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To find your abstract or
an abstract of interest
please use the alphabetical list of
first authors of lectures and posters
starting on next page.

Inhaltsverzeichnis/Contents

Erstautor/First author	Nr. des Vortrages (V) / Posters (P)
Aldirawi M.	P24
Anstötz M.	V28
Antipova V.	P112
Arnold P.	V14
Ascheid D.	P92
Aung T.	P69
Barnerssoi M.	P124
Bartelt-Kirbach B.	P109
Bas-Orth C.	V25
Bauer M.	P94
Beckmann A.	V6
Bhattarai C.	P100
Bielmeier C.	P149
Bittner N.	P126
Blesinger H.	P43
Bock M.	P135
Böing L.	P79
Bonilla-Martinez R.	P18
Brandt N.	P137
Brunner K.	V49
Buck V. U.	P159
Buhrmann C.	P13
Busch M.	V31
Cambridge S.	P99
Caspers S.	V13
Chamas S.	P143
Chunder R.	P153
Claassen H.	P73
Corvace F.	P37
Dahlke E.	P29
Darwisch W.	P31
Devriese A.	P162
Divvela S. S. K.	P49
Domagała Z.	P158
Eckert P.	P56
Egu D. T.	P5
Einhäupl L.	V18
Elashry M. I.	P60
Enders M.	P155
Endle H.	V26
Engelhardt M.	V29
Evers S.	P35
Fanghänel J.	P75
Frabschka A.	P51
Franz H.	V48
Freilinger M.	P38
Frey K. G. S.	V52
Fritsch H.	P47
Froemel F.	P12
Fuchs M.	V55
Gallert S.-M.	V24

Erstautor/First author	Nr. des Vortrages (V) / Posters (P)
García Ponce A.	V7
Gasterich N.	V41
Gasthaus J.	P150
Geyer S.	P41
Gläser A.	P33
Gögele C.	P163
Groeneweg F.	P133
Gross I.	P123
Guy J.	V17
Haenssgen K.	P83
Hafner G.	V19
Hagedorn P.	P87
Hanika S.	P64
Hawlitschka A.	V34
Hecking I.	P117
Heilen L.	P156
Hensel N.	V32
Heun F.	P80
Hilmer J.	P78
Hinganu M.	P72
Hollenbach J.	P62
Hollenhorst M. I.	V39
Hörmann R.	P48, P84
Hu J.	V46
Iliev A.	P103
Islinger M.	V5
Ismail R.	P142
Jahr H.	P1
Jansing J. C.	P66
Jedlicka P.	V16
Johann S.	P138
Joost S.	V11
Kankowski S.	P134
Karnati S.	V15
Kavak D.	P61
Keiler J.	P2
Keller S.	V51
Kemnitz J.	P57
Kleefeldt F.	P9, V54
Kleine A.	P151
Kleinsasser B.	P7
Kliewe F.	V1
Klose L.	P28
Klymiuk M. C.	V45
Kneusels J.	P120
Knickmeyer M.	V23
Knöfler H.	V3
Korf H.-W.	P115
Krueger M.	P101
Kubo H. K.	P88
Kugelmann D.	V2
Kumar P.	P17
Kunke M.	P6
Kwiatkowski J.	P53
Labitzky V.	P59

Erstautor/First author	**Nr. des Vortrages (V) / Posters (P)**
Lambertz J.	P129
Lange T.	V8
Larionov A.	P74
Lehmann J.	P30
Lenz F.	P157
Lenz M.	V27
Li W.	P20
Lienbacher K.	V30
Lindhorst A.	P89
Lobachev O.	P95
Lückstädt W.	P22
Lutz D.	P136
Lutze G.	P42
Mages B.	P132
Mahmoud W.	P67
Malik I. A.	P8
Mann T.	V12
Marschalek A.	P21
Maurer-Gesek B.	P85
Maxeiner S.	P161
Mehlhorn J.	P108
Miroschnikov N.	P105
Müller J.	P152
Mutig K.	V4
Namm A.	P44
Nandigama R.	P26
Neckel P.	V44
Ortug A.	P116
Ortug G.	P82
Oswald J.	P45
Ottone N.	P10, P98
Petkova A.	P114
Pfeiffer V.	P23
Pfitscher K.	P93
Plantera L.	P107
Pous L.	V40
Pruidze P.	P86
Raisch H.	P111
Rana S.	P147
Reissig L. F.	P46
Richard M.	P122
Rietsche M.	V20
Rink S.	P154
Rodewald A.	P104
Rodriguez L. Z.	P25
Ruß T.	P127
Scharr M.	P32
Scheiner O.	P106
Scheld M.	V42
Schicht M.	P34
Schmidt P.	V56
Schnatz A.	V38
Schröder A.	P90
Schropp V.	V50
Schulte H.	P65

Erstautor/First author	Nr. des Vortrages (V) / Posters (P)
Schulze-Tanzil G.	P164
Schumacher S.	P160
Schwaerzer G.	P11
Semikasev E.	P145
Serrano N.	P81
Sharma K.	P76
Shoykhet M.	V43
Sigmund A. M.	P4
Sivukhina E. V.	P58
Slabik A.-K.	P55
Slowik A.	P144
Starzonek S.	P68
Stegemann L. N.	P118
Stein J.	P139
Steiniger B. S.	V10
Stofferin H.	P71, P96, P97, V35
Storsberg S. D.	P113
Sturm M. A.	V37
Stutzkowsky L.	P52
Thomas L.-Z.	P54
Thomson M. N.	V53
Tohidnezhad M.	P3
Torga T.	P63
Trattnig C.	P121
Trinh S. N.-B.	P119
Tsikolia N.	P40
Ullrich N.	P91
Upcin B.	P36
Vasiliev Y.	P70
Voelz C.	V33
Voigt M.	P110
Wagner N.	P14
Wandres M.	P102
Wanuske M.-T.	P27
Washausen S.	P39, P50
Wedel T.	V36
Welss J.	P16
Weninger J.	P148
Wicklein D.	V47
Wieghofer P.	V9
Wiegreffe C.	V21
Winokurow N.	P128
Wolniczak E.	P125
Wörsdörfer P.	V22
Wozniak S.	P77
Wunsch F.	P141
Wurm J.	P130
Xu Y.	P15
Yilmaz D. E.	P19
Zendedel A.	P146
Zhan J.	P140
Zimmermann J.	P131

Vortrag 1:

Titel:
The role of filamins in mechanically stressed podocytes

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Abstract:
Glomerular hypertension induces mechanical load to podocytes, often resulting in podocyte detachment and the development of glomerulosclerosis. Although it is well known that podocytes are mechanosensitive, the mechanosensor is still unknown. Filamin A, an actin-binding protein which is described as a mechanosensor, could be responsible for the outside-in signaling in podocytes.

Mouse podocytes were cultured on silicone membranes that were connected to the stretch apparatus (Fothy, CLS, Eppelheim) for three days (0.5 Hz and 5% extension). To study the role of filamins in cultured podocytes under mechanical stretch, filamin A was knocked down by siRNAs. Additionally, we established a filamin A knockout podocyte cell line (Flna KO) by CRISPR/Cas9. Biopsies of patients suffering from diabetic nephropathy were used to study the expression of filamin A.

We found that filamin A is highly expressed in cultured podocytes and co-localizes with F-actin and the podocyte-specific protein synaptopodin. The knockdown of filamin A changed the F-actin organization and reduced the expression of synaptopodin.

Flna KO podocytes showed an increased cell motility and alterations of focal adhesions and integrins. Surprisingly, Flna KO showed no reduced cell adhesion during mechanical stress. However, the simultaneous knockdown of filamin A and B reduced significantly the adhesion of cultured podocytes after mechanical stress.

Analyzing kidney biopsies of patients suffering from diabetic nephropathy, we found an up-regulation of filamin A in podocytes in contrast to control biopsies.

Filamins play an important role in the adaptation of podocytes to mechanical stretch and could serve as a mechanosensor.

Vortrag 2:

Titel:
Dsg1 and Dsg3 support epidermal integrity by different mechanisms

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Abstract:
Pemphigus is a severe skin blistering disease caused by autoantibodies which interfere with keratinocyte cohesion and thereby impair epidermal integrity and barrier function. The primary targets of pemphigus autoantibodies are the desmosomal cadherins desmoglein (dsg) 1 and 3. These autoantibodies seem to be sufficient for induction of epidermal blistering although the respective contribution of Dsg1 and Dsg3 to epidermal integrity is not entirely clear.

Dsg1 knockout mouse, CriprCas9, Immunostaining, Toluidine dye penetration test, Triton-X100 protein fractionation, Western Blotting, Dissociation Assay, human ex-vivo pemphigus model,

We established a Dsg1 knockout mouse model. Mice with deletion of Dsg1 suffered from lethal skin blistering with superficial cleavage reminiscent of pemphigus foliaceus histology and died within 24h after birth. Immunostaining and toluidine dye penetration test demonstrated a complete epidermal barrier break-down. In contrast, Dsg3-deficient mice displayed mild, self-healing lesions, suggesting different mechanisms of Dsg1- or Dsg3-regulated maintenance of epidermal integrity. In accordance, Dsg1 and Dsg3 autoantibodies in pemphigus engaged different signalling mechanisms. Thus, we established Dsg3-deficient keratinocytes using CrisprCas9. In these cells Ca2+ and Erk signaling were dysregulated although autoantibody-induced loss of keratinocyte adhesion was abolished. Further, p38MAPK and Src are known to be crucial for loss of intercellular adhesion in pemphigus. Interestingly, inhibition of p38MAPK but not of Src was protective in a human ex-vivo pemphigus model.

Taken together, these data indicate that the mechanisms by which Dsg1 and Dsg3 contribute to maintenance of epidermal integrity differ and that different signaling pathways involved in pemphigus are not of equal significance.

Vortrag 3:

Titel:
VHL deletion in proximal tubule epithelial cells of diabetic mice affects renal physiology and architecture

Autoren:
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Abstract:
Diabetic nephropathy (DN) is the leading cause for end stage renal disease. Under normal oxygen levels, the Von-Hippel-Lindau factor (VHL), targets the hypoxia inducible factor 1α (Hif1α) for degradation counteracting its actions on proangiogenic factors, e.g. VEGF. HIF is augmented early in DN and its suppression prevents DN. To analyze in detail the underlying mechanisms regulating DN, local renal VHL ablation was achieved by generating SGLT2Cre/VHLflox mice.

In adult SGLT2Cre/VHLflox mice, diabetes mellitus (DM) was induced by streptozotocin and the knock-out level was determined by BaseScope technology. Glucose levels, electrolytes, renal function parameters and the glomerular filtration rate were analysed. Morphological assessment of renal ultrastuctures was carried out and gene expression profiles, Western Blot and IHC analysis were determined.

Hyperfiltration and hyponatremia were observed in the control DM mice. In DM animals, urinary glucose and electrolytes were altered. DM mice revealed an increase in the area of the glomerular tuft, the Bowman capsule and the mean and total area of glomerular vessels that showed a tendency to further increase in diabetic SGLT2Cre/VHLflox. The area of the glomerular tuft and Bowman capsule were enlarged in DM. The GBM and PTC basement were thickened in control SGLT2Cre/VHLflox as well as in both DM groups.

VHL deletion in PTCs prevents diabetes-induced glomerular hyperfiltration and affects morphological alterations in PTCs and glomeruli. SGLT2Cre/VHLflox animals showed a tendency to increase angiogenesis probably mediated by HIF-induction indicating that VHL deletion affects angiogenesis whereas diabetes might favour mesangial expansion.

Vortrag 4:

Titel:
Calcineurin inhibitors suppress transcellular calcium and magnesium reabsorption in the distal nephron of the kidney

Autoren:
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Abstract:
Calcineurin inhibitors (CnI) such as cyclosporin A (CsA) are widely used for immunosuppression in patients undergoing organ transplantation but may cause hypomagnesemia and hypercalciuria. Previous data suggested that CnI may suppress claudin 16 (Cldn16)-mediated paracellular reabsorption of divalent cations in the thick ascending limb (TAL) of the kidney. To challenge the hypothesis, we studied effects of CsA in wild-type (WT) and Cldn16-deficient (Cldn16-/-) mice.

Kidney performance was evaluated in metabolic cages. Key paracellular and transcellular distal calcium and magnesium transport proteins were assessed by quantitative PCR, immunoblotting and immunofluorescence.

Labeling of Cldn16 produced specific signal in tight junctions of cortical TAL in WT kidneys but not in Cldn16-/- kidneys. Physiological analysis showed baseline hypomagnesaemia and hypercalciuria in Cln16-/- mice compared to controls. Expression and protein abundance of transcellular calcium or magnesium transport proteins downstream of TAL showed compensatory increases, including the transient receptor potential channel TRPM6, calbindin, parvalbumin, and divalent metal cation transporter CNNM2. CsA administration (25 mg/kg i.p. for 7 days) induced hypomagnesaemia and hypercalciuria in WT and aggravated calcium and magnesium wasting in Cldn16-/- mice. Expression of Cldn16 was nor affected by CsA in WT mice, whereas levels of TRPM6, calbindin, parvalbumin and CNNM2 were drastically decreased in both genotypes.

In summary, our data suggest that CsA causes renal calcium and magnesium loss via suppression of transcellular reabsorption pathways, rather than via inhibition of the Cldn16-mediated paracellular transport.

Vortrag 5:

Titel:
Deciphering the role of ACBD5 in peroxisome biology – from molecular function to disease pathology

Autoren:
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Abstract:
Peroxisomes are regularly found in close contact to tubules of the endoplasmic reticulum (ER) implying a close functional cooperation at such membrane contacts. By identifying a tethering complex between the peroxisomal acyl-CoA binding domain containing protein 5 (ACBD5) and the ER resident VAPB, we discovered a molecular basis for the physical interaction between both organelles. To further decipher the protein composition and function of peroxisome-ER membrane contacts and their significance for human health, we combined cellular fractionation experiments with embryonic fibroblast cultures derived from ACBD5-deficient mice.

To analyze the functional significance of ACBD5, pathological alterations at the organ level were linked with subcellular rearrangements as well as changes in lipid metabolism using a mouse model for the human ACBD5 deficiency. To this end, cellular, organellar and molecular alterations were analyzed using immunofluorescence, electron microscopy, cellular fractionation as well as protein and lipid analytical techniques.

ACBD5-deficient mice develop a progressive neurological motor disorder which is accompanied by a decline in cerebellar Purkinje cells. At the subcellular level, ER contacts are drastically reduced in hepatocytes, underlining the significance of ACBD5 for membrane contact formation, while cellular peroxisome numbers are increased. Elevated levels of very long-chain fatty acids in blood plasma and most tissues were observed, implying reduced capacities for peroxisomal β-oxidation.

While a reduction in ER membrane contacts is tolerated with regard to peroxisome biogenesis, metabolic peroxisomal functions appear to decline when interactions with the ER are compromised.

Vortrag 6:

Titel:
Pathologic alteration of astrocytic homeostasis induces changes of Connexin 43 gap junctions encompassing ultrastructure, phosphorylation status and function

Autoren:
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Abstract:
Gap junctional intercellular communication (GJIC) is an important mechanism to regulate brain homeostasis, with Connexin (Cx) 43 being the main astrocytic gap junction (GJ) protein. The capacity of GJs and therewith the extent of GJIC is determined by their morphology and assembly, which, in turn, are influenced by pathologic stimuli. Thus, we investigated GJ ultrastructure and function in two models of astrocytic impairment: model 1 - metabolic inhibition as in the early phase of hypoxia, mimicked by oxygen and glucose deprivation with subsequent reoxygenation (OGD-R) and model 2 - osmotic opening of the blood-brain-barrier (BBB), mimicked by treatment with a hyperosmolar sucrose solution.

Primary cultures of neonatal murine astrocytes were either subjected to 6 h OGD plus 2 h of reoxygenation (model 1) or stimulated with 0.5 M sucrose for 5 min (model 2).

Cell stress, GJIC and Cx43 expression were determined thereafter. Analysis of Cx43-GJ ultrastructure including sizing and a nearest-neighbor-distance (NND) analysis, was performed by freeze-fracture replica immunogold labeling.

OGD-R resulted in activation of ERK1/2. OGD-R as well as sucrose treatment caused downregulation of Cx43 expression and a loosening of Cx43-particle clusters; confirmed by NND analysis. Both treatments resulted in increased phosphorylation of Cx43 at Serine 368.

The relatively short period of OGD affected the viability of astrocytes minimally. Changes in the ultrastructural assembly of Cx43 GJs were observed in both treatments, but were more distinct upon osmotic BBB opening. Both short-term models demonstrated the fast adaptation of the intercellular communication system, visible even at the ultrastructural level.

Vortrag 7:

Titel:
EPAC1 regulates endothelial barrier properties by mechanisms in part independent of Rac1

Autoren:
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Abstract:
Regulation of intercellular junctional complexes is critical for the control of endothelial barrier function and cAMP-mediated Rac1 activation is important in this process. Recently, the exchange protein activated by cAMP (EPAC1) has been shown to serve as a tonic stabilizer of the endothelial barrier. Here, we further elucidate the role of EPAC1 in cAMP- and Rac1-mediated endothelial barrier regulation.

Immortalized myocardial endothelial cells derived from wild type (WT) and EPAC1-knockout (KO) mice were generated. Transendothelial-electrical-resistance (TER) measurements were used to asses barrier function. Western blot, immunofluorescence and G-LISA analyses were performed to determine the protein levels and activity of the small GTPases Rac1 and RhoA as well as phosphodiesterases (PDEs). ELISA assays were employed to quantify intracellular cAMP concentrations.

EPAC1-KO-cells showed significantly reduced baseline TER compared to WT-cells, associated with fragmented VE-cadherin immunostaining. Additionally, protein levels of Rac1 and RhoA were increased similar to basal cAMP concentration. However, baseline activity of Rac1 and RhoA remained unchanged, indicating a defect in GTPases activation. In WT-cells, augmented cAMP concentration mediated by forskolin (F) and rolipram (R) increased TER and induced linearization and enhancement of junctional VE-cadherin immunostaining. In contrast, EPAC1-KO-cells did not respond to F/R treatment although cAMP-induced Rac1 activation was not compromised. Interestingly, when Rac1 and RhoA were activated by CNO4, after a delay, EPAC1-KO-cells responded with TER increase and strengthening of VE-cadherin staining.

Our data show that EPAC1 is crucial for baseline and cAMP-mediated barrier stabilization by mechanisms in part independent of Rac1 regulation.

Vortrag 8:

Titel:
Development of a Spontaneous Bone Metastasis Xenograft Model of Human Cancer

Autoren:
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Abstract:
One major challenge in clinical oncology is the treatment of patients with bone metastases. The mechanisms of bone metastasis formation remain poorly understood, which might be due to a lack of sufficient mouse models that reflect the entire pathophysiology of the disease. Currently used mouse models circumvent early steps of the metastatic cascade due to intracardiac (left ventricle) injection or direct intraosseous inoculation of tumor cells. Our aim was to develop spontaneous bone metastasis mouse models for human cancers that reflect the entire metastatic cascade.

subcutaneous xenograft models, Alu-PCR, primary tumor surgery, post-surgical imaging (MRI, conventional radiography, PET-CT, BLI), corresponding histology, immunohistochemistry, FISH, re-cultivation of primary tumor and bone metastasis cells, functional and molecular characterization

After subcutaneous growth of tumor cells until our classical endpoint (tumor volume: 1.5 cm^3), only single human cells were detectable in the bone marrow (disseminated tumor cells, DTC). Outgrowth of DTC to established bone metastases was only observed, when the overall survival of the mice was prolonged by surgical resection of the primary tumors. The most sensitive technique to detect spontaneous bone metastases was ex vivo-BLI. Re-cultivated bone metastasis cells (BM subline) showed no increased metastatic properties in vitro compared to re-cultivated primary tumor cells (PT subline). After re-injection into mice, there was no difference in the spontaneous bone metastasis incidence between the BM and PT sublines.

The entire pathophysiology of bone metastasis formation of human tumors can be modeled in vivo. Putative pro-metastatic features are not maintained during in vitro re-cultivation.

Vortrag 9:

Titel:
Characterization of innate immune cells in the development of choroidal neovascularization

Autoren:
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Abstract:
Choroidal neovascularization is a hallmark of age-related macular degeneration (AMD) and extensively modulated by the innate immune system. The role of retinal microglia and infiltrating blood-derived myeloid cells are poorly understood in the laser-induced mouse model of choroidal neovascularization (CNV).

Using single-cell RNA Sequencing (scRNA-Seq) and fate mapping, we investigated the CNV-induced presence of resident microglia and peripheral myeloid cells in order to discover new myeloid cell subsets based on altered gene and surface marker expression.

The characterization of CNV-related myeloid cells was conducted using flow cytometry-based scRNA-Seq and validated by confocal microscopy. The experiments were performed with wildtype mice or transgenic reporter and fate mapping mouse models.

Fate mapping of long-lived macrophages by conditional reporter mice revealed microglia to be the most prominent cell population in the CNV model with only a minor contribution of monocyte-derived macrophages. In line with these findings, we identified several subpopulations among microglia but also other infiltrating myeloid cells by scRNA Seq. The loss of microglial homeostatic surface markers, e.g. P2RY12, combined with the aquisition of an inflammatory gene expression profile was observed in a time-dependend manner over the course of the disease model.

During experimental choroidal neovascularization, a key feature of AMD, we recognized disease model-associated macrophage subpopulations with distinct molecular signatures. Our results highlight previously unidentified myeloid subsets and their dynamics providing new insights into the innate immune system of the eye, offering new therapeutic targets for retinal diseases.

Vortrag 10:

Titel:
Human splenic red pulp microvasculature in three dimensions. Visualising capillary sheaths and the open circulation system in virtual reality.

Autoren:
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Abstract:
We wanted to determine whether all post-arteriolar capillaries of the human splenic red pulp possess sheaths and whether the capillary network is connected to venules.

We present a 3D model of red pulp microvessels derived from 120 serial sections of an adult human spleen stained for smooth muscle alpha-actin, CD34, CD271 and CD20, visualising fibroblasts/smooth muscle cells, non-sinusoidal endothelia, resident capillary sheath cells and B-lymphocytes. The model is viewed in virtual reality permitting direct quality control by superimposing each registered section.

Most red pulp capillaries directly following arterioles were covered by CD271+ sheath cells surrounded by CD27-CD20+ B-lymphocytes, before feeding a capillary network with a large number of open ends. The length and diameter of capillary sheaths were extremely variable. Only few post-arteriolar capillaries lacked sheaths. Very few capillaries finally joined venules. Red pulp capillaries were also connected to a capillary net at the surface of follicles located in the area occupied by CD27+ memory B-lymphocytes and MAdCAM-1+ fibroblasts.

Capillary sheaths in the human splenic red pulp may be dynamic structures attracting and arresting naive B-lymphocytes from the open splenic circulation for immigration directed to the follicular mantle zone. The red pulp circulatory system is primarily open with a few direct connections to venules as "emergency exits". CD27+ memory B-lymphocytes occupy both the mantle zone and a more superficial compartment of splenic follicles containing a capillary net fed from the red and white pulp. Both regions may represent a partially spleen-specific migration compartment supporting the survival of special memory B-lymphocytes.

Vortrag 11:

Titel:
Immune cell recruitment in a toxic multiple sclerosis model

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Abstract:
During progressive Multiple Sclerosis (MS), where effective treatment options are limited, peripheral immune cells can be found inside the brain and are suggested to play a functional role during disease progression. Although the underlying mechanisms are unknown, we hypothesize that metabolic oligodendrocyte injury is a potent trigger for peripheral immune cell recruitment in progressive MS.

Metabolic demyelination was induced by Cuprizone administration for up to 5 weeks. Paraffin-embedded brain sections were stained immunohistochemically for PLP (myelin marker), IBA1 (microglia marker), APP (marker for axonal damage), CD3 (global T cell marker), CD4 (T helper cell marker) or CD45R (B cell marker). Glia reactivity in established lesions was additionally analyzed by Positron emission tomography (PET) imaging using ligands targeting the translocator protein (TSPO). CX3CR1+/eGFPxCCR2+/RFP-mice were used to label monocytes.

Cuprizone intoxication leads to severe demyelination accompanied by microgliosis, astrogliosis and axonal damage. This was paralleled by an increase in TSPO-ligand binding. CD3+ and CD4+ cell densities were higher in the Corpus callosum of Cuprizone intoxicated (CD3+: 32.8 ± 15.6 cells/mm²; CD4+: 7.7 ± 4.6 cells/mm²) compared to control mice (CD3+: 0.2 ± 0.3 cells/mm²; CD4+: 0.1 ± 0.3 cells/mm²). T-cell recruitment was most severe in demyelinated brain areas. In parallel, we found the recruitment of CCR2+ monocytes, but not of CD45R+ B-cells.

We demonstrate that metabolic oligodendrocyte injury is an initial trigger of peripheral immune cell recruitment, especially T cells. Further studies now have to show the mode of action and functional consequence of T-cell recruitment in this model.

Vortrag 12:

Titel:
PET/CT analysis in animal models of neurodegeneration

Autoren:
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Abstract:
Neurodegenerative mechanisms during aging and diseases remain largely unclear. Longitudinal studies are, therefore, urgently needed. Positron emission tomography/computed tomography (PET/CT) is a nuclear imaging technology that visualizes metabolic parameters or neurotransmitter receptors in vivo. PET/CT data is commonly standardized on a reference tissue, which is devoid of the target structure. For some targets there is no reference region available.

Here, we hypothesize that continuous blood sampling combined with real time radioactivity measurements is a valid method to quantify and normalize PET/CT data in rat models with the pathologies of PD and HD.

PET/CT scans with [18F]Fallypride to analyze Dopamine-D2/D3-receptor availability and [18F]FDG to detect glucose metabolism were longitudinally performed in hemi-PD rats. Intracerebral Botulinum-Neurotoxin-A (BoNT-A) application was performed to ameliorate PD pathology. To visualize the extent of glia reactivity during physiological aging, the translocator protein 18 kDa (TSPO) tracer [18F]GE180 was used in HD-rats. Blood sampling was performed simultaneously to [18F]FDG and [18F]GE180 measurements via an arteriovenous shunt.

Kinetic modeling with PMOD revealed an increase in the D2/D3-receptor binding potential in hemi-PD rats which was normalized after BoNT-A application. The cerebral metabolic rate in hemi-PD rats as a measure of neuronal activity was slightly decreased compared to controls but not affected by BoNT-A treatment. TSPO binding for microglia activation was increased in older animals which points to a disease-independent but age-dependent effect.

In conclusion, small animal PET/CT imaging enabled the investigation of neurodegenerative mechanisms and the longitudinal monitoring of new preclinical therapy options in animal models of neurodegeneration.

Vortrag 13:

Titel:
How lifestyle affects structure and function in the aging human brain – a multimodal integrative approach

Autoren:
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Abstract:
Human brain aging is influenced by environmental factors, e.g. everyday lifestyle having positive (e.g. sports) or adverse effects (e.g. alcohol consumption, smoking) on cognition and brain health. The interplay between these different factors was not considered so far. We integrated several lifestyle factors per subject and studied their combined influence on brain aging.

Magnetic resonance imaging (MRI) data of brain structure and functional connectivity were obtained in subjects (age 55-85 years) from the population-based 1000BRAINS cohort. Lifestyle data on smoking, alcohol consumption, physical activity, and social integration were integrated into a combined lifestyle risk score per subject. Brain structure was assessed via local gyrification index indicating local brain atrophy, and as local grey matter volume for predicting subject age from brain structure using support-vector machines. Functional connectivity between brain areas was extracted from functional MRI at rest.

First (n=549), we could show that higher combined lifestyle risk was associated with local brain atrophy in premotor (driven by more alcohol consumption and less physical activity) and lateral prefrontal cortex (driven by less social integration). Smoking predominantly influenced functional connectivity of these regions. Secondly (n=622), we showed that combined lifestyle risk accounted for 3.84 months of additional brain aging per unit, smoking alone with additional 0.36 months more brain aging and physical activity with 0.48 months less brain aging.

The complex interplay of different lifestyle factors contributes to understanding of brain aging beyond the single factors. Disentangling individual and combined contributions could help to develop strategies for healthy aging in modern societies.

Vortrag 14:

Titel:
Joined Reconstituted Signaling - a new signaling mode of the IL-6 receptor

Autoren:
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Abstract:
It was long known that the cytokine receptor IL-6 receptor (IL-6R) is present as a full-length version on extracellular vesicles (EVs) in serum samples of mice and men. However, a potential signaling property has not been described so far. Here we show that this EV full-length version signals to distant cells. The described signaling mode was termed joined reconstituted signaling.

Here we use biochemical (western blot) and cell biological (CRSIPR/Cas, cell transfection, Ba/F3 cells for signaling analysis) methods in combination with imaging techniques (CLSM, TEM) to provide detailed insight into the newly described signaling mode.

In cells deficient for the known sheddases ADAM10/17 and meprin ALPHA and BETA we found that a full-length EV bound IL-6R is present in the cell supernatant. Using sequential centrifugation steps we purified the IL-6R containing EVs and assessed the accessibility of the IL-6R for proteases and the overall stability on these EVs. We then used a GFP-tagged version of the IL-6R to show interaction with cells using CLSM and also TEM. To assess signaling properties that these IL-6R containing vesicles may have we used Ba/F3 cells and assessed STAT3 phosphorylation. We could show that EV associated IL-6R has different signaling properties than soluble IL-6R. While the soluble form transduces signals in a range of 15-30 min, we found STAT3 phosphorylation even 240 min after vesicle application.

The newly described JRS signaling mode of the IL-6R depends largely on its trimming at the cell surface, but if present transduces a long term signal in target cells.

Vortrag 15:

Titel:
Super-resolution imaging reveals the PEX14p clusters in the peroxisomal membrane

Autoren:
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Abstract:
Peroxisomes are the dynamic and essential organelles of the eukaryotic cell. Peroxisomes adjust their size, length and shape according to the necessity and metabolic status of the cell. Peroxisomal biogenesis disorders are caused by defects in the peroxisomal matrix protein import, facilitated by peroxisome biogenesis proteins 13 and 14 (PEX13p and PEX14p) forming a docking complex on the peroxisomal membrane. So far, no structural information and distribution patterns are available on these peroxisomal membrane proteins. Moreover, the diameter of peroxisomes is close to the spatial resolution limit of conventional light microscopy and therefore super-resolution microscopy for imaging sub-peroxisomal protein distribution is necessary. Our aim is to quantify and visualize the structural organization of the PEX14p on the peroxisomal membrane.

Innovation: This is the first report of structural organization and distribution of PEX14p on the membrane of lung peroxisomes using a combined approach of laser-scanning confocal microscopy (CLSM), super-resolution Structured Illumination Microscopy (SIM), STimulated Emission Depletion microscopy (STED), and direct STochastic Optical Reconstruction Microscopy (dSTORM) to obtain a better insight into the structure of peroxisomes in C22 cells. Deconvolved CLSM, STED, SR-SIM and dSTORM images were analyzed using Icy, Imaris and custom written cluster analysis software.

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Conclusion: We achieved 20 nm lateral localization precision (FWHM) and our findings showed a structural clustered pattern of PEX14p distribution with different labeling intensities on the peroxisomal membrane.

Vortrag 16:

Titel:
A novel synaptic plasticity rule for detailed model neurons with realistic dendrites

Autoren:
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Abstract:
Spike timing, firing rate and synaptic location have been found to be important factors that dynamically contribute to the outcomes of plasticity induction protocols. While several theoretical models that implement plasticity rules already exist, they have not yet been used in depth to study plasticity in neuron models with detailed morphology.

Here, we extend previous phenomenological voltage-based plasticity rules by developing a new framework based on three signaling pathways. We apply it to a L5 pyramidal cell model with active dendritic properties and realistic propagation of voltage.

We show that our novel rule not only reconciles outcomes of several experiments but also predicts spatiotemporal patterns of plasticity that are characteristic for individual stimulation protocols and their impact on local processes at the synapse, including protocols inducing local plasticity in tuft dendrites. Due to this focus on local voltage signals, our framework can explain synaptic plasticity in the absence of postsynaptic action potentials, as suggested in recent studies. We thereby link experimental results that would intuitively seem to require entirely different rules, showing that a unifying rule might explain the vast majority of experiments in cortical pyramidal cells if key biophysical pathways are taken into account. Ultimately, we can now study how the cell-type specific electrotonic properties can explain differences in emerging plasticity by incorporating our plasticity rule in a variety of existing detailed compartmental models such as models of hippocampal pyramidal or granule cells.

To summarize, a simple plasticity rule that utilizes pre- and postsynaptic plasticity pathways can explain experimental results with a large variety of induction protocols when the plasticity rule is incorporated in the compartmentalized structure of a detailed dendritic model.

Vortrag 17:

Titel:
Circuit imbalances and sensory processing in a model cortex without layers

Autoren:
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Abstract:
Lamination is a well preserved hallmark of the organization of the neocortex, suggesting that distinct layers may fulfill specific functional roles in cortical computations. In the reeler mouse, aberrant neuronal migration during embryonic corticogenesis results in a markedly disorganized cortex, where neurons are scattered across the cortical depth rather than grouped into layers.

We investigated the consequences of mislamination on cortical physiology, using a combination of in vitro and in vivo whole cell electrophysiology, optogenetics and behavioral testing.

Using channelrhodopsin expression targeted to the ventral posteromedial nucleus and in vitro electrophysiology, we show that thalamic fibers send direct but weakened input to their target excitatory cells in the reeler barrel cortex. As a putative compensatory mechanism for a weakened sensory input, paired whole cell recordings in vitro revealed that inhibitory input to excitatory neurons was weakened in the reeler cortex. We next investigated sensory responses in anesthetized reeler animals using in vivo two photon targeted whole cell recordings of excitatory and inhibitory neurons. Although sensory responses were of slightly amplitudes in reeler, they also displayed normal receptive fields with a mild tendency towards higher directional selectivity. Finally, the performance of reeler animals in a novel object recognition task was similar to that of controls, indicating that neither basic perceptual abilities nor recognition memory are grossly impaired.

Overall, our results suggest that physiological abnormalities associated to a loss of cortical lamination compensate rather than compound each other, resulting in the preservation of homeostasis and basic behavior.

Vortrag 18:

Titel:
Localisation and Functional Role of MAGI-1 in Neurons

Autoren:
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Abstract:
MAGI (membrane associated guanylate kinase inverted) proteins belong to the MAGUK-family (membrane associated guanylat kinase) of synaptic scaffolding proteins. They consist of three members: MAGI-1, MAGI-2 (also known as S-SCAM) and MAGI-3. Though MAGI-2 function in the brain has been studied intensively, little information about MAGI-1 function in neuronal tissue is available.

In this study we analyzed the localisation and functional role of MAGI-1 in primary cultured rat hippocampal neurons by using transfection of recombinant MAGI proteins in neurons and immunocytochemical analysis .

Transfection of recombinant MAGI proteins and immunofluorescent analysis in immature neurons revealed diffuse distribution of MAGI-1 and -2 in the cell body and neurites at early DIV stages of the culture system. Synaptic localisation of MAGI-2 was observed at DIV 5 while MAGI-1 was enriched at synapses at later stages of culture development. Both MAGI proteins localise at inhibitory and excitatory synapses. Further, MAGI-2 knockdown neurons display a severe loss of synapses. This phenotype was rescued by increasing MAGI protein levels with recombinant MAGI-1 in MAGI-2 knockdown hippocampal neurons.

Our results suggest that MAGI-1 is competent to substitute for MAGI-2 function during synapse formation while the transport mechanism of these proteins to synaptic sites is temporally different.

Vortrag 19:

Titel:
Differences in callosal connectivity in reeler mice revealed by rabies virus tracing

Autoren:
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Abstract:
In reeler mutant mice cortical neurons are misplaced and fail to form a laminar organization. In the primary somatosensory cortex, both excitatory and inhibitory neurons are dispersed throughout the cortical thickness in a chaotic manner. We wanted to investigate if inhibitory neurons in reeler mice receive the same long-range input as in wildtype despite the absence of layers. Vasoactive intestinal polypeptide (VIP) expressing inhibitory neurons have received attention as major integrators of long-range input. They exhibit a laminar bias towards the upper cortical layers in wildtype. Therefore, we assumed that any alterations in long-range connectivity due to cell displacement would become most strongly apparent for this cell type.

We assessed the long-range input sources of VIP neurons in barrel cortex of wildtype and reeler mice using rabies virus tracing.

VIP neurons received input from the same areas in both genotypes. The major input sources were other sensory cortices, motor cortex, posterior parietal association area, and the thalamus. The proportion of subcortical input was preserved in reeler. However, VIP neurons in reeler mice received a much lower number of ipsilateral cortical inputs and a much higher number of contralateral cortical inputs

We hypothesize that the disorganized arrangement of neurons in reeler compromises the establishment of cell-type specific ipsilateral long-range projections and necessitates compensation by an excess of contralateral inputs. Based on our results we argue that in the absence of a laminar structure, VIP neurons are still maintaining their connectivity with subcortical structures while their cortical connectivity is fundamentally altered.

Vortrag 20:

Titel:
Overexpression of the plasticity-related protein Synaptopodin in the mouse dentate gyrus in vivo increases the density of granule cell spines containing a spine apparatus organelle

Autoren:
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Abstract:
Dendritic spines are sites of synaptic plasticity and important for memory trace formation. A subpopulation of spines contains the actin-binding protein Synaptopodin (SP), which is necessary for the formation of a spine apparatus organelle (SA). Functionally, SP/SA have been linked to different forms of synaptic plasticity. Mechanistically, SP/SA are considered part of downstream pathways executing changes in synaptic strength.

Here we analyzed adult male transgenic mice overexpressing CFP-tagged SP (CFP-SPtg) under the control of the Thy1-promoter. Using laser-microdissection and quantitative-PCR we could show that CFP-SP mRNA is 4.5 times more abundant in transgenic granule cells compared to wildtype controls; at the protein level SP was 2.5 times higher. We then employed intracellular injection of Alexa 568 in fixed slices to label single granule cells (dorsal hippocampus, suprapyramidal blade) of transgenic and control mice. Sections were resliced, immunolabeled for SP and investigated using confocal microscopy.

Compared to controls, the ratio of SP+ granule cell spines almost doubled in CFP-SPtg mice. Within both groups, SP+ spines had significantly larger spine heads than SP- spines. Spine density, mean spine head size and mean SP-cluster size were not significantly different.

We conclude that neuronal overexpression of SP increases the density of spines containing SA. Furthermore, our data reveal a tight correlation between SP-cluster size and spine head size. Since functional data link SP to spine plasticity and spine head size to synaptic strength, we speculate that a higher density of SP-positive spines could make neurons more adaptive (supported by DFG).

Vortrag 21:

Titel:
Bcl11a and Bcl11b are required for neocortex formation

Autoren:
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Abstract:
Neocortical projection neurons (PNs) are generated in the ventricular/subventricular zones and transit through the intermediate zone (IZ) before entering the cortical plate (CP). During embryogenesis the majority of PNs transiently co-express the closely related transcription factors Bcl11a and Bcl11b, which belong to the BAF chromatin remodeling complex and later become restricted to different neuron populations. Yet, cooperative functions of Bcl11a and Bcl11b in neurons have not been determined.

We used immunohistochemistry, RNA in situ hybridization, and a transcriptomic approach to analyze the phenotype of embryonic and early postnatal mice with a forebrain-specific deletion of both Bcl11a and Bcl11b.

We found a severe reduction in cortical thickness, impaired neuronal migration, defects in major axon tracts and defective cortical lamination in Bcl11a/b double mutant mice in comparison to controls. Our transcriptomic data revealed a significant downregulation of CP enriched genes, while many IZ enriched genes were significantly upregulated in double mutant neocortex.

We provide experimental evidence demonstrating both factors to synergistically control CP formation. Thus, Bcl11a and Bcl11b, together, exert essential functions during early cortical differentiation, before their expression becomes restricted to discrete neuron populations in superficial and deep cortical layers, respectively.

Vortrag 22:

Titel:
Generation of human vascularized organoids

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Abstract:
Organoids derived from pluripotent stem cells (PSCs) are interesting models to study mechanisms of development and morphogenesis. Furthermore, organoids serve as drug testing and disease modeling platforms. Several models were reported, recapitulating the development of different organ`s epithelial structures. However, they remain inherently incomplete as they lack stroma, vasculature and tissue resident immune cells. We propose, that the directed incorporation of PSC-derived mesodermal progenitors (MPCs) into organoids will overcome the aforementioned limitations.

First, we generated MPCs from PSCs by adding the GSK3 inhibitor Chir99210 and BMP4 to the culture medium. The MPCs were characterized and co-cultured in 3D-aggregates with either tumor cells or iPSC-derived neuroepithelial cells. The resulting organoids were analyzed regarding vascular network formation using immunofluorescence analyses, tissue clearing and electron microscopy. Moreover, tumor organoids were transplanted on the chick chorioallantoic membrane to test vessel function in vivo.

We show that the co-culture of PSC-derived MPCs with either neural spheroids or tumor cells leads to the formation of vascularized organoids. The formed blood vessels display a hierarchic organization. In addition, pericytes/smooth muscle cells are assembled into the vessel wall indicating maturation. Moreover, we observed a typical ultrastructure including junctional complexes, a basement membrane, caveolae as well as myoendothelial junctions. Remarkably, MPCs also delivered macrophages/microglial cells. To demonstrate the feasibility of the method, we generated human vascularized tumor as well as neural organoids.

Our data demonstrate the generation of vascularized complex organoids resembling the in vivo situation more closely than previously reported models.

Vortrag 23:

Titel:
Induced BMP signaling disrupts optic chiasm formation in zebrafish (Danio rerio)

Autoren:
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Abstract:
In previous studies, we observed that bmp4 overexpression during optic cup formation in zebrafish results in morphogenetic defects affecting the ventral optic cup and the optic stalk. This includes the area of the prospective optic nerve head. Therefore, we became interested in how these defects affect the guidance of retinal ganglion cell (RGC) axons and the formation of the optic nerve.

We use transgenic embryos tg(hsp70l:bmp4, pou4f3:mGFP) as well as DiI/DiO injections for in vivo imaging of RGC projections. We identify candidate genes downstream of bmp4 by RNA microarray analyses and address the expression of these target genes by in situ hybridization. Consecutively we are going to analyze distinct candidate genes using transgenic overexpression or CRISPR/Cas9-mediated knock-out.

RGC axon projections within the optic cup appear not to be defective, indicated by the formation of a single optic nerve exiting the eye at the location of the persisting optic stalk. This optic nerve, however, aberrantly projects to the ipsilateral optic tectum, rather than crossing to the contralateral side, as would be expected for all RGC axons in wildtype zebrafish. Thus, no optic chiasm forms. Transcriptomic analysis of forebrain and eye in combination with whole mount in situ hybridization revealed a loss of gene expression of potential axonal guidance cues in the optic stalk and midline.

We show for the first time that ectopic BMP signaling is affecting the formation of the optic chiasm. Based on our transcriptomics data and morphological analyses we propose a diencephalic patterning defect affecting axonal guidance cues.

Vortrag 24:

Titel:
Modelling Duchenne Muscular Dystrophy with patient - derived induced pluripotent stem cells

Autoren:
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Abstract:
Duchenne Muscular Dystrophy (DMD) is one of the most common hereditary skeletal muscle diseases affecting 1 in 5000 newborn males. DMD patient – derived induced pluripotent stem cells (iPSC) can be utilized to model and study the pathogenesis of this disease in vitro.

Reprogrammed iPSC from three patients with DMD in comparison to iPSC from healthy control patients are subjected to multi-step myogenic differentiation protocols to induce skeletal muscle precursors and muscle cells, which include 2-dimensional as well as 3-dimensional differentiation protocols. Pax7+ satellite-like cells and maturated skeletal muscle cells are identified and quantified by immunohistochemistry. Cell extracts from distinct time points are subjected to mass-spectrometric analysis for protein expression profiling.

Human skeletal muscle organoids have been established as a novel 3D multi-step differentiation protocol from human iPSC, which incorporate satellite-like cells as well as electrophysiology responsive mature skeletal muscle. DMD patient iPSC can be efficiently converted to skeletal muscle cells using these 2D and 3D differentiation protocols. By mass spectrometry over 2000 proteins from iPSC derived skeletal muscle cells are identified, including components of the cytoskeleton (Titin, Myosin-superfamily, intermediate filaments), dystrophin-associated glycoprotein complex and extracellular matrix (Collagen, Tenascin). DMD iPSC derived skeletal muscle cells in comparison to control cell line show differences depending on the time points and between the different cell lines.

Our studies explore DMD patient derived iPSC and novel in vitro differentiation strategies as a genuine human model system to study the pathogenesis of the most common congenital muscular dystrophy.

Vortrag 25:

Titel:
Mitochondrial calcium signaling is crucial for synchronization of fast neuronal network oscillations

Autoren:
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Abstract:
Synaptic activity acutely stimulates mitochondrial energy metabolism to meet increased energy demand. Such neurometabolic coupling is especially relevant for fast neuronal network activities like gamma oscillations that associate with perception, attention and memory in vivo. Different mechanisms underlying neurometabolic coupling have been suggested. They include stimulation of mitochondrial substrate carriers by cytosolic calcium, and stimulation of TCA cycle dehydrogenases by mitochondrial matrix calcium. The physiological relevance of these mechanisms, however, has remained unclear. To address this question, we studied the role of mitochondrial calcium in neuronal network oscillations.

We used acute hippocampal slices from a newly generated mouse line that lacks expression of the mitochondrial calcium uniporter (Mcu) in excitatory neurons of the forebrain. TCA cycle stimulation was measured by immunofluorescence-based quantification of pyruvate dehydrogenase dephosphorylation. Cholinergically-induced gamma oscillations and spontaneous sharp wave-ripples were analyzed by local field potential recordings, including fast Fourier transform. Gene expression was analyzed by RT2 Profiler PCR Arrays.

In Mcu knock-out animals we found (i) decreased activity-dependent stimulation of mitochondrial energy metabolism, (ii) gamma oscillations with lower power and synchrony, including less precise spiking, and (iii) sharp waves with lower incidence and ripple frequency. We found (iv) no compensatory adaptations in gene expression related to mitochondrial function and glucose metabolism, suggesting that basal mitochondrial function and energy metabolism were not affected in Mcu mutants.

These findings suggest that the neuronal Mcu is crucial for proper generation of fast network oscillations, most likely by adapting oxidative phosphorylation and controlling cytoplasmic calcium homeostasis.

Vortrag 26:

Titel:
Metabolic changes alter brain lipids and cortical function involved in food intake control

Autoren:
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Abstract:
Recently, we showed that synaptic phospholipids regulate glutamatergic transmission and cortical network excitability. Since metabolic changes affect body phospholipid levels, we aimed to understand how dietary metabolic changes affect brain phospholipid composition and thereby modulate cortical excitability and behavior.

In this study we combined measurements of phospholipid composition in the blood and in the CSF (measured by mass spectrometry) with electrophysiological and behavioral analyses.

After overnight fasting, lysophosphatidic acid (LPA) levels were increased in murine blood plasma. However, in the cerebrospinal fluid, only specific LPA-subtypes (16:0 and 18:1) were elevated leading to higher cortical excitability as shown by single-cell electrophysiology and by augmented cortex-related exploratory behaviors. Interestingly, LPA-related cortical excitability induced significant rebound hyperphagia after fasting. However, this hyperphagia was significantly decreased after inhibition of LPA synthesis by the ATX inhibitor PF-8380 or by loss-of-function of specific elements of the synaptic LPA signaling pathway in genetic deletion models, as it was the case with cortex-related exploratory behaviors. Moreover, we detected a significant increase in the body mass index (BMI) and a higher prevalence of diabetes type 2 in human individuals carrying a single-nucleotide polymorphism (SNP) leading to increased synaptic lipid signaling and higher cortical excitability.

Our findings unraveled a direct influence of dietary metabolic changes on cortical excitability via changes in synaptic LPA levels, which affected cortex-related behaviors independent of classical hypothalamic mechanisms. The metabolic influence on cortical excitability altered food intake behaviors in mouse and man and revealed a yet unknown control of cortical function on food intake. Since cortical overactivation and rebound hyperphagia were significantly reduced by decreasing synaptic LPA, we propose to target synaptic LPA signaling by ATX-inhibition to control food intake.

Vortrag 27:

Titel:
All-Trans Retinoic Acid modulates the ability of dentate granule cells to express synaptic plasticity through intracellular calcium stores

Autoren:
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Abstract:
All-trans retinoic acid (atRA) has been suggested to modulate the ability of neurons to express synaptic plasticity. Although recent studies have shown that atRA mediates the accumulation of AMPA-receptors at excitatory postsynaptic sites, the mechanisms through which atRA mediates its effects on plasticity remain not well understood.

In this study, we tested for the role of synaptopodin-associated intracellular calcium stores in atRA-mediated excitatory synaptic plasticity in mouse entorhino-hippocampal tissue cultures and adult mice. To address this question, we used whole-cell patch-clamp and extracellular recordings of dentate granule cells as well as immunohistochemistry and ultrastructural analysis of excitatory synapses.

Our electrophysiological recordings demonstrate, that 1 µM atRA exposure for 3 days leads to an accumulation of AMPA-receptors at excitatory postsynapses of dentate granule cells. These atRA-mediated changes in excitatory neurotransmission are not observed in synaptopodin-deficient mice, which do not form spine apparatus organelles. Consistent with this observation changes in synaptopodin expression and ultrastructural changes of spine apparatuses are observed in response to atRA. Finally, we show that the ability to express in vivo perforant path LTP is improved upon systemic atRA injection in wildtype but not synaptopodin-deficient mice.

We conclude that atRA promotes metaplasticity, i.e., it improves the ability of neurons to express plasticity via a synaptopodin/spine apparatus-dependent mechanism. Thus, the results provide a biological basis for the use of atRA as a plasticity enhancing therapeutic strategy in neurological diseases associated with alterations in synaptopodin expression and synaptic plasticity.

(supported by DFG, CRC1080)

Vortrag 28:

Titel:
Glutamate released by Cajal-Retzius cells regulates morphology of pyramidal neurons in the hippocampus

Autoren:
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Abstract:
Cajal-Retzius cells (CR-cells) are believed to play critical roles in the developmental organization of neocortical and hippocampal circuits due to their secretion of the glycoprotein reelin. However, the reelin-independent signaling of CR-cells and their function in neuronal circuits is less understood. Using in-vitro electrophysiological techniques, we have shown that CR-cells are excitatory neurons that activate both AMPA- and NMDA receptors on postsynaptic hippocampal interneurons and pyramidal cells. Here, we explore the possibility that glutamate released by CR-cells may affect the structural development of postsynaptic dendrites and/or spines.

To address this question, we created a conditional knock-out mouse combining ChannelRhodopsin2 expression with the specific silencing of synaptic transmission from CR-cells to their target neurons.

Optogenetic stimulation of CR-cells in slices prepared from these animals triggered photocurrents of the same size observed in control animals (expressing only ChR2). Evoked postsynaptic responses on their target neurons, however, were reduced by 90% in knock-outs compared to control, thus confirming a successful functional inhibition of glutamate release from CR-cells. We are now investigating the developmental impact of this type of functional inactivation by comparing morphological parameters, such as dendritic spine density in knock-outs vs. controls. Golgi stain preparations revealed a strong layer specific difference in the spine density of CA1 pyramidal cells. In particular, spine density in the molecular layers decreased by 53% in knock-out vs. control mice.

In conclusion, our results suggest that CR-cells may govern the development/maturation of postsynaptic spines in their postsynaptic targets, which has important implications for hippocampal integration and signaling.

Vortrag 29:

Titel:
Cortical remodeling after spinal cord injury

Autoren:
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Abstract:
Spinal lesions are mostly studied regarding their dramatic effect on the injury site and how local circuits are compromised after injury. However, recent data also suggests that spinal cord injury can have significant effects on cortical network function particularly in primary motor cortex (M1). Therefore, we studied cortical neuron remodeling after distal spinal cord lesion in a rat model in vivo.

Adult rats underwent laminectomy at C4 and wire-knife lesion of the dorsal corticospinal tract (CST). To test how distal axotomy impacts M1 layer V CST pyramidal neurons, we focused on parameters of single cell excitability and morphology by applying multi-channel immunofluorescence, confocal microscopy, patch-clamp recordings, and surface reconstruction of axo-axonic synaptic complexes 3, 5 and 7 days after lesion. Lesioned CST neurons were visualized via retrograde tracer injection using hydroxystilbamidine (FluoroGold).

Data show that axotomized M1 layer V pyramidal neurons have significantly longer axon initial segments (AIS) than non-lesioned controls, indicating higher intrinsic excitability. Furthermore, the number of axo-axonic GABAergic synapses at the AIS of axotomized neurons is significantly reduced (up to 50%). Patch-clamp recordings indicate axotomized CST neurons have a significantly depolarized resting membrane potential and show a stronger rebound depolarization (Ih sag) at hyperpolarized potentials typically mediated by hyperpolarization-activated cyclic nucleotide gated channels.

Taken together, our results suggest that after distal lesion, M1 layer V circuits undergo significant remodeling and individual pyramidal neurons may undergo active modification of their channel architecture to adapt their intrinsic excitability to acute changes in network state.

Vortrag 30:

Titel:
The C-group of the oculomotor nucleus and its two different cell groups

Autoren:
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Abstract:
Extraocular muscles (EOM) perform high speed contractions during saccades, but also fine eye alignment during fixation of a visual target. Palisade endings (PE) in the myotendinous junction may contribute to eye stabilization. To further specify their function we investigated, whether PE contain the calcium-binding protein calretinin (CR) present in proprioceptive afferents and localized their cell bodies in monkey and human.

EOM of Rhesus monkey and human were studied for CR-immunoreactivity in PE, multiple endings and en-plaque endings. The innervation was visualized with antibodies against synaptosomal-associated protein 25 and combined with CR-immunofluorescence. With tracer injections (choleratoxin subunit B or wheat germ agglutinin) into different parts of the medial (MR) or inferior rectus muscle (IR) in monkey in combination with CR-immunofluorescence the PE cell bodies were identified in the midbrain.

In both species only in MR and IR a subdivision of PE and multiple endings was CR-immunoreactive, whereas en-plaque endings targeting twitch muscles fibres lacked CR. Accordingly, in the oculomotor nucleus C-group CR-positive and CR-negative neurons were present. A homologue group of CR-positive putative PE neurons was found in human.

Our data revealed a specialized property of PEs and multiple endings in those eye muscles being most active in vergence. We propose that the CR-negative PEs along with multiple endings represent a basic set of endings controlling fine alignment in all EOM during all eye movements, whereas the CR-positive counterparts have a specialized function in convergence. Malfunction of the PE system may contribute to strabismus or congenital nystagmus.

Vortrag 31:

Titel:
Tumor suppressor function of TFFs in human retinoblastoma cells are triggered by the p53/caspase pathway and miRNA deregulation

Autoren:
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Abstract:
Trefoil factor family (TFF) peptides play pivotal roles in different cancers. In different approaches, we set out to analyze TFF1`s and TFF3`s tumor suppressive functions in retinoblastoma (RB), the most common malignant intraocular tumor in early childhood.

Effects of TFFs on RB cells were revealed by WST-1 and TUNEL assays as well as BrdU and DAPI cell counts. Involvement of caspases in apoptosis induction were investigated by caspase inhibitor studies. p53 activity was measured by luciferase reporter assays and WB analyses and gene expression changes by expression array analyses. Effects on tumorigenicity were analysed using the in vivo CAM assay. TFF1 expression in primary RB tumor material was investigated by IHC staining.

TFF1 and TFF3 overexpression decreases RB cells` viability, proliferation and growth and significantly increases apoptosis via cleaved caspase-3. Induced apoptosis is mediated by p53 activation with concomitant regulation of miR-34a or miR-18a expression. In vivo CAM assays revealed that TFFs overexpression significantly influences tumor sizes and invasive potential of RB cells. Differentially expressed genes and pathways involved in cancer progression were identified after TFFs overexpression in Y79 cells. Functional analyses of the identified TFF3 target EMP1 support the tumor suppressive function of TFF3 in RB progression. In addition, TFF1 expression significantly correlates with clinical parameters.

Our studies revealed that TFFs induce apoptosis and decrease proliferation and in vivo tumor growth of RB cells and thus, for the first time demonstrated the tumor suppressive function of TFF1 and TFF3 in retinoblastoma.

Vortrag 32:

Titel:
A network-biology approach for the development of combinatorial treatments for the motoneuron-disease Spinal Muscular Atrophy (SMA)

Autoren:
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Abstract:
Spinal Muscular Atrophy is a motoneuron disease caused by low levels of functional Survival of Motoneuron (SMN) protein. Strategies to enhance the protein production have been followed for a long time in experimental SMA-models. Those efforts led to the approval of the first SMA-specific treatment in 2017, Nusinersen or Spinraza©, which was followed by the gene-therapy Zolgensma© in 2019. Although resulting in impressive benefits, there are a substantial number of non-responders. Since SMN has already been enhanced in those patients other complementary approaches are needed. Those are termed SMN-independent approaches. Previously, we and others reported a number of altered signaling pathways with different potentials as SMN-independent treatment targets. However, signaling pathways act as a network and this property has been neglected in the SMA-field so far.

Here, we present novel data on a systems-biology approach towards altered signaling in SMA. We used antibody arrays able to detect over 1300 signaling molecule species screening SMA-mice spinal cords. We generated a network of altered pathways from this data allowing an informed decision for highly connected targets.

Among those, we identified B-Raf, which was pre-symptomatically down-regulated in SMA-mice. This down-regulation localizes to lower motoneurons in the spinal cord. Importantly, we could rescue a C. elegans SMA-model with a neuronal over-expression of a B-Raf orthologue.

B-Raf is a neuronal Raf family kinase which propagates neurotrophic factor signaling in motoneurons. Thus, our findings may point towards a novel mechanism of motoneuron-degeneration which could be used as an SMN-independent, AAV-based approach for future combinatorial treatment regimens.

Vortrag 33:

Titel:
Regional expression patterns of altered miRNAs after experimental stroke in rats

Autoren:
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Abstract:
MicroRNAs (miRNAs) are short, non-coding sequences with the ability to silence gene expression by complementary sequence binding. miRNAs are playing an important role in a variety of diseases, such as cancer, cardiovascular diseases and pathological alterations in the central nervous system (CNS)

Using the middle cerebral artery occlusion (MCAO) model for ischemic stroke in rats, we studied several altered miRNAs in different parts of the CNS, blood serum and peripheral organs such as kidney, liver, spleen and duodenum by quantitative real-time PCR.

Predicted gene targets for distinct miRNAs regulate the cell-cycle, inflammatory responses or are involved in post stroke depression. miRNA-223 showed an increased expression after 24 and 72 h in the peri-infarct area of the cerebral cortex-, but additionally in non-affected regions such as thalamus and amygdala. Further, changes of miR-223 and -451-5p levels in the blood serum were found during the first 12 h after ischemia onset.

The release of miRNAs into the circulation and transport to peripheral organs appears to be an important mechanism to influence body functions under an ischemic challenge. This could initiate signalling from the periphery to the CNS to stabilize brain functions. Further research is required to better understand such reciprocal interactions between the CNS and body after brain ischemia.

Vortrag 34:

Titel:
Intrastriatal BoNT-A injection for experimental treatment of hemiparkinsonism in rodents – an overview

Autoren:
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Abstract:
Parkinson's disease (PD) is the second most neurodegenerative disease. All current forms of PD treatment show various shortcomings. PD therapy with centrally acting anticholinergics effectively combats PD symptoms. However, it is afflicted with particularly strong side effects and has therefore fallen behind in recent years.

The aim of our group is to achieve a local anticholinergic therapy by injecting BoNT-A directly into the striatum, avoiding all peripheral and almost all central side effects.

For our experiments we performed stereotactic injections of BoNT-A directly into the striatum of rats and mice and tested its effects in naïve as well as in hemiparkinsonian animals. Various behaviours were characterised using drug-induced rotation tests, tests for forced and spontaneous motor behaviours, cognition tests and tests for anxiety. Subsequently, brains were analysed by histological, immunohistochemical and stereological methods and receptor autoradiography for cell loss, inflammatory reactions and changes of receptor densities.

In hemi-PD rats BoNT-A inhibits pathological apomorphine-induced rotations and leads partially to an improvement of spontaneous motor behaviour and a neglect of the contralateral side for up to six months.

Intrastriatal application of BoNT-A causes a downregulation of D2 receptors in hemi-PD rats and induces swellings of cholinergic and catecholaminergic nerve fibres. However, BoNT-A seemingly is not cytotoxic. There is no neuronal loss or inflammation reactions in the brain.

Intrastriatal applied BoNT-A can counteract consequences of 6-OHDA-induced hemiparkinsonism by modulating neuronal transmission of striatal circuits. Therefore, BoNT-A treatment might well be suitable as a new therapy option for PD, while maintaining good tolerability, reversibility and absence of cytotoxicity.

Vortrag 35:

Titel:
A novel dissection course with assessed competency skills: evaluation and perception

Autoren:
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Abstract:
The discussion about the optimal teaching approach in anatomy is ongoing since a recent meta-analysis found no difference in short-term anatomy knowledge. A general agreement exists on creating a multimodal learning environment to prime future doctors optimally for clinical practice. We aimed to compose a dissection course were professional skills have a significant impact on the final score and how medical students evaluate and perceive such an approach.

Graduates dissected one body donor for 184 hours in two groups. Students needed to fulfill assessed dissection tasks and assemble new teams every week, comprising one-third of the final score. We implemented structured oral exams, near-peer teaching, visits from clinical specialists, and a portfolio. All assessments followed a strict evaluation grid.

The course was evaluated with a 15-item questionnaire and free-text comments. We also compared the grades between the current and the previous year to estimate the impact of the intervention.

Of the 384 questionnaires, 353 were returned. The study population comprised of 46.7 % males, 52.1 % females, and 0.6 % transgender.

Graduates assessed items 1 to 11 in median with “agree” and item 12 “I grade the overall composition of the dissection course:” with a median of “good” (Cronbach’s Alpha = 0.754). The cohort of 2017 completed the dissection course in median with “good”, the one from 2018 with “excellent”.

We demonstrate that students appreciate a dissection course with assessed competency-skills and encourage the implementation of similar concepts in medical education.

Vortrag 36:

Titel:
Topographic anatomy of perirectal fasciae and pelvic autonomic nerves revisited - an anatomical "roadmap" for novel techniques in rectal cancer surgery

Autoren:
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Abstract:
The architecture of perirectal fasciae and its relation to pelvic autonomic nerves is complex as mirrored by several different anatomical concepts. Novel techniques for rectal cancer surgery (e.g. transanal total mesorectal excision, TaTME) require a subtle knowledge of the perirectal anatomy to achieve both optimal oncological and functional results.

For comprehensive visualization of perirectal fasciae and pelvic autonomic nerves macroscopic dissections were carried out in 13 formalin preserved body donors (67-92 years). Key-structures were studied by histological/immunohistochemical stainings. Data were compared to intraoperative findings during rectal surgery.

The retrorectal space extended between the mesorectal fascia and the parietal pelvic fascia composed of two lamellae ensheathing the pelvic autonomic nerves. Between the parietal pelvic fascia and presacral fascia extended the presacral space covering sacral arteries and veins. Approximately at the 4th sacral vertebra all fascial layers fused in the midline and were connected to the rectum via the rectosacral ligament. Anterolaterally, the neurovascular bundles were closely related to the anorectal junction and the rectogenital septum.

The correct dissection plane during rectal mobilization in TaTME should follow the retrorectal space and not the presacral space to avoid injury of sacral blood vessels and to preserve the autonomic pelvic nerves mediating urogenital functions. Dorsally, the rectosacral ligament must be sharply divided to enter the retrorectal space. Anterolaterally, nerve fibers for the internal anal sphincter must be preserved and dissection should respect the rectogenital septum to avoid urethral injury in males or vaginal lesion in females.

Vortrag 37:

Titel:
GPER1 signaling promotes migration of V-SVZ derived cells

Autoren:
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Abstract:
In the rodent ventricular-subventricular zone (V-SVZ) neurons are generated throughout life. They migrate along the rostral migratory stream (RMS) into the olfactory bulb and differentiate into local circuit interneurons. Estrogen receptors (ERs) are steroid hormone receptors with important functions in development, growth, and reproduction. The membrane-bound estrogen receptor GPER1 mediates rapid actions of estrogens via various protein-kinase cascades and regulates rapid transcriptional activation of genes as well. However, little attention has been paid to a potential role of ERs in modulating olfactory neurogenesis. Therefore we aimed in analyzing the expression and possible function of GPER1 in the V-SVZ.

Immunohistochemistry was used to analyze the expression of GPER1 and to identify the GPER1-positive cell types. We blocked signaling of GPER1 with its antagonist G15 in V-SVZ derived Matrigel cultures and analyzed the migration of cells. To identify parts of the signaling cascade that are downstream of GPER1 signaling we used qPCR and western blotting.

We show that GPER1 is expressed in subsets of cells within the V-SVZ and the RMS, and provide evidence for a local estrogen source with aromatase-positive astrocytes. Blocking of GPER1 impairs the migration of V-SVZ derived cells, suggesting a role of GPER1 in neuronal migration. Further analysis revealed GPER1 mediated regulation of cofilin phosphorylation and an involvement of the p21-ras pathway.

We show for the first time that GPER1 can control neuroblast migration and identified Ras-mediated signaling mechanisms and p21 dependent modulation of cofilin as being crucial for this process.

Vortrag 38:

Storno

Vortrag 39:

Titel:
Activation of the bitter taste signalling cascade in tracheal brush cells induces neurogenic inflammation

Autoren:
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Abstract:
Tracheal brush cells (BC) emerged as candidates for detection of molecules displaying bitter characteristics, such as quorum-sensing molecules secreted by inhaled bacteria. We recently showed that BC release acetylcholine dependent on bitter taste signalling. Here we investigated if activation of BC-dependent taste signalling is responsible for induction of protective local effects e.g. neurogenic inflammation, a process characterized by activation of sensory nerve endings followed by peptide release, plasma extravasation and neutrophil recruitment.

Peptide-release was studied with ELISA in stimulated explanted tracheas. We established an in vivo mouse model to monitor neurogenic inflammation. Evans blue extravasation after inhalation of bitter or bacterial substances was assessed in tracheal cryosections. Neutrophil recruitment was monitored using in vivo two-photon microscopy in Ly6-eGFP mice and further quantified in tracheal sections.

Stimulation with 20 mM denatonium evoked calcitonin gene-related peptide-release from explanted tracheas. Denatonium (1, 10, 20 mM), Pseudomonas aeruginosa N-3-oxo-dodecanoyl-L-homoserine-lactone (1, 10 mM) and Streptococcus pneumoniae–supernatants (D39WT and PN36) induced Evans blue extravasation and neutrophil recruitment in the trachea 30 min after inhalation. These effects were completely abolished at low dose of denatonium and N-3-oxo-dodecanoyl-L-homoserine-lactone in TRPM5-deficient mice and significantly reduced at maximum dose. In substance P-deficient mice and in TRPM5-DTA

mice (BC-deficient) denatonium-dependent Evans blue extravasation and neutrophil recruitment was abolished.

Denatonium as well as P. aeruginosa and S. pneumoniae signal molecules induce BC- and TRPM5-dependent neurogenic inflammation. Thus, selective activation of BC could be an important alternative for non-antibiotic treatment of airway infections.

Vortrag 40:

Titel:
Fibrinogen deposition in the subventricular zone stem cell niche induces neural stem cell differentiation into astrocytes via BMP receptor signaling

Autoren:
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Abstract:
Neural stem/progenitor cells (NSPCs) originating from the subventricular zone (SVZ) contribute to brain repair in CNS disease. The microenvironment within the SVZ stem cell niche controls NSPC fate. However, the identity of extracellular factors within the NSPC environment triggering astrogenesis over neurogenesis in CNS disease remains unknown.

Here we show that the blood-derived protein fibrinogen leaks into the distant SVZ stem cell niche environment upon cortical injury and has a potent astrogenic effect on NSPCs using mouse models for cortical ischemic stroke (photothrombotic ischemia) and cortical brain trauma (stab wound injury).

Fibrinogen inhibited neuronal differentiation in SVZ and hippocampal NSPCs while promoting astrogenesis via activation of the BMP receptor and upregulation of the downstream transcriptional regulator Id3. Fibrinogen acts as a ligand of beta1 integrin to induce the BMP signaling pathway via its αC-terminus in NSPCs. Fibrinogen-mediated NSPC differentiation into astrocytes is reversed by blocking either beta1 integrin or by using fibrinogen isolates lacking the RGD containing αC-terminus. Genetic or pharmacologic depletion of fibrinogen reduced the number of newborn SVZ astrocytes. Using genetic tagging, we showed that pharmacologic depletion of fibrinogen reduced the contribution of SVZ-derived reactive astrocytes to the lesion scar formation.

We propose that in CNS disease, fibrinogen serves as a critical mediator of SVZ astrogenesis in the NSPC niche by engaging BMP receptor signaling.

Vortrag 41:

Titel:
Lipocalin 2 as a setscrew for neuroinflammation and astrocyte responsiveness

Autoren:
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Abstract:
Reactive astrocytes are central players in brain inflammation. Once activated, they undergo morphological and functional changes to differentiate either into a detrimental (A1, pro-inflammatory) or protective (A2, anti-inflammatory) phenotype. Using mouse models for multiple sclerosis (MS), we recently identified a astrocyte subpopulation in the vicinity of inflammatory lesions that is characterized by high lipocalin 2 (LCN2) expression. LCN2 appears to be a critical regulator of astrocyte chemokine expression and thus might be involved in the initiation and progression of inflammatory brain lesions.

In this study, the primary neurodegenerative cuprizone model (Cup) and an autoimmune MS animal model (combinatory Cup/EAE) were conducted on LCN2-deficient mice. Then, we analyzed the histopathological characteristics compared to wild type controls. In addition, we studied inflammatory responses and the activation state of LCN2-deficient astrocytes in vitro in response to detrimental stimuli.

LCN2-deficient and wild type mice showed comparable gliosis, oligodendrocyte loss and axonal damage in the cuprizone model. In the autoimmune Cup/EAE model, number and amount of inflammatory infiltrates were increased, resulting in higher axonal damage. LCN2-deficient astroglia revealed a dampened responsiveness to inflammatory stimuli.

Taken together, our data indicate a critical role of LCN2 for the development of inflammatory lesions in the CNS. We are currently including astrocyte-specific LCN2-deficent mice in our studies. This will help us to better understand the precise role of astroglia-derived LCN2 in the context of MS-related inflammation in the CNS.

Vortrag 42:

Titel:
Modulatory effect of estrogen on myeloid cell infiltration in the rat brain after ischemic stroke

Autoren:
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Abstract:
Ischemic stroke results from an abrupt reduction of local cerebral blood flow. As a consequence, post-ischemic neuroinflammation mainly contributes to secondary tissue damage. Brain intrinsic inflammation involves microgliosis and astrogliosis as well as the infiltration of circulating immune cells. Since estrogen shows neuroprotective effects after ischemic brain damage, we assume that this steroid can influence neuroinflammatory processes.

12-week-old male Wistar rats underwent an experimental ischemia by occluding the middle cerebral artery (tMCAO) for 1 h. Rats subjected to tMCAO were randomly assigned to receive subcutaneous estrogen or vehicle treatment immediately after the catheter withdrawal and every 12 h later. Animals were sacrificed 72 h post-tMCAO, transcardially perfused, and the brains proceeded either for TTC staining (infarct volume) and gene expression analysis or ex vivo flow cytometry.

Treatment with estrogen significantly reduced the cortical infarct volume and restored behavioral deficits. Flow cytometry revealed that CD45+CD11b+CD11c+ microglia/monocytes were massively increased in tMCAO animals. Microglia/monocyte cell numbers were significantly reduced to basal levels after estrogen substitution. Gene expression analysis further showed a time-dependent up-regulation of the microglia/monocyte activation markers CD40 and CCR2 which were clearly abrogated after estrogen substitution.

Our data reveal that estrogen affects the composition of immune cells in the peri-infarct area after ischemia. This technical approach further shows that ex vivo flow cytometry allows to detect microglia/monocytes in a quantitative and qualitative manner. Additional cell sorting after flow cytometry will extend the method and offer the possibility to characterize the activation state of immune cells as well as their cellular composition more precisely.

Vortrag 43:

Titel:
ERK1/2 is critical for signaling mechanisms to enhance cardiomyocyte adhesion.

Autoren:
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Abstract:
Arrhythmogenic cardiomyopathy (ACM) is a heart disease caused by mutations in genes encoding for desmosomal proteins, such as desmoglein 2 (Dsg2), desmocollin 2 (Dsc2), plakoglobin (Pg) and desmoplakin (Dp). We have previously shown that adrenergic signaling and PKC enhances cardiomyocyte adhesion, and we explored further the role of other signaling pathways known to regulate desmosomal adhesion including p38 MAPK and ERK1/2.

Immunostaining, Western blot, dissociation assay and hyperadhesion assay were applied in HL-1 cells and murine cardiac slice cultures.

Dissociation assays in HL-1 cells and cardiac slices showed that, similar to increased cAMP, activation of PKC and inhibition of p38 MAPK enhanced cardiomyocyte adhesion and induced hyperadhesion, which renders cardiomyocyte adhesion independent of Ca2+. Western blot analyses showed an increase in pERK1/2-levels when adhesion was enhanced, suggesting a role of the above mentioned signaling pathways in ERK1/2-activation. However, inhibition of ERK1/2 alone did not affect both baseline adhesion and hyperadhesion, but diminished PKC-, cAMP- and p38 MAPK-mediated effects on basal cell adhesion but not on hyperadhesion. This was paralleled by a decrease of Dsg2 at cell borders in HL-1 cells.

Increased cAMP levels, activation of PKC as well as inhibition of p38 MAPK enhances cardiomyocyte basal adhesion and induces hyperadhesion, the former of which is dependent on ERK1/2.

Vortrag 44:

Titel:
Wnt and Frizzled-4 – breadcrumbs on the search for specific enteric nervous progenitor cell markers

Autoren:
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Abstract:
Neural progenitor cells from the enteric nervous system are a potential source for cell-replacement therapies. Yet, we know little about the molecular mechanisms regulating this cell pool, let alone specific markers to identify neural progenitors in the ENS, especially in the human intestine. Here, we hypothesize that the canonical Wnt-pathway plays a central role in ENS-progenitor proliferation and that the Wnt-receptor Frizzled-4 is expressed by human ENS-progenitor cells.

We investigated the influence of Wnt-signaling on the proliferation of isolated postnatal ENS-progenitors from murine and human intestine using gene-chip analysis, BrdU-incorporation assays, immunohistochemistry, western blot, and RT-PCR experiments. We used FACS analysis, immunohistochemistry, and patch-clamping to characterize Frizzled-4-expressing cells from human intestine. human intestine.

Here we present sound evidence that the activation of the canonical Wnt pathway increases the proliferation of enteric neural progenitors and leads to a higher yield of differentiated neurons in vitro, both in humans and mouse models. We identified the Wnt-receptor Frizzled-4 as a novel marker expressed on human postnatal ENS-progenitor cells: Frizzled-4positive cells gave rise to neurosphere-like bodies, expressed Nav, Kv and BK channels, and, hence, differentiated into functional neurons. In Frizzled-4negative cultures, we did not detect any neural cells.

Canonical Wnt-signaling has stimulating effects on the proliferation of ENS-progenitors in mice and man. Frizzled-4 is expressed by human neural progenitors and can be used for isolation and purification. Frizzled-4positive cells are capable of proliferation and functional neuronal differentiation in vitro. Therefore, Wnt-signaling and its molecular pathway components are likely to be an important part of ENS-progenitor regulation.

Vortrag 45:

Titel:
Mesenchymal stem cell derived exosomes reduce secretion of inflammatory cytokines of chondrocytes after IL1BETA stimulation

Autoren:
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Abstract:
Orthopedic diseases originating from joint inflammation are common in pets and humans. Treatment options for these diseases are still not satisfying. Recently, not only the use of mesenchymal stem cells but also exosomes secreted by stem cells were becoming a subject of further investigations to support the healing process during e.g. osteoarthritis (OA) thus providing a better quality of life. The beneficial effect of exosomes secreted by equine mesenchymal stem cells (eqMSC) was investigated in an in vitro OA model by challenging equine chondrocytes by interleukin 1 BETA(IL1BETA).

eqMSC derived exosomes of were harvested after 72h incubation in an exosome free medium and were concentrated by ultrafiltration. These exosomes were supplemented to initially IL1BETA challenged equine chondrocytes for 24 hours. Afterwards chondrocytes were incubated for additional 24 hours until supernatant was collected for the analysis of IL6 and TNFalpha. Additionally, cells were analyzed for the expression of COL2A1, COX2, IL6, iNOS, MMP9 and PTGES using RT-qPCR.

Levels of IL-6 and TNFalpha were notably lowering at presence of eqMSC derived exosomes. COX2, IL6, iNOS, MMP9 and PTGES were remarkably increased after IL1BETA challenge and could be significantly decreased in the presence of eqMSC derived exosomes. Additionally, the expression of COL2A1 was reduced after IL1BETA stimulation and was slightly recovered under addition of eqMSC derived exosomes.

In our study we could show that eqMSC derived exosomes provide a significant effect on the secretion of inflammatory cytokines in an OA in vitro model and are thus a successful cell free treatment option for inflammatory joint diseases.

Vortrag 46:

Titel:
Differential control of COX-2 expression in macula densa cells under calcineurin inhibition by cyclosporine A

Autoren:
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Abstract:
Cyclosporine A (CsA) is used to prevent rejection of transplanted organs. Despite positive outcomes, side effects such as overall functional and structural deterioration may affect the kidney. We studied how CsA may cause dysregulation of key juxtagomerular signaling components.

Wistar rats received 25mg CsA/kg b.w./d for 2 weeks. Kidneys were perfusion-fixed and evaluated histologically. Cultured macula densa (MD) cells were treated with CsA (5 µM) and angiotensin II (AngII; 1 µM). Tissues or cells were immunohistochemically analyzed for renin, COX-2, NFAT 1 to 4, p38 MAPK, CREB, NF-kB, and activating phosphorylation of p38 MAPK and CREB. Inhibitors to p38 MAPK (10 µM) and NF-kB (5 µM) were applied to cells.

CsA caused upregulation of renin and complete downregulation of COX-2 in vivo. An assumed link between NFAT and COX-2 within MD could not be established. In cultured MD cells, CsA caused a rise in COX-2 abundance after 6 or 24 h (2-fold each); p38 MAPK phosphorylation was increased in parallel (1.9-fold). Inhibition of p38 MAPK attenuated CsA-induced COX-2 upregulation by 50%. Under the same conditions, NF-kB revealed its nuclear translocation (+50%). Inhibitor to NF-kB (6h) blunted COX-2 stimulation by 35%. CREB phosphorylation was increased 2- to 4-fold upon CsA (6h). AngII decreased expression of baseline COX-2 by 40% (6h) and blunted CsA-induced COX-2 stimulation by 30% (6h). All data were significant (min. $p<0.05$).

The discovery of a novel, regulatory synergism of calcineurin and angiotensin in kidney macula densa, governing COX-2 biosynthesis, may serve to address juxtaglomerular dysregulation under CsA treatment.

Vortrag 47:

Titel:
The role of selectins and integrins in xenograft models of human intraperitoneal carcinomatosis

Autoren:
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Abstract:
Intraperitoneal carcinomatosis is a common form of progression in abdominal cancers, which leads to a dismal prognosis for the affected patients. New therapeutical strategies for selective inhibition of the mechanisms underlying the development of intraperitoneal carcinomatosis are thus desperately needed.

As the mesothelial cells of the peritoneal epithelium and the endothelial cells of blood vessels are closely related embryologically, we hypothesized that tumor cells spreading in the peritoneal cavity use similar mechanisms as in hematogenous metastasis.

We used xenograft models for intraperitoneal carcinomatosis formation in E- and P-selectin deficient mice by human ovarian and pancreatic cancer cell lines with and without stable shRNA mediated knockdown of integrins.

Carcinomatosis formation was significantly reduced in E- and P-selectin deficient mice prolonging the animals' survival, however, even in selectin deficient animals, intraperitoneal carcinomatosis still developed. We thus compared gene expression in carcinomatoses formed by human pancreatic carcinoma cells in E-/P-selectin deficient and selectin competent mice. Interestingly, integrins, especially integrin αV, were upregulated in the intraperitoneal carcinomatoses from selectin k.o. animals. Stable shRNA mediated knockdown of integrin αV in human pancreatic carcinoma cells significantly reduced carcinomatosis formation in immunodeficient mice and caused morphological changes in the remaining tumors. Additionally, the knockdown showed an additive effect when combined with E- and P-selectin deficiency in the animals.

Currently, we are trying to elucidate the role of other integrins during carcinomatosis development in our xenograft models (e.g. α6 and β4).

Selectins and Integrins might be interesting targets for future anti-carcinomatosis therapies.

Vortrag 48:

Titel:
Nr2f1 transcriptional gradient in the developing mouse cerebral cortex depends on histone demethylase KDM1a activity

Autoren:
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Abstract:
Arealisation of the murine cerebral cortex depends on morphogenetic gradients such as SHH (sonic hedgehog), FGFs (fibroblast growth factors) and BMPs (bone morphogenetic proteins), which induce subsequently gradients of transcription factors. Further regionalized gene activities result in functionally specialized areas of the neocortex. Here we present data showing that methylation of lysine 4 of histone H3 (H3K4me) occur in a caudal-high to rostral-low gradient in the E14.5 developing mouse cerebral cortex. Both, H3K4 di-(me2) and tri-methylation (me3) activate gene transcription.

We determined genome-wide distribution of H3K4me3 in the rostral and caudal E14.5 developing cortex and correlated this data to transcriptional alterations between both brain regions.

We identified twelve developmental genes as differentially methylated and expressed between rostral and caudal regions, including the transcription factor Nrf2f1 (Nuclear Receptor Subfamily 2 Group F Member 1). Nrf2f1 was transcribed in a rostral-low to caudal-high gradient. Among the H3K4 modifying enzymes, activity of which could be responsible for the differential H3K4me2/me3 along the rostro-caudal axis, we identified Kdm1a, an H3K4me2 demethylase. Kdm1a was expressed in an opposing gradient, with rostral-high to caudal-low levels, both in mRNA and protein. Chromatin-immunoprecipitation followed by quantitative real-time PCR confirmed KDM1A location at the promotor of Nr2f1. KDM1A levels were lower in the rostral compared to the caudal telencephalon. Pharmacological inhibition of KDM1A in vivo during mouse brain development disrupted the Nr2f1 transcriptional gradient at E14.5.

Together our data suggest an important role of KDM1A enzymatic function during corticogenesis by regulating Nr2f1 expression in a rostro-caudal gradient.

Vortrag 49:

Titel:
A polycystin-2 mutant protein with modified channel properties leads to an increased diameter of renal tubules and to polycystic kidney disease in mice

Autoren:
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Abstract:
15% of patients with autosomal-dominant polycystic kidney disease (ADPKD) carry mutations in the PKD2 gene, which encodes polycystin-2, a non-selective cation channel of the TRP family. So far, however, the underlying mechanism of cyst formation is unknown. With the help of model organisms, we investigated the pore region of polycystin-2.

Eleven amino acids in the pore region of polycystin-2 were exchanged by that of the related protein polycystin-2L1 to generate the mutant polycystin-2poreL1. Homology modeling was used to shed light on potential conformational changes after this substitution. Polycystin-2poreL1 and wild-type polycystin-2 were expressed in Xenopus oocytes to detect differences in channel conductivity. Knock-in mice were generated to express polycystin-2poreL1 in vivo. Collecting ducts from the knock-in and wild-type mice were examined for their Ca2+ response after stimulation with vasopressin using Fura-2 imaging.

Electrophysiological experiments showed increased Ca2+ currents in oocytes expressing polycystin-2poreL1 compared to wild-type polycystin-2. In silico homology modeling indicated an enlarged selectivity filter in polycystin-2poreL1 compared to wild-type polycystin-2 explaining the higher conductivity of Ca2+ in the mutant. Polycystin-2poreL1 led to the enlargement of collecting ducts, to cyst formation and to elongated cilia in homozygous knock-in mice. In Ca2+ imaging assays of knock-in mice increased intracellular Ca2+ levels could be detected.

Selective replacement of the pore region of polycystin-2 results in increased conductivity for Ca2+. This leads to lumen enlargement and cyst formation in collecting ducts of mice which

emphasizes the relevance of the pore region for the maintenance of tubular geometry in the kidneys.

Vortrag 50:

Titel:
Contribution of LTi and TH17 cells to B cell aggregate formation in the central nervous system in a mouse model of multiple sclerosis

Autoren:
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Abstract:
In a subgroup of patients suffering from progressive multiple sclerosis (MS), the occurrence of B cell aggregates within the meninges was associated with a more severe disease course and cortical histopathology. We have developed the B cell-dependent MP4-induced experimental autoimmune encephalomyelitis (EAE) as a mouse model to mimic this trait of the human disease. The aim of this study was to determine a potential role of lymphoid tissue inducer (LTi) and TH17 cells in the process of B cell aggregate formation in the MP4 model.

We performed flow cytometry of cerebellar and splenic tissue of MP4-immunized mice in the acute and chronic stage of the disease to analyze the presence of CD3-CD5-CD4+RORγt+ LTi and CD3+CD5+CD4+RORγt+ TH17 cells. We further determined the gene expression profile of B cell aggregates using laser capture microdissection, followed by RNA sequencing.

There was no evidence for the existence of LTi cells in acute or chronic EAE in neither of the two models. Yet, we detected CD3-CD5-CD4-RORγt+ innate lymphoid cells (ILCs) and TH17 cells in the CNS, the latter especially in the chronic stage of MP4-induced EAE. Moreover, we observed a unique gene signature in CNS B cell aggregates compared to control tissue.

The absence of LTi cells in the cerebellum suggests that other cells might take over the function as an initiator of lymphoid tissue formation in the CNS. Overall, we propose some potential candidates, which might be involved in the formation of B cell aggregates in the CNS of MP4-immunized mice.

Vortrag 51:

Titel:
Brain tumor-specific EGFRvIII as a new old target in precision medicine

Autoren:
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Abstract:
Glioblastoma multiforme (GBM) is one of the most malignant types of brain tumors with a median survival of 15 months. In spite of multimodal treatments GBMs remain incurable and alternative treatments are desperately needed. About 40 percent of primary GBM display an amplification of erbb1 encoding for the epidermal growth factor receptor (EGFR). Half of these cells co-express an oncogenic constitutive active mutant termed EGFRvIII, which correlates with a poor prognosis in glioma patients.

EGFRvIII is a promising anti-tumor target as its expression is restricted to tumor cells. Therapies directed against EGFRvIII such as ABT-414 or mAb806 reduced tumor growth and increased survival in experimental glioma models. However, trials targeting EGFR or EGFRvIII were not as convincing as expected therefore the molecular mechanisms allowing EGFRvIII to escape tumor therapy have been analyzed in this study. In particular, intracellular trafficking of endogenous EGFR and EGFRvIII as well as receptor-specific signaling have been studied by immunocytochemistry, mass spectrometry and RNA-Seq. Further, experimental glioma models have been applied in vivo, i.e., animals were treated by a combination of chemotherapy and EGFR/EGFRvIII-inhibiting antibodies.

In conclusion, EGFR and EGFRvIII significantly differed in intracellular trafficking and signaling. Glioma growth depended to a large extend on endogenous EGFRvIII and experimental glioma bearing mice treated with chemotherapy and an anti-EGFRvIII blocking antibody displayed an increased average survival time as compared to animals treated with isotype control.

These findings open up new avenues in how to improve anti-EGFR glioma treatment to render existing therapies more effective.

Vortrag 52:

Titel:
Human iPSC derived olfactory placode

Autoren:
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Abstract:
The development of the human olfactory epithelium largely remains an uncharted field of study due to its challenging accessibility. To shine a light on these yet hidden processes we have established an in vitro protocol, to differentiate human induced pluripotent stem cells (iPSCs) into cells of the olfactory placode (OP), being the direct precursors of the olfactory receptor neurons in the olfactory epithelium.

The induced pluripotent stem cells (iPSC) are generated by the Sendai-viral reprogramming of human keratinocytes which are obtained from the outer root sheath of plucked hair. Using optimized differentiation protocols in cell culture, the iPSCs are then differentiated towards the OP and analyzed by immunofluorescence staining, qPCR and FACS. Furthermore, our group is analyzing the CRISPR/Cas method to generate reporter- and knock-out lines for certain markers and other genes of interest to gain more insights into their specific role in the developmental process.

We can report the successful and robust differentiation of iPSC into cells of the olfactory placode and further insight into the development of the human olfactory epithelium.

Our findings show the possibility to generate cells of the OP and possibly even an olfactory epithelium in vitro. Further efforts will need to be made in the future with the goal to generate a functioning in vitro model of the human olfactory epithelium, that will open new possibilities for disease modeling and drug testing.

Vortrag 53:

Titel:
WNK bodies are novel organelles linking plasma potassium to renal salt handling

Autoren:
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Abstract:
The renal distal convoluted tubule (DCT) is vital for potassium homeostasis. Low plasma [K+] stimulates its apical Na+,Cl--cotransporter (NCC), limiting K+ loss in the downstream nephron but increasing NaCl retention and blood pressure. NCC is activated by phosphorylation via a kinase cascade comprising with no lysine (WNK) kinases upstream of two homologous Ste-20-related kinases, SPAK and OSR1. During hypokalemic NCC activation, these kinases accumulate in membraneless, cytoplasmic structures of unclear function, termed WNK bodies. We hypothesized that WNK bodies perform SPAK/OSR1 activation by phosphorylation.

We analyzed cellular distribution and phosphorylation of SPAK/OSR1 using high-resolution immunofluorescence and electron microscopy in different rodent models of hypokalemia, namely dietary K+ deprivation, genetic WNK4 deletion, and furosemide treatment.

Feeding mice a K+-deficient diet induced formation of WNK bodies enriched in phosphorylated (p) WNK and pSPAK/OSR1 along with increased abundance of pSPAK/OSR1 at the apical DCT membrane. In contrast, WNK bodies of WNK4-deficient mice contained only unphosphorylated kinases. The WNK bodies in the WNK4-deficient DCT were enlarged, whereas apical abundance of SPAK/OSR1 was reduced, suggesting that WNK4-dependent phosphorylation within WNK bodies facilitates apical trafficking of SPAK/OSR1. Accordingly, ultrastructural analysis showed close association of WNK bodies and the microtubular trafficking apparatus. Disruption of microtubules using colchicine in furosemide-treated rats led to accumulation of pSPAK/OSR1 in WNK bodies.

In sum, our results indicate that WNK bodies are membraneless organelles performing SPAK/OSR1 activation for their subsequent apical trafficking, thereby linking plasma [K+] to NCC phosphorylation, NaCl balance and blood pressure.

Vortrag 54:

Titel:
Aging-related CEACAM1 signaling promotes vascular dysfunction

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Abstract:
Aging is an independent risk factor for cardiovascular diseases and therefore of particular interest for the prevention of cardiovascular events. However, the mechanisms underlying vascular aging are not well understood. Since CEACAM1 is crucially involved in vascular homeostasis, we sought to identify the role of CEACAM1 in vascular aging.

Specimen of human internal thoracic artery as well as aorta of WT and CEACAM1 knockout mice were analyzed by immunohistochemistry, immunoblotting, RT-PCR and functional analyses, i.e. permeability assay. Furthermore, the effect of CEACAM1 on endothelial expression of pro-inflammatory and pro-fibrotic genes was analyzed in vitro by siRNA-mediated CEACAM1 knockdown in human endothelial cells.

We show that CEACAM1 is upregulated in the course of vascular aging. Further analyses demonstrated that TNF-α is CEACAM1-dependently upregulated in the aging vasculature. Vice versa, TNF-α induces CEACAM1 expression. This results in a feedforward loop in the aging vasculature that maintains a chronic pro-inflammatory milieu. Furthermore, we demonstrate that age-associated vascular alterations, i.e. increased oxidative stress and vascular fibrosis due to increased medial collagen deposition crucially depend on the presence of CEACAM1. Additionally, age-dependent upregulation of vascular CEACAM1 expression contributes to endothelial barrier impairment, putatively via increased VEGF/VEGFR-2 signaling. Consequently, aging-related upregulation of vascular CEACAM1 expression results in endothelial dysfunction that may promote atherosclerotic plaque formation in the presence of additional risk factors.

Our data suggest that CEACAM1 might represent an attractive target in order to delay physiological aging and thereby the transition from physiological aging into vascular disorders like atherosclerosis.

Vortrag 55:

Titel:
Desmosomal hyper-adhesion requires desmoglein 3

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Abstract:
Intercellular adhesion of keratinocytes is critically dependent on desmosomes which, during maturation, acquire a hyper-adhesive state at which desmosomes become independent from Calcium (Ca2+). However, the mechanisms underlying desmosomal hyper-adhesion are not yet elucidated. Thus, we here characterized the roles of desmoglein (Dsg) 1 and 3 in desmosomal hyper-adhesion dependent on the desmosomal plaque proteins plakophilins (Pkp) 1 and 3.

Atomic force microscopy (AFM), ex-vivo human skin model, keratinocytes dissociation assay, Western blot, Extracellular Crosslinking

We probed Ca2+-dependency of Dsg 1 and 3 in an ex-vivo human skin model. Interestingly, immunostaining was reduced after 24h of treatment with the Ca2+-chelator EGTA for Dsg1 but not for Dsg3, suggesting that hyper-adhesion may be Dsg isoform-dependent. In accordance, murine keratinocytes lacking Dsg3 failed to acquire a hyper-adhesive state in contrast to wt in dissociation assays. During this process, wt keratinocytes increased levels of Dsg3-oligomers, which correlated with desmosomal hyper-adhesion. Next, we used murine keratinocytes lacking Pkp1 or Pkp3. Loss of Pkps drastically reduced Ca2+-independency and Pkp1-deficiency abolished formation of Ca2+-independent Dsg3 oligomers. Further, in AFM experiments Dsg3 single molecule binding strength was increased under hyper-adhesive conditions in wt, but not in Pkp-deficient keratinocytes whereas Dsg1 binding strength were unaltered in all cell lines.

Taken together, the data indicate that hyper-adhesion may not be a state acquired by entire desmosomes but rather a phenomenon provided by certain desmosomal cadherin isoforms, which during acquisition of hyper-adhesion change their binding properties in a Pkp-dependent manner.

Vortrag 56:

Titel:
Sugar sensing in the urethra

Autoren:
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Abstract:
Urethral cholinergic chemosensory cells (UCCC) serve as sentinels utilizing taste receptors to monitor the urethral lumen for potential harmful substances and triggering protective reflex mechanisms. Increased urinary glucose concentration promotes bacterial growth and diabetes mellitus patients are at high risk for urinary tract infection. This study aimed to clarify whether UCCC functionally respond to sugars and artificial sweeteners.

UCCC were isolated from ChAT-eGFP reporter mice (ChAT=choline acetyltransferase) and Tas1R3-/- (sweet receptor) deficient mice for recording of intracellular [Ca2+] by CLSM. Stimuli were various sugars and artificial sweeteners, gurmarin served as Tas1R3 inhibitor. UCCC from wild-type mice were analyzed by RT-PCR and urodynamic responses to urethral sucrose application were recorded in anesthetized rats.

Intracellular [Ca2+] increase was observed in response to the sugars sucrose (26/38 UCCC; 76%), fructose (13/20; 65%), glucose (11/18 61%) mannose (3/21; 14%) maltose (6/20; 30%), lactose (5/18; 28%) and to the artificial sweeteners sorbitol (1/16; 6%), saccharin (5/15; 27%), sodium cyclamate (2/11; 18%). In Tas1R3-/- mice, the reaction to sucrose is significantly reduced and less UCCC respond (10/26; 38%). Gurmarin also significantly attenuated, but not abolished the responses. RT-PCR revealed the expression of Tas1R3 and components of an alternative sugar recognition pathway. Intraurethral application of sucrose reflexively increased activity of the bladder detrusor muscle measured by cystometry.

UCCC respond to various sugars and artificial sweetener in a Tas1R3-dependent and an additional Tas1R3-independent manner. Intraurethral sucrose triggers a protective reflex mechanism. We, thus, consider sweet as a urethral danger signal to be monitored and counteracted by UCCC.