouse. The homozygous knockout in females leads to a defect of placenta development, which reveals a completely novel and very complex phenotype.

First the spatiotemporal expression of Math6 was studied in placenta by in-situ hybridization and quantitative PCR. Furthermore, placental tissues of different developmental stages were investigated by morphometric and immunohistochemically and differential gene expression analysis to show differences between wild type and mutant mice.

Math6 is highly expressed during early pregnancy whereas the expression level decreases in later stages dramatically. The Math6 expression is localized in all placental layers, but only in a few cells of the decidua a strong and very specific expression remains during hole pregnancy. There are significant differences detectable in morphology of Math6 knockout placenta, for example vascularization and size and shape of decidua. Additionally, the number of uterine natural killer cells is significantly decreased and expression of placental markers confirm differences. At day 13.5 of pregnancy, a massive bleeding in uterus and placenta leads to death of the fetus. The majority of placentas and fetuses degrade.

The results of this work show that the maternal expression of the Math6 is essential for the development of placenta. It is assumed that the miscarriage is a consequence of a disordered decidualization and remodeling that leads to a defective regulation of maternal-fetal cross-talk and results in disturbed reproduction.

Vortrag 32:

Titel: Visualization of contractile function in the prostate reveals different effects of PDE5 inhibitors used for the treatment of benign prostatic hyperplasia

Autoren/Adressen: Andrea Mietens (Justus-Liebig-University), Robert Kügler (Justus-Liebig-University), Mathias Seidensticker (Justus-Liebig-University), Sabine Tasch (Justus-Liebig-University), Florian Wagenlehner (Justus-Liebig-University), Claudia Tomczyk (Justus-Liebig-University), Daniela Beyer (Justus-Liebig-University), Gail Risbridger (Department of Anatomy and Developmental Biology), Stuart Ellem (Department of Anatomy and Developmental Biology), Betty Exintaris (Monash Institute of Pharmaceutical Sciences), Ralf Middendorff (Justus-Liebig-University);
andrea.mietens@anatomie.med.uni-giessen.de

Abstract:
Pharmacologic treatment strategies for benign prostatic hyperplasia target and relax smooth muscle cells to reduce prostate muscle tone. Classical alpha1-adrenoreceptor antagonists like tamsulosin are often accompanied by disorders of emission whereas PDE5 inhibitors, a recently emerged therapeutic option, may be devoid of such unwanted effects. These divergent drug profiles suggest distinct local smooth muscle cell (SMC) compartments warranting detailed investigation.

Vital prostate tissue from patients and isolated glands and duct from rat were investigated by CLARITY and immunohistochemistry to localize PDE5. Effects of PDE5 inhibition on contractile function were directly visualized by novel time-lapse imaging.

In man, two distinct SMC compartments could be identified in intact prostate tissue. SMCs were interspersed around prostatic glands, additionally, we found a high number of PDE5-expressing SMCs in prostate excretory ducts. CLARITY illustrated the three-dimensional architecture of PDE5 positive SMCs in the prostate. Moreover, we directly visualized spontaneous contractile activity of intact human prostate tissue which was strongly decreased by PDE5 inhibitor sildenafil. Comparative experiments with rat tissues allowed to analyze contractility of single glands and to allocate spontaneous contractions to glandular function. In contrast, isolated excretory ducts lacked spontaneous contractility, but pronounced contractions were elicited by noradrenaline. PDE5 inhibition did not affect these induced contractions.

In the prostate, SMCs are localized in the interstitial (periglandular) compartment and prostatic ducts, both express PDE5. The interstitial localization might explain beneficial effects of PDE5 inhibitors by decreasing intraprostatic muscle tone. Meanwhile, our data regarding the ducts suggest that (noradrenaline-induced) contractions of prostatic ducts to emit secretions remain unaltered.

Vortrag 33:

Titel: Blood-brain barrier breakdown following experimental cerebral ischemia is associated with endothelial degeneration along the different segments of the vascular tree

Autoren/Adressen: Martin Krueger (University Leipzig), Bianca Mages (University Leipzig), Wolfgang Härtig (University Leipzig), Ingo Bechmann (University Leipzig), Dominik Michalski (University Leipzig); martin.krueger@medizin.uni-leipzig.de

Abstract:
Recanalization of occluded arteries still represents the only causal and therefore central treatment of ischemic stroke. However, restoration of local blood flow further impairs the ischemia-affected vasculature, with an increased risk for hemorrhagic transformation and intracranial bleeding. Moreover, the ischemia-related brain edema is aggravated by the loss of blood-brain barrier (BBB) function, which impairs the outcome of concerned patients.

To systematically investigate vascular effects of ischemia/reperfusion, filament-based models of permanent (pMCAO) and transient middle cerebral artery occlusion (tMCAO) were applied in mice. Further, the translationally relevant model of embolic middle cerebral artery occlusion (eMCAO) was applied in rats. Areas showing BBB breakdown were identified by intravenous application of FITC-conjugated albumin 1 hour prior to sacrifice at 24h after ischemia induction.

Fluorescence microscopy revealed that BBB breakdown was apparent in each segment of the vascular tree, with veins exhibiting the highest ratio of leaky vessels, whereas the most wide-spread tracer extravasations were shown for capillaries. Although the arterial segment is facing higher velocities and blood pressures, electron microscopy revealed comparable patterns of endothelial degeneration including edema and loss of endothelial cells for capillaries, arteries and veins. However, structural loss of arterial smooth muscle cells was only observed in the model of eMCAO.

Ischemia-related BBB breakdown is associated with severe alterations of the endothelial layer in capillaries, arteries and veins. Of note, these alterations are comparable in each of the applied models, including the reperfusion scenario. Thus, addressing endothelial protection may turn out as a promising approach for future neuroprotective strategies.

Vortrag 34:

Stockmann R. IFA, Berlin

Ergebnisse aus dem Formaldehyd-Projekt der Unfallversicherungsträger

Vortrag 35:

Hirt B. Tübingen
Formaldehyd und wie geht es weiter mit der Fixierung anatomischer Präparate?

Vortrag 36:

Titel: Neuronal responses to distributed synaptic inputs are independent of dendritic length and branching structure

Autoren/Adressen: Peter Jedlicka (Goethe University Frankfurt), Hermann Cuntz (Ernst Strüngmann Institute (ESI)), Alexander Bird (Ernst Strüngmann Institute (ESI)), Marcel Beining (Goethe University Frankfurt), Felix Hoffmann (Ernst Strüngmann Institute (ESI)), Steffen Platschek (Goethe University Frankfurt), Thomas Deller (Goethe University Frankfurt); jedlicka@em.uni-frankfurt.de

Abstract:
Alterations in dendritic morphology have a profound impact on the spiking behavior of neurons. Reducing neuronal size results in less cell membrane and lower input conductance. This increases the excitability of smaller neurons and alters their input-output function in response to current injections in the soma. However, the general consequences of this relation for responses to distributed synaptic inputs have not yet been studied in detail.

To assess the effects of dendritic morphology on synaptic input-output function, we used analytical cable theory, detailed compartmental modeling in reconstructed dendrites and morphological modeling.

Surprisingly, our results show that, in contrast to somatic current injections, spiking rate of neurons is independent of the length or branching of dendrites when distributed synaptic inputs are stochastically activated. Assuming a given synaptic density, voltage responses only depend on the average diameter and the specific membrane conductance in the dendrite. We show in compartmental simulations that this relation holds for large datasets of 3D-reconstructed morphologies and is reflected in invariant numbers of spikes for a given synaptic input frequency. This mechanism enables any dendritic tree, which undergoes structural remodeling including extension or shortening of its dendrites to adjust its responsiveness to synaptic activation.

Principles of neural architecture support neural input-output functions that are independent of dendrite length and shape within the range of most realistic biological neurons. This represents a homeostatic dendritic input-output relation that dendritic non-linearities and synaptic plasticity can modulate.

Vortrag 37:

Titel: Intrinsic properties of VIP neurons: insights into morphology, electrophysiology, and the influence of neuromodulation

Autoren/Adressen: Alvar Prönneke (Universitätsmedizin Göttingen), Martin Möck (Universitätsmedizin Göttingen), Mirko Witte (Universitätsmedizin Göttingen), Jochen Staiger (Universitätsmedizin Göttingen); alvar.proenneke@med.uni-goettingen.de

Abstract:
The neocortical GABAergic interneuron subpopulation of VIP-expressing cells operates disinhibitory circuit motifs which are activated during various behaviors. To understand these circuit motifs in detail, it is crucial to elucidate the intrinsic properties of individual VIP neurons in terms of electrophysiology, morphology, and responsiveness to neuromodulation.

Using whole-cell patch clamp recordings in acute brain slices of the primary somatosensory (barrel) cortex in transgenic Vip-ires-cre mice we targeted VIP neurons in all layers to extract their electrophysiological profile, reconstruct their morphology, and, for layer II/III, understand their responsiveness to acetylcholine (ACh) and serotonin (5HT).

Unsupervised cluster analysis based on adaptation rates and frequency spectrum of firing patterns revealed 5 electrophysiological types of VIP neurons. Additionally, the morphology of these neurons was dependent on the location of their somata. Four electrophysiological types did not correlate with differences in morphology, only bursting VIP neuron were predominantly found in upper layer II/III which corresponds to a specific morphological type. VIP neurons in layer II/III were depolarized via nicotinic non-a7-subunit-containing AChR. Despite being classified as 5HT3aR-expressing interneurons, only 50% of VIP neurons displayed 5HT3aR mediated currents. Interestingly, all VIP neurons were depolarized via 5HT2R.

In conclusion, VIP neurons are capable of integrating brain states caused by neuromodulation in a differential manner because response patterns to ACh and 5HT differ throughout the population. In combination with differences in the distribution of neurites throughout the cortical depth and a variety of electrophysiological types VIP neurons show the diversity necessary to influence cortical networks during various behaviors.

Vortrag 38:

Titel: Reactive changes in murine optic nerve astrocytes are mediated by growth factors and increasing substratum stiffness

Autoren/Adressen: Andrea E. Dillinger (University of Regensburg), Matthias Mayer (Ostbayerische Technische Hochschule Regensburg), Magdalena Schneider (University of Regensburg), Gregor R. Weber (University of Regensburg), Corinna Goeppner (Max-Delbrück-Centrum für Molekulare Medizin), Ernst R. Tamm (University of Regensburg), Mikhail Shamonin (Ostbayerische Technische Hochschule Regensburg), Gareth J. Monkman (Ostbayerische Technische Hochschule Regensburg), Rudolf Fuchshofer (University of Regensburg);
andrea.dillinger@vkl.uni-regensburg.de

Abstract:
Patients with primary open-angle glaucoma have a stiffer peripapillary sclera, reactive astrocytes and a remodeled lamina cribrosa. TGF-beta2 and its downstream mediator CTGF mediate pathologic changes. In this study, we investigate the glial lamina of our murine glaucoma model (beta-b1CTGF), and changes of astrocytes in response to CTGF, TGF-beta2 as well as increasing substratum stiffness.

Tangential sections of the glial lamina of 4-and 8-week old beta-b1CTGF mice and wild-type littermates were phalloidin-labeled and stained against GFAP, CTGF and fibronectin. CTGF and GFAP mRNA expression was investigated in optic nerve (ON) and optic nerve head (ONH) of beta-b1CTGF mice at both ages. Astrocytes were treated with TGF-beta2 and CTGF or seeded on PDMS substrata with different stiffness. Cells were analyzed by Western blotting, real-time RT-PCR and immunohistochemistry. Wound healing assays were performed to analyze migration rate.

Beta-b1CTGF mice showed an increase in CTGF and GFAP in the glial lamina and an increase in fibronectin-staining and phalloidin-labeled actin in the peripapillary sclera compared to wild-type littermates. CTGF and GFAP mRNA was significantly increased in the ONH of TG mice compared to controls. The expression was unaltered in the ON. Murine ON astrocytes reacted on increased substratum stiffness by increasing reactivation and CTGF synthesis. TGF-beta2 and CTGF treatment led to an enhanced migration rate and an increase in extracellular matrix.

We conclude that remodeling of the lamina cribrosa and the peripapillary sclera alter the biomechanical properties and induce reactive changes in astrocytes. The reactivated astrocytes, in turn, contribute to the pathogenesis of glaucoma.

Vortrag 39:

Titel: Single hippocampal mossy fiber synapses control hilar mossy cell firing

Autoren/Adressen: Alexander Drakew (Zentrum für Molekulare Neurobiologie Hamburg, Universitätsklinikum Hamburg-Eppendorf), Urban Maier (Zentrum für Molekulare Neurobiologie Hamburg, Universitätsklinikum Hamburg-Eppendorf), Michael Frotscher (Zentrum für Molekulare Neurobiologie Hamburg, Universitätsklinikum Hamburg-Eppendorf); alexander.drakew@zmnh.uni-hamburg.de

Abstract:
The axons of the dentate granule cells form the hippocampal mossy fiber (MF) projection to the CA3-region of the hippocampus proper. En passent they provide the main excitatory input to hilar mossy cells that in turn innervate dentate granule cells directly and indirectly via inhibitory interneurons. This feed-back loop modulates entorhino-hippocampal information transfer by the dentate gyrus. However, it is not clear how the very sparsely firing dentate granule cells procure the high AP firing rate of mossy cells. We analysed the impact of single MF synapses on mossy cell activity combining single-bouton stimulation and two-photon imaging of spines postsynaptic to the stimulated MF bouton.

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

Single bouton stimulation evoked very heterogeneous synaptic responses ranging from subthreshold postsynaptic potentials to suprathreshold events resulting in direct AP firing of the mossy cell. The majority of synapses showed both, subthreshold and suprathreshold responses. The fraction of suprathreshold responses changed in response to a defined potentiation protocol depending on the initially encountered synaptic state.

These results suggest that single MF synapses are capable to elicit AP firing of hilar mossy cells thereby controlling the functional connectivity of the dentate gyrus.

Vortrag 40:

Titel: CD44 as a trigger for inflammatory lesion formation

Autoren/Adressen: Tanja Hochstrasser (Ludwig-Maximilians-University of Munich), Christin Rebecca Reinbach (Ludwig-Maximilians-University of Munich), Nicolas Pröbstl (Ludwig-Maximilians-University of Munich), Christoph Schmitz (Ludwig-Maximilians-University of Munich), Markus Kipp (Ludwig-Maximilians-University of Munich); tanja.hochstrasser@med.uni-muenchen.de

Abstract:
During brain inflammation, immune cells first have to cross the endothelial barrier to gain access into the perivascular space, and second break through the glia limitans. This second step is poorly understood. CD44 is expressed by immune, as well as cells of the luminal and adluminal site. Thus, CD44 interaction might play a role during immune cell progression. In this project, we aim to investigate the relevance of CD44 for immune cell recruitment.

Activation of microglia and astrocytes was induced by cuprizone intoxication. Encephalitogenic immune cells in the peripheral lymphoid organs were induced by active immunization with the myelin protein peptide MOG35-55 (i.e. experimental autoimmune encephalomyelitis (EAE)). The liaison of innate and adaptive immunity was realized by combining the cuprizone model with active EAE (i.e. Cup/EAE). CX3CR1+/eGFP CCR2+/RFP transgenic mice were used to label microglia and monocytes.

In cuprizone-treated mice, CD44 was strongly induced at adluminal sites. The observed staining pattern suggested either expression of CD44 at the most distal astrocyte processes and/or secreted soluble CD44 in the extracellular space. To test, whether this brain intrinsic CD44 expression triggers peripheral immune cell recruitment into the brain, both models were combined. The liaison of innate and adaptive immunity resulted in massive recruitment of peripheral immune cells. Now, CD44 was expressed at the luminal and adluminal site. Detailed phenotypic characterization revealed that CD44 is expressed by both, CD3+ lymphocytes and RFP+ monocytes.

Our results suggest that CD44 is important for the progression of immune cells over the perivascular glia limitans.

Vortrag 41:

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), Johannes Knabbe (University of Heidelberg), Thomas Kuner (University of Heidelberg), Sidney Cambridge (University of Heidelberg); cambridge@ana.uni-heidelberg.de

Abstract:
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. Thus, these neurons can be analyzed before, during, and after induced gene expression to precisely correlate phenotypic changes to the genetic manipulation of neuronal excitability.

For transgene expression with high spatio-temporal control in living mice, we optimized a virus-based version of the inducible Tetracycline (TetOn) system to achieve substantially reduced background expression. In addition, the TetOn system was made Cre-dependent to allow cell-type specific manipulation in transgenic Parvalbumin-Cre (PV-Cre) mice. Co-transduction with GCamP6 viruses allowed repeated monitoring the activity of the same cortical neurons for up to four days using in vivo two-photon microscopy.

By silencing a subset of neurons within Kir2.1, an inward rectifying potassium channel, the consequences on neuronal network homeostasis of larger ensembles could be investigated. Induced genetic inhibition of the inhibitory PV neurons with Kir2.1 reduced PV activity and correlation between PV cells. Simultaneously, global neuronal activity of surrounding neurons rapidly increased within 2-3 hours after doxycycline injection and peaked after about 12 hours. Some of the increased activity was due to large epileptiform discharges, a likely consequence of the reduced PV inhibition. After peaking, global activity decreased again, possibly because of global homeostatic network mechanisms that strived to counteract the acute increase in overall activity.

We are now in the process of characterizing the molecular, cellular, and network contributions to the observed changes in activity.

Vortrag 42:

Titel: Studying neuronal mechanisms of hepatic encephalopathy (HE) in CA1 pyramidal neurons of entorhino-hippocampal slice cultures

Autoren/Adressen: Maximilian Lenz (Institute of Anatomy and Cell Biology), Andreas Vlachos (Institute of Anatomy and Cell Biology); maximilian.lenz@anat.uni-freiburg.de

Abstract:
Hepatic Encephalopathy (HE) is the most common neuropsychiatric complication of acute or chronic liver failure. It is well-established that increased levels of ammonia play an important role in the pathogenesis of HE, which includes symptoms of cognitive and motor dysfunction. However, the neuronal mechanisms underlying HE-associated alterations in learning and memory remain not well understood.

We here employed three weeks old mouse entorhino-hippocampal slice cultures and combined single cell electrophysiological recordings of CA1 pyramidal neurons, qPCR analysis and immunostainings to investigate the effects of high ammonia treatment (5mM NH_4Cl; 72 h) on synaptic transmission and plasticity.

We report that 5mM NH_4Cl induces a strong glial activation within 72 h. Both the astrocytic marker GFAP and the microglial marker Iba-1 are considerably increased upon ammonia treatment. These changes are accompanied by structural and functional remodeling of CA1 excitatory synapses. Moreover, increased neuronal oxidative stress is observed, as reflected by an increase in the number of neuronal 8-OHdG-oxRNA aggregates. Interestingly, oxRNA aggregates are observed in close association with the actin-modulating molecule synaptopodin, which is a marker for the spine apparatus organelle.

Considering the role of synaptopodin and the spine apparatus in synaptic plasticity, we propose a model in which HE-associated glial activation mediates its effects on neural plasticity in a synaptopodin-dependent manner, which may involve oxidation of mRNA and alterations in local protein synthesis. (supported by DFG-CRC974)

Vortrag 43:

Titel: Sex-specificity of dendritic spine type density in the hippocampus

Autoren/Adressen: Nicola Brandt (University Oldenburg), Tobias Löffler (University Medical Center Hamburg-Eppendorf), Gabriele M. Rune (University Medical Center Hamburg-Eppendorf); nicola.brandt@uni-oldenburg.de

Abstract:
Sex differences in hippocampal memory tasks suggest the existence of sex-dependent differences in hippocampal connectivity.

To address this question, we analyzed the densities of various spine types along hippocampal dendrites in CA1 region of male and female Thy1-GFP mice allowing us to study spine density "in vivo".

In previous studies, in which primary dissociated hippocampal neurons were used, we found that the proportion of mushroom spines, which are considered to be mature, stable spines, was larger in cultures originating from female animals than in cultures generated from male animals. Spine and spine synapse density depends on sex steroid hormones, such as estradiol and testosterone, originating from the gonads and/or from local synthesis in the hippocampus. Considering the average spine numbers of the estrus cycle stages in female mice we compared them with those of male mice. As a result, while the total number of spines was similar in both sexes, the density of "mushroom" spines differed in male and female animals in vivo. Consistent with sex-dependency of mushroom spine density, NMDA-R1 and NMDA-R2A/B expression were different in adult male animals as compared to adult female animals.

Our data indicate that hippocampal neurons are differentiated in a sex-dependent manner.

Vortrag 44:

Titel: Four dimensional in vivo imaging of glomerular dynamics in a zebrafish podocyte injury model

Autoren/Adressen: Florian Siegerist (University Medicine Greifswald), Weibin Zhou (University of Michigan), Antje Blumenthal (University Medicine Greifswald), Karlhans Endlich (University Medicine Greifswald), Nicole Endlich (University Medicine Greifswald); florian.siegerist@stud.uni-greifswald.de

Abstract:
In the past, it was postulated that the highly branched podocytes are able to migrate along the glomerular basement membrane (GBM), especially in podocytopathies. In this study, we wanted to clarify whether podocytes exhibit a migratory phenotype after the induction of podocyte injury.

We used a zebrafish strain expressing mCherry and the prokaryotic nitroreductase (NTR) which converts the prodrug metronidazole (MTZ) into a cytotoxin specifically in podocytes (Zhou et al. 2012). To characterize the phenotype of this strain subsequently to MTZ exposition, we performed qRT-PCR, immunofluorescence, histology, transmission electron microscopy and long-term in vivo two-photon microscopy (2PM).

The application of MTZ induced cell death which was demonstrated by TUNEL assay. We found decreased levels of nephrin and podocin in immunofluorescence stainings and by qRT-PCR. Moreover, we observed a widespread denudation of the GBM shown by histology and electron microscopy. In a time-dependent 3-D reconstruction (4-D) of glomeruli of up to 31 larvae at one time we found that podocytes retracted their major processes, Bowman’s space dilated significantly, pseudocysts formed and podocytes detached. However, no podocyte migration on the denuded GBM was observed during 24 h in zebrafish larvae (n=61).

Taken together, we show that in vivo 2PM is a feasible technique to follow dynamics of the glomerular filtration barrier and podocytes in the state of podocyte injury. Our results indicate that podocytes do not migrate along the naked GBM in our zebrafish injury model during the early time period of 24 h.

Vortrag 45:

Titel: Influence of angiogenesis in the pathogenesis of fibrotic pulmonary disease

Autoren/Adressen: Maximilian Ackermann (University Medical Center of the Johannes Gutenberg-University), Axel Walch (Helmholtz Zentrum Muenchen), Danny Jonigk (Hannover Medical School), Steven Mentzer (Laboratory of Adaptive and Regenerative Biology); maximilian.ackermann@uni-mainz.de

Abstract:
The classification of interstitial pulmonary diseases is difficult because more than 200 distinct disease patterns are characterized by diffuse, parenchymatous pulmonary disease, either as a primary disease or as an important part of a multiorgan disease such as systemic sclerosis. Neoangiogenesis plays an important pivotal role in the fibrogenesis of these inflammatory and granulomatous reactions.

The vascular architecture of explanted human lung tissue was analyzed three-dimensionally by microvascular corrosion casting using the scanning electron microscopy as well as synchrotron X-ray microtomography. The microvascular changes in idiopathic pulmonary fibrosis and systemic sclerosis are complemented by molecular pathology and MALDI-TOF Imaging.

Altered vascular architecture occurred predominantly in the subpleural and peribronchial regions. Microvascular corrosion casting revealed the appearance of both forms of angiogenesis, namely sprouting angiogenesis and intussusceptive angiogensis. MALDI-TOF Imaging could show the different distribution of extracellular matrix proteins in the fibrotic lesions. Digital multiplexed gene expression analysis using the NanoString nCounter System could show altered expression of proangiogenic genes.

In summary, our study provides the first evidence that the formation of fibrotic lesions is accompanied by sprouting and intussusceptive angiogenesis. We suggest that pulmonary fibrogenesis and neoangiogenesis interact in the progression of pulmonary fibrosis and systemic sclerosis.

Vortrag 46:

Titel: Beneficial therapeutic effects of the L-type calcium channel antagonist nimodipine in experimental autoimmune encephalomyelitis – an animal model for multiple sclerosis

Autoren/Adressen: Andrea Schampel (University of Wuerzburg), Stefanie Kürten (University of Erlangen);
schampelandrea@googlemail.com

Abstract:
Multiple sclerosis (MS) is the most prevalent neurological disease of the central nervous system (CNS) in young adults and is characterized by inflammation, demyelination and axonal pathology that result in multiple neurological and cognitive deficits. The focus of MS research remains on modulating the immune response, but common therapeutic strategies are only effective in slowing down disease progression and attenuating the symptoms. Developing an option to prevent neurodegeneration early on would be a valuable addition to the current standard of care for MS.

For this study we performed detailed analyses of neurodegeneration in experimental autoimmune encephalomyelitis (EAE), an animal model of MS, and in in vitro experiments regarding the effect of the clinically well-established L-type calcium channel antagonist nimodipine.

Nimodipine treatment attenuated the course of EAE and spinal cord histopathology. Furthermore, it promoted remyelination. The latter could be due to the protective effect on oligodendrocytes and oligodendrocyte precursor cells (OPCs) we observed in response to nimodipine treatment. To our surprise, we observed calcium channel-independent effects on microglia, resulting in apoptosis. These effects were cell type-specific and independent of microglia polarization. Apoptosis was accompanied by decreased levels of nitric oxide (NO) and inducible NO synthase (iNOS) in cell culture as well as decreased iNOS expression and reactive oxygen species (ROS) activity in EAE.

Overall, application of nimodipine seems to generate a favorable environment for regenerative processes and could therefore be a novel treatment option for MS, combining immunomodulatory effects while promoting neuroregeneration.

Vortrag 47:

Titel: Functional relevance of Cajal-Retzius cells in adult hippocampus

Autoren/Adressen: Anstötz M[1,2], Lee SK[2], Neblett TI[2], Maccaferri G[2], Frotscher M[3], Rune GM ; (1)Institute for Neuroanatomy, University/University Hospital Hamburg, 20246 Hamburg, Germany; (2)Department of Physiology, Northwestern University, Feinberg School of Medicine, Chicago, IL, USA; (3)Center for Molecular Neurobiology Hamburg (ZMNH), Institute for Structural Neurobiology, University Medical Center Hamburg-Eppendorf Hamburg, Germany; m.anstoetz@uke.de

Abstract:
Cajal-Retzius Cells (CR cells) are early-born neurons of brain, which are known to 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 revealed a lifelong persistence of CR cells in the hippocampus, while neocortical CR cells vanished soon after the second postnatal week. Moreover, mice held in an enriched environment, which was shown to stimulate the hippocampal network, displayed 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 hippocampus-specific intense connectivity. Finally, optogenetic stimulation of CR cells revealed various types of as yet unknown target neurons such as GABAergic interneurons and granule cells.

In conclusion, our results indicate that hippocampal CR cells remain integrated in the mature hippocampal circuit and suggest that they play additional, non-developmental roles that are modified by environmental experience. Hence, their excitatory signals could strongly modulate adult neurogenesis, explaining their lifelong expression in the hippocampus.

Vortrag 48:

Titel: Plakoglobin regulates cell-cell adhesion and desmoglein 3 binding properties

Autoren/Adressen: Marie-Therès Wanuske (Ludwig-Maximilians-Universität München), Elias Walter (Ludwig-Maximilians-Universität München), Franziska Vielmuth (Ludwig-Maximilians-Universität München), Jens Waschke (Ludwig-Maximilians-Universität München), Volker Spindler (Ludwig-Maximilians-Universität München); volker.spindler@med.uni-muenchen.de

Abstract:
Structural integrity of the epidermis depends on desmosomes. In these complex supramolecular structures, the cadherin-type adhesion molecules desmogleins (Dsg) and desmocollins are linked to the intermediate filament cytoskeleton by plakoglobin (Pg), desmoplakin and the plakophilin family. We here tested the relevance of Pg for cell-cell adhesion and on functionality of Dsg3, a desmosomal cadherin indispensable for keratinocyte cohesion.

Pg knockout cell lines were generated using the CRISPR/Cas9 system in human HaCaT keratinocytes. Knockout and wildtype control lines were characterized by western blot analysis and immunostainings. Cell cohesion was quantified by dispase-based-dissociation assays and single molecule Dsg3 binding properties were measured by atomic force microscopy (AFM) experiments.

Cellular cohesion was severely impaired by Pg knockout. The levels and localization of most desmosomal adhesion molecules were unaltered; however the amount of Dsg2 was reduced and AFM experiments revealed a reduction in the numbers of Dsg3-mediated binding events. Interestingly, the forces of individual Dsg3-mediated bonds were increased in Pg knockout cells. In line with reduced cell cohesion, keratin filaments did not expand to the cell membrane under knockout conditions and the keratin-anchoring molecule desmoplakin appeared internalized and reduced at the membrane. In contrast, the Pg homolog β-catenin was upregulated and enhanced at cell-cell borders.

Loss of Pg abrogates strong cell cohesion and leads to mislocalization of desmosomal molecules. Furthermore, Pg deficiency resulted in altered Dsg3 binding properties, demonstrating the importance of a normal desmosome composition for the function of individual adhesion molecules.

Vortrag 49:

Titel: Origin, fate and dynamics of macrophages at central nervous system interfaces

Autoren/Adressen: Peter Wieghofer (University of Leipzig), Tobias Goldmann (University Medical Center Freiburg), Marta Joana Costa Jordao (University Medical Center Freiburg), Fabiola Prutek (University Medical Center Freiburg), Nora Hagemeyer (University Medical Center Freiburg), Ori Staszewski (University Medical Center Freiburg), Martin Krüger (University of Leipzig), Josef Priller (Charité – Universitätsmedizin Berlin), Ingo Bechmann (University of Leipzig), Steffen Jung (The Weizmann Institute of Science), Marco Prinz (University Medical Center Freiburg);
peter.wieghofer@medizin.uni-leipzig.de

Abstract:
Perivascular, subdural meningeal and choroid plexus macrophages are non-parenchymal macrophages that mediate immune responses at brain boundaries. Although the origin of parenchymal microglia has recently been elucidated, much less is known about the precursors, the underlying transcriptional program and the dynamics of the other macrophages in the central nervous system (CNS). By taking advantage of recently developed methods and mouse models we challenged the traditional view of a steady replenishment of these cells under homeostatic conditions.

The origin and turn-over of microglia, perivascular, subdural meningeal and choroid plexus macrophages were investigated by embryonic and adult fate-mapping experiments (Cx3cr1-CreER, Flt3-Cre), parabiosis and several knock-out strains. An in-depth characterization of these macrophages was further performed by unbiased single cell RNA seq, flow cytometry, confocal and electron microscopy.

The macrophages at CNS interfaces show only a limited relationship to peripheral blood monocytes but originate mainly from erythro-myeloid precursors in the yolk sac. They are consequently ontogenetically related to microglia and share a common origin. Only choroid plexus macrophages show some contribution of monocytes under steady-state conditions.

Since the 1980s it was believed that macrophages in the CNS-associated tissues are blood-borne. This assumption was made from experiments with whole-body irradiated and bone-marrow reconstituted mice. Here, the non-physiological effects of irradiation combined with the presence of undifferentiated progenitor cells from the bone marrow in the blood led to this conclusion. In contrast to this, our findings disproved the current view and represent a major conceptional change in the field of neuroimmunology.

Vortrag 50:

Titel: Neurons re-arrange synapses under nutrient starvation

Autoren/Adressen: Alberto Catanese (Universität Ulm), Débora Garrido (Universität Ulm), Tobias Böckers (Universität Ulm);
tobias.boeckers@uni-ulm.de

Abstract:
In neurons, the synaptic micro-environment continuously undergoes structural and molecular changes to allow the correct maintenance of synaptic activity and plasticity. These changes include the turnover of scaffold proteins and receptors, suggesting that autophagy could strongly contribute in orchestrating synaptic transmission. Given these considerations, our study aims to clarify if induction of autophagy through nutrient starvation could lead to structural and molecular changes at the synapses.

Autophagy was induced in primary rat hippocampal neurons through 5 hours of starvation in Hank's Balanced Salt Solution. Cells were then fixed and processed for immunohistochemical stainings or cryo-fixated for Electron Microscopy.

We observed a strong down-regulation of Shank2- and Bassoon clusters sizes and number along the dendrites of starved neurons in comparison to control. Moreover, the cells showed decreased size of Homer- and Gephyrin clusters and an increase in the size of NMDAR1 signals. In addition, a Synaptotagmin assay showed a dramatic decrease of positive puncta in starved neurons compared to untreated cells, suggesting a decreased synaptic activity. Furthermore, ultrastructural analysis highlighted significant changes induced by starvation at the synapses. Starved neurons showed decreased PSD´s volume and number of presynaptic vesicles, together with increased width of the synaptic cleft. Finally, spine morphology analysis highlighted a reduced spine length in starved neurons.

Our in vitro experiments show that nutrient deprivation modifies synapses both structurally and molecularly. This might be interpreted by the hypothesis that neurons detect the lack of nutrients and decrease synaptic activity in order to survive the period of stress.