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Vortrag 1

Titel:
Neuronal IL-4R signaling in CNS repair

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Abstract:
Cytokines are known for their role in the immune system, however, recent work suggests that some cytokines also act directly on neurons. The interleukin-4 receptor (IL-4R) is expressed on numerous neurons in the brain, especially in areas involved in locomotion. Our previous experiments showed a role for T helper 2 cells and IL-4 in CNS regeneration after traumatic injury. The objective of this study was to investigate axon repair in neuroinflammatory disorders.

IL-4 treatment was applied during the chronic phase of experimental auotimmune encephalomyelitis (EAE), the mouse model for multiple sclerosis (MS), via lumbar intrathecal injection or nasal application. The neuronal IL-4R signaling pathway was investigated using cultured cortical neurons.

We demonstrate that intrathecal IL-4 treatment during the chronic phase of several experimental autoimmune encephalomyelitis models reversed disease progression without affecting inflammation. Amelioration of disability was abrogated upon neuronal deletion of IL-4R.(Vogelaar et al, 2018). We identified a fast and direct neuronal signaling pathway that leads to cytoskeletal remodeling and thereby axonal repair. For better clinical translation, nasal treatment with IL-4 was equally effective.

IL-4 treatment is able to reverse disease progression during chronic neuroinflammation through neuroprotection and axon repair. Targeting neuronal IL-4 signaling may offer new therapeutic strategies to halt disability Progression in MS and possibly also neurodegenerative conditions. We are currently investigating the role of IL-4 in neuronal homeostasis.

Vogelaar CF*, Mandal S*, Lerch S*, at al. 2018 Fast direct neuronal signaling via the IL-4 receptor as therapeutic target in neuroinflammation. Science Translational Medicine, DOI: 10.1126/scitranslmed.aao2304

Vortrag 2

Titel:
Axonal transport deficits are driven by inflammation rather than demyelination

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Abstract:
Axonal damage is the main factor contributing to disease progression in Multiple sclerosis (MS). In neuropathological investigations, axonal damage is most often identified using immunohistochemical staining for the β-amyloid precursor protein (APP). Although it is assumed that axonal damage injury results in disturbances of APP transport leading to accumulation of vesicles and subsequent formation of APP+ spheroids, the reliability of this method to detect axonal injury was not systematically tested. The objective of this study was to determine the utility of APP as immunohistochemical marker of axonal damage in demyelinating and neuroinflammatory conditions.

Inflammatory and toxic demyelination was induced by MOG35-55 immunization, cuprizone-intoxication or stereotactic LPC-injection. The morphology of axonal injury was investigated by serial block-face scanning electron microscopy (3D-EM)and immunfluorescence microscopy. Measurements of the electrically evoked compound action potential were performed to correlate morphological with functional changes.

3D-EM revealed axonal swellings in early demyelinated white matter tissues. Such swellings contained numerous mitochondria, synaptic and dense core vesicles. Densities and volumes of ultrastructural axonal swellings correlated with densities and volumes of APP+ spheroids, detected by immunohistochemistry. Spheroids were as well positive for mitochondrial and synaptic proteins. Such morphological alterations were paralleled by an impaired propagation of the action potential. Of note is that in various demyelination models, densities of axonal spheroids best correlated with microglia activation, but not demyelination.

In conclusion, our data suggest that the axonal accumulation of synaptic and mitochondrial proteins is a reliable marker of axonal damage, but is driven by inflammation rather than demyelination.

Vortrag 3

Titel:
The phosphatase inhibitor CPI17 regulates demyelination and autoimmune inflammation in the brain

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Abstract:
We recently found that the C-kinase-activated protein phosphatase-1 (PP1) inhibitor of 17 kDa (CPI-17), a phosphorylation-dependent inhibitory protein for myosin-associated PP1 holoenzyme, is expressed not just by smooth muscle cells but as well by mature oligodendrocytes. The role of CPI-17 for oligodendrocyte physiology and pathology remains unknown. Here, we investigated the relevance of CPI-17 in two commonly used MS animal models.

Toxin-induced oligodendrocyte apoptosis with subsequent demyelination was induced by feeding CPI-17-/- and wildtype C57/BL6J mice a diet containing 0.25 % cuprizone for up to 3 weeks. Autoimmune demyelination was induced by MOG35-55 immunization (experimental autoimmune encephalomyelitis; EAE). Densities of apoptotic oligodendrocytes were quantified in H&E-stained sections. Myelination was assessed in LFB/PAS and anti-PLP stained sections. Microglia, astrocyte, and oligodendrocyte densities were analyzed using anti-IBA1, anti-GFAP and anti-OLIG2 antibodies. Axonal injury was analyzed in anti-APP stained sections.

Densities of CPI-17-expressing cells were reduced in experimental demyelination and multiple sclerosis post mortem tissues. At week 1, the extent of early oligodendrocyte apoptosis was slightly higher in CPI-17-/- compared to wildtype mice. At week 3, microglia activation was slightly more pronounced in CPI-17-/- mice. At week 5, at the peak of active demyelination, myelin degeneration and microgliosis were significantly ameliorated in CPI-17-/- compared to wildtype mice. Furthermore, clinical recovery after experimental autoimmune encephalomyelitis was more pronounced in CPI-17-/- compared to wildtype mice.

In this study, we identified a novel regulator of oligodendrocyte degeneration. Pharmacological blockade of the CPI-17 pathway might stabilize oligodendrocytes and, therefore, halt disease progression in MS patients.

Vortrag 4

Titel:
The cuprizone model as a tool to study the relevance of Nrf2-activation for lesion development and progression in Multiple sclerosis

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Abstract:
Multiple sclerosis (MS) is an inflammatory disease of the CNS. Mitochondrial dysfunction and oxidative stress are critically involved in lesion development and disease progression. The antioxidant responsive element (ARE) is an enhancer element that initiates the transcription of genes encoding detoxification and factors essential for neuronal survival. ARE is activated through the binding of its transcription factor Nrf2 (NF-E2-related factor 2). In the MS mouse model cuprizone, impaired mitochondria lead to increased oxidative stress and subsequent activation of the Nrf2/ARE system. This allows investigating the potential of this model to study oxidative stress and Nrf2/ARE signaling in the scope of MS and in relevance for oligodendroglial responses against mitochondrial dysfunctions.

Nrf2-deficient and wild type controls were fed 0.25% cuprizone for one and three weeks, mimicking beginning and acute demyelination. Micro- and astrogliosis, myelin status (PLP and LFB) and oligodendrocyte loss were assessed by immunohistochemistry. Oligodendroglial OliNeu cells were used for shRNA silencing of Nrf2. Mitochondrial dysfunctions were induced by sodium acid (SA) treatment.

Reactive gliosis and demyelination were evident in the acute demyelination group (three weeks). Microglia activation and axonal damage (indicated by increased numbers of APP- positive axonal bulbs) were more severe in cuprizone-fed Nrf2-deficient mice when compared to cuprizone fed WT animals. Nrf2 knockdown in vitro decreased metabolic activity and cell viability and increased the amount of depolarized mitochondria in response to SA.

Our results highlight the cuprizone model as possible tool to study pharmacological interventions that aim to stabilize mitochondrial functions and/or to reduce oxidative challenge

Vortrag 5

Titel:
Cuprizone-induced oligodendrocyte degeneration triggers peripheral immune cell recruitment into the forebrain

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Abstract:
Brain-intrinsic degenerative cascades are a proposed factor driving inflammatory lesion formation in multiple sclerosis (MS) patients. We recently showed that encephalitogenic lymphocytes are recruited to the sites of active demyelination in a mouse model of MS. Here, we investigated whether cuprizone-induced oligodendrocyte apoptosis is sufficient to trigger peripheral immune cell recruitment into the forebrain.

Different groups of C57BL/6 mice were fed cuprizone followed by subsequent immunization with myelin oligodendrocyte glycoprotein peptide (MOG35-55). Brains were histochemically evaluated for the presence of perivascular inflammatory infiltrates. Furthermore, brains were stained by immunohistochemistry for microglia, astrocyte, demyelination and axonal damage.

We show that early cuprizone-induced white matter lesions display a striking similarity to early MS lesions, i.e., oligodendrocyte degeneration, microglia activation and absence of lymphocytes. Such early cuprizone lesions are sufficient to trigger peripheral immune cell recruitment. The lesions are characterized by discontinuation of the perivascular glia limitans, focal axonal damage, and perivascular astrocyte pathology. Time course studies showed that the severity of cuprizone-induced lesions positively correlates with the extent of peripheral immune cell recruitment. Furthermore, results of genome-wide array analyses suggest that moesin is integral for early microglia activation in cuprizone and MS lesions.

This study underpins the significance of brain-intrinsic degenerative cascades for immune cell recruitment and, in consequence, MS lesion formation.

Vortrag 6

Titel:
Targeting myelin lipid metabolism as a therapeutic strategy in a model of CMT1A neuropathy

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Abstract:
Charcot-Marie-Tooth disease type 1A (CMT1A) is the most common inherited neuropathy. Affected patients display aberrant developmental myelination of peripheral nerves and secondary axonal loss during adult life that leads to slowly progressive muscular atrophy and sensory impairment. A therapy is not available.

We used a transgenic rat model for CMT1A (CMT rats) that closely mimicks the human disease. In order to gain insight into the still poorly understood pathomechanism and to be able to develop a therapeutic strategy for CMT1A, we performed RNA and lipid profiling of peripheral nerves as well as molecular, histological and functional analyses in vitro and in vivo.

We show that myelinating Schwann cells in CMT rats exhibit a developmental defect that includes the reduced transcription of genes required for myelin lipid biosynthesis. Consequently, lipid incorporation into myelin is reduced, leading to an overall distorted stoichiometry of myelin proteins and lipids with ultrastructural changes of the myelin sheath. Importantly, we also found that the substitution of phosphatidylcholine and phosphatidylethanolamine in the diet is sufficient to overcome the myelination deficit of affected Schwann cells in vivo.

Phospholipid treatment rescues the number of myelinated axons in peripheral nerves of CMT rats and leads to a marked amelioration of their clinical phenotype. We propose that lipid supplementation is an easily translatable therapeutic approach in CMT1A and possibly other dysmyelinating neuropathies.

Vortrag 7

Titel:
Intra-articular sprifermin reduces cartilage loss in addition to increasing cartilage gain in a randomized, placebo-controlled phase II clinical trial

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Abstract:
Osteoarthritis (OA) is the most common disease of human joints, with no disease-modifying therapy yet approved. A recent randomized, placebo-controlled phase II clinical trial (FORWARD[1]) suggests that intra-articular injection of recombinant human fibroblast growth factor 18, sprifermin, increases mean cartilage thickness in patients with knee OA. The current post-hoc analysis [1] aimed to determine whether sprifermin also reduces cartilage loss, wherever it occurs in the knee joint.

Patients with symptomatic, radiographic knee OA (age 40–85 years) were randomized (1:1:1:1:1) to receive intra-articular injections with placebo, 30 or 100 µg sprifermin every 6 or 12 months [1]. MR imaging was used to determine cartilage thickness change over 24 months in 16 femorotibial subregions. The changes in cartilage thickness were measured in each knee, with the thickening score defined as the sum of positive changes and the thinning score as the sum of negative changes across all subregions.

The modified intention-to-treat population included 494 patients [1]: Compared with healthy controls from an observational cohort (OAI) and the placebo patients, the thickening score was almost doubled with 100µg sprifermin. The thinning score was -414 (95%CI, -477; -351) in healthy reference controls and was -766µm (95%CI, -972; -560) in placebo patients, whereas in patients treated with 100µg sprifermin every 6 months, the score was -459 µm (95%CI, -550; -368).

Intra-articular sprifermin increases cartilage thickening and almost normalizes cartilage thinning to the level observed in healthy controls.

Vortrag 8

Titel:
CEACAM1 promotes melanoma metastasis and is involved in the regulation of the EMT associated gene network in melanoma cells

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Abstract:
Despite significant recent progress in the therapy of metastasized melanoma, there are still no therapeutic options for a considerable number of patients. Additional therapeutical functional targets are thus urgently needed.

We investigated the functional role of CEACAM1 in a spontaneous metastasis xenograft model of human melanoma using the BRAF wildtype cell line MeWo with and without RNAi mediated knockdown of CEACAM1 and scid mice, lacking functional T and B cells. Tumors from the xenograft model were subjected to whole genome expression analysis and metastasis was quantified histologically. Results and identified markers were verified using tissue samples of over 100 melanoma patients.

Knockdown of CEACAM1 prolonged the animals' survival by significantly reducing subcutaneous growth of MeWo tumors and spontaneous lung metastasis independently of T cell responses. Whole genome expression arrays revealed a strong influence of CEACAM1 knockdown on the network of EMT associated genes in the xenograft tumors. IGFBP7 and Latexin (highest up- and downregulated expression) were found to be associated with longer and shorter survival, respectively, in a cohort of melanoma patients. High FOSL1 and altered TWIST1 expression were found to be correlated with shortened survival in the cohort of melanoma patients. After a stepwise selection procedure combining above markers, multivariate analysis revealed IGFBP7, Latexin and altered TWIST to be prognostic markers for death.

These results show that CEACAM1 could be an interesting target for melanoma therapy as an alternative to (or in combination with) immune checkpoint and BRAF inhibitors.

Vortrag 9

Titel:
Intraperitoneal spreading of ovarian cancer cells depends on integrin-β4 expression in xenograft model

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Abstract:
The major clinical problem of ovarian cancer is its early intraperitoneal spread. From hematogenous metastasis, it is known that adhesion of tumour cells to vascular endothelium is essential for metastasis formation. Since endothelium and peritoneal mesothelium, both derived from the mesoderm, share morphologic and functional similarities, we hypothesized that intraperitoneal spread of ovarian cancer cells might be driven by adhesion molecules involved in endothelial adhesion such as integrins like integrin-β4 (ITGB4).

To test this hypothesis, a stable (shRNA-mediated) ITGB4-knockdown (KD) was established in the human ovarian cancer cell line SKOV3. These cells and their control transfectants were xeno-engrafted intraperitoneally into immunodeficient mice. First, potential pro-survival effects of the ITGB4-KD were tested. Secondly, tumour development at the injection site, peritoneal carcinosis, tumour spheres in the peritoneal fluid and distant metastasis were compared in ITGB4-KD vs. control xenografts after similar growth periods (endpoint experiment). Thirdly, the prognostic value of ITGB4 was determined in ovarian cancer patients.

Mice injected with ITGB4-KD cells showed an improved survival compared to mice injected with control cells. In the endpoint experiments, the ITGB4-KD resulted in smaller tumours, less solid intraperitoneal carcinosis, less tumour spheres floating in the peritoneal fluid, and a lower count of lung metastases. Accordingly, low ITGB4 expression predicted a better prognosis of ovarian cancer patients upon tumour-free surgery.

The adhesion molecule ITGB4 is functionally involved in the peritoneal spreading of ovarian cancer in vivo and has prognostic impact for clinical ovarian cancer. Therefore, ITGB4 could be a promising target for ovarian cancer therapy.

Vortrag 10

Titel:
CHD1 deletion is an independent predictor of poor prognosis by increased metastasis in both PTEN-deficient and -intact prostate cancer

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Abstract:
Chromosomal deletions characterize different subsets of human prostate cancer (PCa) with PTEN and CHD1 being the most frequently affected genes. Both events indicate earlier tumor marker relapse after surgery in univariate analyses. While PTEN has been the focus of extensive research, the functional consequences of deleted CHD1, which is a chromatin modifier, remain largely unknown. We aimed to determine whether and by which mechanisms CHD1 depletion alters metastasis formation in vivo and to test CHD1 as an independent predictor of poor prognosis in the worldwide largest PCa patient cohort (n=6,883).

Knockdown of CHD1 in PC-3 (PTEN-/-) and ARCAP-M cells (PTEN-wt), FISH, WB, spontaneous metastasis xenograft models, histology, liquid biopsy (miRNA arrays), 3D cell culture, RNA-seq, bio(statistics)

CHD1 depletion leads to increased spontaneous lung metastasis formation in both tested models irrespective of the PTEN-status, which appeared to be due to improved metastatic outgrowth (increased number of multicellular lung colonies in vivo and improved colony forming capacity in vitro). This improved colonization could be the consequence of altered expression of cell cycle genes as indicated by miRNA microarray and RNA-seq data. CHD1 deletion affects 10 % of patients and is an independent predictor of earlier biochemical relapse, increased risk of metastasis and increased cancer-specific mortality. Most interestingly, CHD1 deletion predicts metastasis independently of the PTEN-status.

Loss of CHD1 impacts on metastatic progression in PCa. Candidate genes or miRNAs epigenetically regulated by CHD1 remain to be functionally validated. Testing CHD1 in PCa tissues could influence therapeutic strategies in the future.

Vortrag 11

Titel:
Integrin β4 knockdown in tumor cells synergizes with E- and P-selectin knockout in mice to suppress tumor growth: Unravelling the molecular mechanisms

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Abstract:
Scientific evidence has accumulated showing that adhesion molecules like selectins and integrins contribute to cancer metastasis. In particular, upregulation of integrin β4 (ITGB4) is associated with tumor progression in various tumor entities. Therefore, we aimed at detecting a potential crosstalk between selectins and integrins in the process of cancer formation and metastasis.

Knockdown of ITGB4 in PaCa5061 and PC3 cells, proliferation and colony forming assays, ELISA, WB, 3D chemotaxis assays with human macrophages, qRT-PCR arrays, tumor initiation assays in vivo, immunohistochemistry

We showed that ITGB4-knockdown in PaCa5061 and PC3 cells delayed tumor growth in vivo which was much more pronounced in E-/P-selectin-deficient than wildtype mice. Selectin-knockout alone had no effect. The delay in tumor formation was associated with increased apoptosis. Immunostainings further revealed an enhanced immune cell infiltration in ITGB4-knockdown tumors grown in wildtype mice. This effect was impaired in E-/P-selectin-deficient mice, where leukocytes remained at the outer tumor periphery despite ITGB4-knockdown. The increased leukocyte infiltration in ITGB4-knockdown tumors in wildtype mice appeared to result from a greater chemotactic potential of ITGB4-knockdown cells. CCL5 and CCL20 were identified as potential chemotactic factors upregulated upon ITGB4-knockdown in PaCa5061 cells while the chemoattractant of ITGB4-knockdown PC3 cells remains to be determined.

Our results suggest that ITGB4-knockdown tumor initiation depends on pro-tumoral signals from attracted tumor-associated leukocytes. These leukocytes appear to depend on endothelial selectins for infiltration of the tumor stroma. Investigating how leukocytes actually stabilize the survival of ITGB4-knockdown xenografts is the subject of ongoing experiments.

Vortrag 12

Titel:
Cancer cells use different classes of ligands for the adhesion to human or murine E- and P-selectin under static or dynamic binding conditions

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Abstract:
One critical step of metastasis formation is the interaction of endothelial E- and P-selectins with carbohydrate ligands on circulating tumor cells (CTCs), prior to their extravasation into distant organs. It is still unclear whether these interactions take place only under dynamic (blood flow-dependent) or also under static conditions and whether species-specific differences in the ligands of human vs. murine E- and P-selectins exist. This question is relevant since metastasis is widely studied in xenograft models.

We analyzed potential differences in the functional ligands of human vs. murine E- and P selectin under dynamic vs. static conditions. Three subsets of human cancer cell lines, categorized by their canonical E-selectin ligand status (sialyl-Lewis A and X +/+, -/+, -/-), were compared after different treatments, including cleavage of sialic acid residues or glycoproteins or inhibition of O- or N-glycosylation.

Static tumor cell adhesion to E-selectin required the presence of the aforementioned canonical ligands. Other (non-canonical) ligands must exist that are functional under dynamic conditions only. Murine selectins are less selective and more diverse regarding their ligands than human selectins. Cleaving sialic acid residues and inhibiting O-glycosylation significantly impaired static binding of human E-selectin. However, glycolipid ligands must be considered as well since the tumor cells commonly showed glycoprotein-independent selectin binding. Most treatments affected either dynamic or static selectin binding again indicating different classes of ligands.

The molecular interaction between CTCs and selectins is more complex than widely assumed. Our findings encourage future studies on static vs. dynamic selectin binding in more physiologic metastasis assays.

Vortrag 13

Titel:
Comprehensive evaluation of AAV2/DJ-mediated alpha-synuclein overexpression in the rat substantia nigra

Autoren/Adressen:
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Abstract:
Parkinson's disease (PD) is pathologically characterized by the progressive loss of dopaminergic (DA) neurons in the substantia nigra pars compacta (SNpc) and alpha-synucleinopathy. Viral vector mediated overexpression of the alpha-synuclein (α-syn) protein mimics the disease pathology in rats.

Either the human aSyn wildtype (aSyn-wt) or the E46K mutant form (aSyn-E46K) were overexpressed in DA neurons of the SN in adult rats using AAV2/DJ for the first time. Transduction efficiency was compared to an equal virus titer expressing the green fluorescent protein (GFP). Motor skills of all animals were evaluated in the cylinder and amphetamine-induced rotation test over a total time period of 12 weeks. Additionally, stereological quantification of DA cells and striatal fiber density measurements were performed every four weeks after injection.

In the α-syn-WT group, animals showed a progressive loss of DA neurons with 40% reduction after 12 weeks accompanied by a greater loss of striatal fibers. The α-syn-E46K group already showed this reduction after four weeks without further progress. All α-syn overexpressing animals displayed α-syn positive cytoplasmic inclusions. In addition, both α-syn groups developed a characteristic worsening of the rotational behavior over time. However, only the α-syn-WT group reached statistically significant different values in the cylinder test. Moderate correlation between the morphometric evaluation and the behavioral changes were found after α-syn-WT overexpression.

Summarizing these effects, we established a suitable animal model of PD with AAV2/DJ. Overexpressing α-syn-E46K mimicked a rather pre-symptomatic stage, while the α-syn-WT overexpressing animals imitated an early symptomatic stage of PD.

Vortrag 14

Titel:
Sprouty2 knockdown promotes axon regeneration

Autoren/Adressen:
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Abstract:
The Sprouty (Spry) proteins are endogenous modulators of receptor tyrosine kinase (RTK) signaling. Their assigned major role is the inhibition of the extracellular signal-regulated kinase (ERK) pathway. Four functionally conserved Spry isoforms (1-4) exist in mice and humans, which are expressed in the nervous system during development as well as in the adult stage.

Axon growth of adult neuron cultures from dorsal root ganglia (DRG) was analyzed using MetaMorph software. Axon regeneration of heterozygous Spry2+/- knockout mice was evaluated in response to a sciatic nerve crush.

We found that Spry2 is the isoform with the highest expression in adult DRG neurons. Downregulation of Spry2 with shRNA promoted elongative axon growth of adult DRG neuron cultures. Furthermore, DRG cultures dissociated from Spry2 deficient mice revealed enhanced axon outgrowth with prominent axonal elongation of heterozygous Spry2+/- neurons, whereas homozygous Spry2-/- neurons exhibited a branching phenotype. Downregulation of phosphatase and tensin homolog deleted on chromosome 10 (PTEN), an inhibitor of the phosphatidylinositol-3-kinase (PI3K)/Akt pathway, promoted axon elongation of homozygous Spry2-/- DRG cultures. Following sciatic nerve crush, Spry2+/- mice recovered faster in motor but not sensory testing paradigms and increased levels of GAP-43 mRNA were observed in the regenerating sciatic nerve of Spry2+/- mice.

Together our results demonstrate the important role of the endogenous inhibitors of RTK signaling, Spry2 and PTEN, in axon regeneration.

Vortrag 15

Titel:
Impaired ROS Metabolism in Wobbler Mice, a Model of Sporadic Amyotrophic Lateral Sclerosis

Autoren/Adressen:
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Abstract:
Amyotrophic lateral sclerosis is a fatal motor neuron disease and to this day not curable. Only 5-10% of all ALS cases are inherited (fALS), whereas 90-95% appear sporadically (sALS). Several cellular processes are deregulated in ALS, however a single causative dysfunction has not been found yet. Many studies revealed an involvement of the reactive oxygen species (ROS) in the disease. Not only in fALS, which has a genetic dysfunction in the detoxification of ROS, but also in sALS ROS homeostasis seems to be disturbed.

The wobbler mouse resembles almost all phenotypical hallmarks of human sALS patients and is therefore an excellent motor neuron disease model. We focus on ROS metabolism and related signaling pathways. Here, we have gained new mechanistically insights into the development of sALS by using cellular assays, quantitative analysis of gene and protein expression as well as immunohistochemical and -cytochemical stainings.

In our project, we showed an increased ROS level in the spinal cord, more specifically in motor neurons, of the wobbler mouse compared to the wild type counterparts. We were also able to uncover related mechanisms that may lead to a deregulated ROS homeostasis by using the sALS wobbler mouse model (Röderer et al., 2018, Molecular Neurobiology).

Even in the sporadic form of ALS, deregulated ROS homeostasis appears to be a basis for increased oxidative stress in motor neurons. It turns out that ALS seems to be a disease with impaired metabolic causes. Thus, the metabolism of ALS patients should move into the focus of research.

Vortrag 16

Titel:
Neural surface antigen profiling reveals transferrin receptor protein-1 (CD71) as a novel neuronal selection marker for human stem cell paradigms

Autoren/Adressen:
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Abstract:
The inherent heterogeneity of stem cell-derived progeny remains an impediment to biomedical applications. Cell surface molecules are critical mediators of cellular interactions and can serve as lineage- and stage-specific identifiers. As an approach complemental to microscopic study, flow cytometric analysis offers the simultaneous, immediately quantitative detection of multiple markers per single cell in real time.

We performed a comprehensive expression analysis of cluster-of-differentiation (CD) antigens on neural cell types, exploiting human pluripotent stem cell-derived neural cell systems. Using high-throughput multiwell screening approaches followed by in depth validation of expression patterns and dynamics, we exemplify a strategy for resolving cellular heterogeneity.

In addition to providing a catalogue of surface antigens expressed in the neural lineage, we report neuronal differentiation to be associated with a prominent decrease of the transferrin receptor protein-1 (CD71). We identified a role for the proto-oncogene MYCN in maintaining CD71 expression in proliferating neural cells, while in vitro human stem cell-derived neurons lack CD71 expression. Based on near-ubiquitous expression of CD71 in other cell types, we demonstrate its utility as negative selection marker to eliminate unwanted contaminants from neuronal cell preparations by FACS and immunomagnetic selection. Moreover, flow cytometric readout of CD71 allowed for monitoring the number of neurons in toxicity studies.

The extensive surface antigen expression data may prove useful for studying neural stem cell niches, intercellular and host-pathogen interactions. CD71 is identified as a novel, clinically applicable single marker to select human pluripotent stem cell-derived neurons, with utility for biomedical assays and neural therapeutic cell preparations.

Vortrag 17

Titel:
Altered enteric neuro-inflammatory signature in patients with Parkinson's disease – A pilot study

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Abstract:
Neuroinflammation is a key process in the pathogenesis of Parkinson's disease (PD). However, characterization of neuroinflammatory pathways is limited by the impossibility to study biopsies from central nervous system in living patients. Accumulating data report that the enteric nervous system (ENS) is also affected in PD and an analysis of intestinal biopsies obtained from living PD patients indicates that enteric neuroinflammation is also taking place in PD. However, a detailed characterization of these enteric neuroinflammation pathways occurring in PD is still missing.

Deep colonic biopsies, containing mucosal and submucosal nerve tissue, were obtained from 12 PD patients and 12 controls. Quantitative PCR and nCounter technology were used to quantify the expression levels of a panel of 770 genes involved in neuroinflammation and neuropathological disorders.

Expression of 24 genes was altered in PD patients in comparison to controls ($p < 0.01$, FDR adjusted p-value ≤ 0.2). Expression of 4 genes was highly significantly increased in biopsies from PD patients in comparison to controls, whereas expression of 4 genes was highly significantly down-regulated ($p < 0.001$, FDR adjusted p-value ≤ 0.06). Expression of these 8 genes defined a neuroinflammatory signature that was observed in 10 out of 12 PD patients.

These results confirm that enteric neuroinflammatory processes are taking place during the course of PD and suggest that the altered gene expression pattern may be specific for this disease. Further characterization of this neuroinflammatory signature might help to identify novel biomarkers and potential new therapeutic targets for PD.

Vortrag 18

Titel:
Pharmacological modulation of vascularization and visual dysfunction in a murine degenerative hypoxic ischemic retinopathy model

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Abstract:
In the present study we investigated the efficacy of Aflibercept (AFL), a broad-spectrum VEGF-decoy receptor, in promoting the morpho-functional recovery of ischemic retinal tissue in the murine oxygen-induced retinopathy (OIR) model. The latter represents the most widely studied pre-clinical model of retinal ischemic retinopathies (e.g. diabetic retinopathy), characterized by tissue hypoxia, compensatory neovascularization, inflammation and neural dysfunction.

Vaso-obliteration (VO) and pathologic neovascularization (NV) was induced exposing post-natal mice to hyperoxia followed by normoxia (relative hypoxia). Retinae from mice subjected to OIR (control) and such after receiving VEGF-Trap were analyzed by morphological, biochemical and electrophysiological methods, and gene expression profiling was performed at specific time-points.

Besides inhibiting the occurrence of microvascular aberrations and modulating retinal inflammatory reactions associated with ischemia, AFL application significantly improves light responsiveness in a dose-dependent manner as revealed by electroretinographic examination. Furthermore, AFL tightly regulates the expression of VEGFR-2 (transcript and protein). As revealed by global gene expression profiling, AFL normalizes the expression of a "core angiogenic gene signature", whose dysregulation has also been partially implicated in tumor neovascularization processes. Notably, AFL administration also reduces the hypoxia-associated loss of inner retinal cells.

Altogether, we provide evidence that AFL acts at multiple levels and possesses marked anti-angiogenic, anti-inflammatory and neuroprotective properties that lead to partial functional recovery of the visual system. Thus, multimodal/broad-spectrum therapies might help overcome the shortcomings associated with conventional anti-VEGF-A monotherapies and, thereby, represent promising therapeutic substitutes in the context of ischemic retinopathies characterized by abnormalities of the inner retinal vasculature and functional deficits.

Vortrag 19

Titel:
Macroscopic anatomy of the nasolacrimal system

Autoren/Adressen:
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Abstract:
Objective
Dacryoendoscopy and minimally invasive therapeutic techniques are established for observation and treatment of lacrimal duct obstructions. In order to gain more detailed insights into the nasolacrimal system, especially valves or plicae of saccus lacrimalis and ductus nasolacrimalis whose existence is still under debate, we started to assess the normal morphology of the lumina of the human nasolacrimal system via micro-computer tomography (µCT) post mortem.
Methods
For reconstruction of the lacrimal excretory system, a tissue block containing the nasolacrimal system was dissected from a human corpse fixed via ethanolic formaldehyde perfusion. µCT scans with a Zeiss xRadia 410 versa were done as moist scan (70% EtOH; resolution: 41 µm/voxel) followed by decalcification with EDTA and critical point drying (Leica CPD300) for a subsequent dry scan (resolution: 16 µm/voxel) to assess the detailed morphology of lumina and plicae. 3D-reconstruction of the virtual volumes was done with Imaris (bitplane).
Results
3D-reconstruction of the post mortem human tissue resulted in very good visualization of the luminal structure of the lacrimal excretory system. Especially size and shape of major plicae (e.g. "valves" of Rosenmüller and Krause) could be clearly evaluated and visualized in fine detail.
Conclusions
In this prove of principle study we showed that µCT-based 3D-reconstruction of the lacrimal excretory system can give improved and new insights especially when the evaluation of the valves is concerned. Future studies on an increased number of specimens should broaden the knowledge of size and shape of the individual valves.

Vortrag 20

Titel:
Educational training in gynecological laparoscopic surgery based on ethanol-glycerol preserved body donors

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Abstract:
Educational training of laparoscopic surgical skills performed on body donors is considered the gold standard prior to surgery on real patients. However, appropriate, harmless and easy-to-handle fixation of body donors provding true-to-life conditions of tissues is essential for professional training workshops. Here we describe and evaluate a modified ethanol-glycerol based fixation technique.

Body donors were fixed by perfusion (ca. 20 l: 70% ethanol, 30% glycerol, lysoformin) via femoral artery, stored in humid atmosphere (1% thymol) at 4°C and then used for gynecological laparoscopic interventions. Technical equipment included mobile operating table, endoscopy system with gas insufflation, suction/irrigation pump, standard and electrosurgical instruments. Tissue properties of ethanol-glycerol fixed body donors and its suitability for laparoscopic surgery were tested and compared to the in vivo situation.

Modified ethanol-glycerol fixation was a simple, cost-efficient and hazard-free procedure resulting in near-to-life tissue conditions regarding consistency and flexibility, while moderate discoloration and viscosity of organs were observed. Key laparoscopic procedures (e.g. trocar handling, pneumoperitoneum, blunt/sharp dissection, partial/total removal or organs, bi/monopolar electrosurgery, suturing techniques) could be carried without difficulty. Multiple reuse of body donors was feasable over one year. Compared to the in vivo situation, gas insufflation and energy for electrosurgery had to be increased in body donors.

Modified ethanol-glycerol fixation applied to body donors allowed laparoscopic surgery in a realistic and practical manner. Due to its logistic advantages this technique provides appropriate conditions for educational surgical workshops to train laparoscopic skills and implement novel minimal-invasive approaches.

Vortrag 21

Titel:
Sopranes vs tenors

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Abstract:
The objectives are to highlight the morpho-functional changes occurring at the level of the superior vocal formant, in the case of the opera singers and their comparison on gender-related criteria and the type of vocal training.

We conducted this study on a group of subjects composed of 3 tenors and 3 sopranos. In each subgroup, a subject is a student of the singing faculty (voice in training), the second is the experienced soloist and the third has the voice formed in the theater. Each subject has been explored with 3D CBCT in 3 different situations: sustained phonation with “Ə, I” vocals and mimic phonation.

The results show significant differences within the same subgroup, depending on the type and period of training, but also similarities between the two subgroups. These similarities occur only in the context of the same type of vocal training.

The most common types of opera voices - soprano and tenor - show morpho-functional features that differ depending on time and type of vocal training. Within a similar time and type of training, the two types of voices have common characters. An important practice is the fact that the theater voice and of the professional opera soloist is depicted by the opposite morphological features. This aspect has a distinct resonance in phoniatry and divides our study into two directions - the theater and the opera voices.

Vortrag 22

Titel:
A topographical investigation of the human symphysis pubis with measurements of the pubic ligaments

Autoren/Adressen:
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Abstract:
Besides traumatic symphyseal disruptions, the sympyhsis pubis is considered as a cause of pain, especially in sports medicine. Anatomical descriptions of this region differ and impair the interpretation of radiological data. Additionally, quantitative data of the sympyhseal ligaments for biomechanical observations with subsequent stabilization concepts would further improve fixation procedures.

70 hemipelves were dissected and relationships of the symphysis pubis and its ligaments to the surrounding muscles were documented. Furthermore, the symphyseal ligaments were measured and analyzed using ImageJ.

The pyramidal muscle and inguinal ligament were connected to each other and reached the superficial fibers of the adductor longus (AL), rectus abdominis (RA) and the anterior pubic ligament (APL). The adductor brevis muscle had a connection to the superficial and deep parts of the APL. The AL and RA were directly connected to each other and to the APL. The gracilis muscle was linked to the anterior and inferior pubic ligament (IPL). The posterior pubic ligament (PPL) was connected to the pubovesical/puboprostatic ligament. The PPL yielded the smallest thickness (1.51 ± 0.51 mm) and area (45.60 ± 17.76 mm2). Gender specific differences were present in the thickness of the superior and inferior pubic ligaments as their values were higher in men than in women.

In symphyseal pathologies, the deeply connected muscles (AL and RA) should be evaluated. Furthermore, the available data provide evidence that especially the APL seems to play an important role for the stability of the symphysis pubis due to its muscular relationships.

Vortrag 23

Titel:
Effects of the highly COX-2-selective analgesic NSAID etoricoxib on the rate of orthodontic tooth movement

Autoren/Adressen:
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Abstract:
NSAID analgesics are widely used in the treatment of pain, inflammation and fever. The highly COX-2-selective NSAID etoricoxib has shown a favourable side effect profile and excellent analgesic efficacy, particularly for dental and orthodontic pain. However, potential side effects on the rate of orthodontic tooth movement (OTM) and cranial growth, relevant for clinical usability in orthodontics, have not yet been investigated.

40 male Fisher344 rats were randomly assigned to 4 groups: (1) 1.5ml/day isotone saline by oral gavage for 5 weeks (controls); additional 7.8mg etoricoxib/kg/day for 3d/week (2) and 7d/week (3) and 13.1mg/kg for 7d/week (4) with serum bioavailability assessed by LC-MS. After 7d of premedication, the first upper left molars (M1) were moved orthodontically in anterior direction for 4 weeks using a closed NiTi coil spring (0.25N). OTM and sagittal cranial growth were quantified cephalometrically by CBCT imaging.

OTM, measured as anterior metric tipping of M1, was significantly inhibited by about 33% only in rats receiving high-dose etoricoxib 7d/week ($p = 0.046$) with a respective, but insignificant tendency also detectable for the normal dosages, whereas sagittal cranial growth was by tendency slightly increased with rising etoricoxib dosages, reflected by corresponding steady-state serum concentrations, confirming bioavailability.

An etoricoxib-induced clinically relevant deceleration of OTM is not to be expected at dosage regimens used in clinical practise to treat dental or orthodontic pain in contrast to a continuously administered high dosage. Etoricoxib should thus be a clinically valid alternative to the current standard orthodontic analgesic acetaminophen with its associated higher risk profile.

Vortrag 24

Titel:
An ultrasound evaluation of radial artery in young adults

Autoren/Adressen:
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Abstract:
The radial artery is an important route of access to coronary vessels. There is a lot of data indicating the presence of anatomical difficulties associated with that procedure. The aim of the study is to assess the diameter of radial artery and its distance to skin in young adults and to determine the presence of a relationship between the diameter and position of the artery and basic anthropometric parameters or body composition.

102 participants (36.25% of men) with an average age of 20.445 years (SD-1.77) were qualified for the study, which was approved by the local bioethics commission. To assess the position of the artery, an ALOKA Prosound 6 ultrasound machine equipped with a linear head with the power doppler option was used. The study was conducted on both limbs in three anatomical locations within the forearm. The evaluation of basic anthropometric parameters was carried out using certified measuring tools.

The diameter of the artery assessed at level of processus styloideus radii was 1.42 mm on the right side and 1.47 mm on the left side. It gradually moves father from the skin surface and increase in diameter. We proved and statistically significant correlation between arterial diameter and basic anthropological measurements. We observed also some dimorphic and bilateral differences.

The radial artery has an increase in diameter and gradually moves father from the skin surface relative to the decreasing distance to the heart.
A relationship between the diameter of the artery, some anthropological measurements and body composition was demonstrated.

Vortrag 25

Titel:
Ontogeny and transcriptional heterogeneity of eye macrophages on single-cell level

Autoren/Adressen:
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Abstract:
The eye is a complex organ composed of various compartments inhabited by myeloid cells. The access of pathogens to the distinct compartments of the eye range from strictly protected, like the retina, to continuously exposed, like the cornea, which implies different demands for macrophages inhabiting these compartments.
To decipher the complexity of eye macrophages we applied single-cell RNA Sequencing, embryonic fate mapping and several reporter mouse models to investigate the origin and turnover, transcriptomic signature and surface marker expression.

The characterization of eye macrophages was conducted using bulk and single-cell RNA Seq, confocal microscopy and flow cytometry. The experiments were performed with wildtype mice or transgenic reporter and fate mapping mouse models.

By single-cell RNA Seq, we revealed a close relationship between macrophages in the cornea and ciliary body both separating from retinal microglia. Cornea macrophages and bone marrow-derived monocytes were found to share a molecular signature that differed substantially from both retinal and brain microglia. Using fate mapping and transgenic reporter mice, this study demonstrates that retinal microglia are exclusively yolk sac-derived and long-lived while macrophages in the ciliary body and cornea originate from both primitive and definitive hematopoiesis, being constantly renewed.

Resident myeloid cells in the retina, ciliary body and cornea display distinct transcriptomic profiles under homeostatic conditions reflecting their environmental challenges and differ in their origin and turnover behaviour. The identification of new compartment-specific myeloid subpopulations is the first step to the investigation of disease-specific macrophage populations in the retina, ciliary body and cornea.

Vortrag 26

Titel:
A novel computational model of mature and adult-born dentate granule cells provides insights into their structure-function relationships

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Abstract:
We developed a new computational model that mimics electrophysiological behavior of mature granule cells (GCs) and young adult-born GCs (abGCs) in mouse and rat (Beining et al. eLife 2017).

We used compartmental and morphological modeling (T2N - TREES-to-NEURON toolbox).

The model has five improvements when compared to previous models: (1) It is the first compartmental GC model – and one of the first neuron models overall – which remains robust across a wide variety of reconstructed and synthetic morphologies. (2) The model contains only conductances of channels that are currently known to exist in GCs and accurately implements their kinetics. (3) The model is capable of reproducing findings and experiments from several different studies. (4) After adjustment of channel densities, the model reproduced electrophysiological behavior of both rat and mouse mature GCs indicating that these species might share similar active channels. (5) The adapted model for young abGCs represents the first available data-driven compartmental model of these neurons. The model predicted the impact of differences in intrinsic properties between young abGCs and mature GCs on the temporal summation of synaptic input. We found that higher intrinsic excitability allows young abGCs to integrate synaptic inputs in a broader time window compared to mature GCs.

Our GC simulations provide important insights and tools for the hippocampus research field in general and the adult neurogenesis field in particular. Our study builds the cornerstone for future GC modeling approaches, by providing a model with which hypotheses on the impact of structural and functional alterations can be tested.

Vortrag 27

Titel:
PLPPR-4/PRG-1 acts as a neuronal lipidporin

Autoren/Adressen:
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Abstract:
Various specific channels, transporters or porins within the cytoplasmic membrane of neurons have been characterized for different types of molecules. However, a mechanism how neurons can support rapid transmembrane transport of bioactive lipids, shown to critically modulate glutamatergic signaling at the synapse, has remained enigmatic.

Here, we combined 2-photon live-imaging, superresolution as well as electrophysiological and biochemical methods to elucidate the molecular mechanism of bioactive lipid uptake into neurons.

Using 2-photon live-Imaging and superresolution at the spine, our data provide the first evidence that the membrane protein PLPPR-4/PRG-1 (PRG-1) serves as a lipidporin allowing for a rapid LPA-uptake from the synaptic cleft into the spine. The kinetics of this selective LPA-uptake by PRG depend on its molecular interaction with either calmodulin or the phosphatase PP2A, in turn regulated by neuronal activity in a calcium-dependent fashion.

Our finding of rapid LPA-uptake mediated by PRG-1, which appears to be modulated by neuronal activity, is the first example for the existence of a selective lipidporin allowing for transmembrane transport of a polar bioactive phospholipid in the body.

Vortrag 28

Titel:
A role for the axon initial segment in rapid modulation of neuronal input-output parameters in mouse barrel cortex

Autoren/Adressen:
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Abstract:
The axon initial segment, strategically positioned at the proximal axon and essential for action potential (AP) initiation, has recently been identified as a key regulator of neuronal excitability by fine-tuning neuronal activity depending on network state. Previously, we showed that in rodent barrel cortex, the AIS undergoes activity-dependent structural plasticity during development and retains plasticity in adult circuits after bilateral whisker-trimming. AIS length increase was observed within time-frames of days to weeks post deprivation. These structural changes had functional consequences: neurons with longer AIS exhibited reduced AP thresholds and increased firing rates, while passive properties remained unchanged. Here we ask whether sudden changes in network state can elicit rapid AIS plasticity (time-scale within hours).

Adult mice were subjected to unilateral whisker-trimming and exposed to enriched environment (EE) for 1, 3 and 6 hours, triggering increased stimulation of the remaining whisker-to-barrel pathway. AIS modulations and cellular responses were analysed using multichannel immunofluorescence, confocal microscopy and whole-cell patch-clamp recordings in acute slices.

Increased stimulation of the barrel network was indicated by upregulation of the immediate-early gene c-fos in layer II/III pyramidal neurons within 3 hours. These cells showed a significant AIS length reduction, an effect that could no longer be observed 6 hours after EE. Cellular excitability also changed significantly in this 3 hour time-frame: AP threshold was elevated and cells showed reduced excitability to appropriate stimuli.

These data suggest that rapid AIS plasticity could serve as a fast modulator of neuronal activity in excited networks until a homeostatic balance is achieved.

Vortrag 29

Titel:
Novel and sex-specific effects of G-protein-coupled estrogen receptor GPER1 signaling in hippocampus

Autoren/Adressen:
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Abstract:
17-beta-estradiol (E2) is a modulator of hippocampal plasticity. Its functions are mediated by cytosolic receptors (ERalpha, ERbeta), but rapid effects may be triggered via membrane-bound receptors, including G-protein-coupled receptor GPER1, which in hippocampus is concentrated in CA1 stratum lacunosum-moleculare (SLM). Here we examined functions of GPER1 by determining its role (a) for the enrichment of HCN channels and (b) for dendritic spine formation in SLM.

Organotypic entorhino-hippocampal cultures were used. For (a), cultures from 5-day-old rats were incubated from DIV 5-10 with E2, agonists for ERalpha (PPN), ERbeta (DPN), GPER1 (G1), or GPER1 antagonist G36 + E2. For (b), cultures from 7-day-old mice expressing GFP under control of the Thy1-promoter were treated with E2, G1 or E2+G36 for 4, 24 or 48 hours. HCN1 immunosignal and spine densities in SLM were analyzed using confocal microscopy.

E2 promoted HCN1-enrichment in SLM. Results were mirrored by GPER1 agonist G1, but not agonists for the cytosolic receptors. Further, co-application of G36 abolished the E2-effects, identifying GPER1 as the responsible receptor. Effects were significant in both sexes. Dendritic spine density in SLM was enhanced after G1-treatment. However,these effects were observed only if the cultures derived from females.

As GPER1-agonist G1 influenced HCN channel enrichment and dendritic spine density in SLM, GPER1 likely plays a role for the processing of information from entorhinal cortex via the perforant path. Importantly, a sex difference was observed with respect to G1 effects on spine formation. Whether this difference is conserved into adulthood, is currently under investigation.

Vortrag 30

Titel:
The Presynaptic Protein Mover is Differentially Expressed across Brain Areas and Synapse Types

Autoren/Adressen:
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Abstract:
The assembly and function of presynaptic nerve terminals relies on evolutionarily conserved proteins. A small number of presynaptic proteins occurs only in vertebrates. These proteins may add specialized functions to certain synapses, thus increasing synaptic heterogeneity. We show that the vertebrate-specific synaptic vesicle protein Mover is differentially distributed in the forebrain and cerebellum of the adult mouse.

Using a quantitative immunofluorescence approach, we compare the expression of Mover to the expression of the general synaptic vesicle marker Synaptophysin in sixteen brain areas.

Within the hippocampus, Mover is predominantly associated with excitatory synapses. Its levels are low in layers that receive afferent input from the entorhinal cortex, and high in layers harboring intra-hippocampal circuits. In contrast, Mover levels are high in all nuclei of the amygdala, and Mover is associated with inhibitory synapses in the medioposterior amygdala.

Our data reveal a striking heterogeneity in the abundance of Mover on three levels, i.e. between brain areas, within individual brain areas and between synapse types. This distribution suggests a role for Mover in providing specialization to subsets of synapses, thereby contributing to the functional diversity of brain areas.

Vortrag 31

Titel:
E-cadherin but not desmosomal cadherins is critical for intercellular cohesion in Meibomian gland cells

Autoren/Adressen:
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Abstract:
Meibomian glands are modified, holocrine sebaceous glands within the eyelid important for the ocular surface integrity. It was described that in human Meibomian glands the number of desmosomes increased with differentiation. Patients suffering from pemphigus vulgaris (PV) caused by autoantibodies against desmosomal cadherins, often have dry eye syndrome. Therefore, we studied cell cohesion in human Meibomian gland epithelial cells (HMGEC).

Using immunostaining and Western blot analysis expression of adhesion molecules in HMGEC was examined. Lipid production was monitored by LipidTox-staining. Intercellular cohesion was measured using dispase-based dissociation assay. Immunostaining of desmoglein (Dsg) 3-deficient mice eyelid sections.

During Ca2+-induced differentiation for 1d up to 6d, HMGEC drastically enhanced intercellular cohesion whereas lipid production did not change. The expression profile of desmosomal cadherins as well as of plaque proteins was dependent on the presence of Ca2+ but did not change over time whereas the adherens junction (AJ) component E-cadherin was similar under all conditions. Surprisingly, after 1d Ca2+-supply but not after 6d differentiation, an inhibitory antibody against E-cadherin caused enormous loss of cell-cell cohesion and blocked lipid production of HMGEC. In contrast, antibodies against desmosomal cadherins including pemphigus autoantibodies had no effect on monolayer integrity and lipid production under conditions where they reduced cell cohesion of keratinocytes. This is supported by the observation that in eyelids from Dsg3-deficient mice lipid production was not altered.

These data demonstrate that cell cohesion is maintained differently in Meibomian gland cells and indicate that AJ rather than desmosomes are critical for cell cohesion and lipid formation.

Vortrag 32

Titel:
Mutations in GATM causing renal Fanconi syndrome and kidney failure

Autoren/Adressen:
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Abstract:
Chronic kidney disease is a worldwide health problem, and the underlying pathogenesis is complex and in many cases unknown. Here, we describe in five extended families a novel form of autosomal dominant kidney disease. The disease is characterized by renal Fanconi syndrome early in life followed by progression to renal failure in middle adulthood.

We performed genome-wide linkage analysis, sequencing, and expression studies in kidney biopsy specimens and renal cells along with knockout mouse studies, evaluations of mitochondrial morphology and function and in silico structural analyses.

The renal disease in these patients resulted from monoallelic mutations in the gene encoding glycine amidinotransferase (GATM), a renal proximal tubular enzyme in the creatine biosynthetic pathway. Gatm knockout mice showed no renal abnormalities, confirming the dominant nature of the heterozygous mutations. Immunofluorescence of genetically modified renal proximal tubular cells demonstrated elongated and enlarged mitochondria containing fibrillary structures. Electron microscopy of patient renal biopsies showed similar abnormal findings. In silico analysis showed that the particular GATM mutations create an additional interaction interface within the GATM protein and likely cause the linear aggregation of GATM. GATM aggregates-containing mitochondria were elongated and associated with increased ROS production, activation of the NLRP3 inflammasome, enhanced expression of the profibrotic cytokine IL-18, and increased cell death.

In this novel genetic disorder, heterozygous missense mutations in GATM trigger intramitochondrial fibrillary deposition of GATM and lead to elongated and abnormal mitochondria. We assume that this mitochondrial pathology initiates a response from the inflammasome, with subsequent development of kidney fibrosis.

Vortrag 33

Titel:
Keratin-dependent desmoglein1 redistribution during loss of intercellular adhesion in pemphigus is mediated by p38MAPK

Autoren/Adressen:
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Abstract:
In pemphigus, autoantibodies directed against the extracellular domains of desmoglein (Dsg) 1 and 3 lead to loss of keratinocyte cohesion and keratin alterations. We recently showed that keratins differentially regulate Dsg1 and 3 binding properties and control p38MAPK activity. However, the molecular mechanisms how autoantibodies weaken Dsg1 adhesion in pemphigus have not been elucidated yet.

AFM, Immunostaining, Western blot

In keratin-deficient keratinocytes (k.o.), in which basal adhesion is severely impaired, pemphigus autoantibodies have only minor effects on intercellular adhesion. Nevertheless, autoantibodies caused direct inhibition of Dsg3 but not of Dsg1 interactions, the latter of which were weakened after autoantibody treatment in wt and faster in keratin-deficient keratinocytes. Wildtype (wt) keratinocytes show dense Dsg1 clusters along cell borders which are not present after incubation with aDsg1 autoantibodies as well as in keratin-deficient keratinocytes, indicating that autoantibodies induce Dsg1 redistribution in keratin-dependent manner. Inhibition of p38MAPK restored intercellular adhesion in both wt and keratin-deficient keratinocytes and abolished Dsg1 redistribution after autoantibody treatment. Conversely, activation of p38MAPK via anisomycin impaired intercellular adhesion in both wt and keratin-deficient keratinocytes and caused keratin retraction in wt keratinocytes. It further led to redistribution of Dsg1 and 3 binding events away from cell borders in wt but not in k.o. keratinocytes, showing that p38MAPK contributes to keratin-dependent regulation of Dsg binding events.

The data propose that in addition to direct inhibition of Dsg3 interactions a sequence of keratin- and p38MAPK-dependent redistribution and weakening of Dsg1 may contribute to loss of intercellular adhesion in pemphigus.

Vortrag 34

Titel:
TRPV4 ion channel as potential regulator of cyst growth in cyst-forming collecting duct cells

Autoren/Adressen:
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Abstract:
Autosomal dominant polycystic kidney disease (ADPKD), the most common inherited renal cystic disease, is characterized by the development of renal cysts and is caused by mutations in polycystin-1 or polycystin-2. The TRPV4 (transient receptor potential vanilloid 4) ion channel may be involved in the pathophysiology of polycystic kidney disease which is incompletely understood (Köttgen et al., 2008; Gradilone et al., 2010; Zaika et al., 2013). This study aims to study the subcellular localization of TRPV4 in ADPKD cyst-lining cells and to investigate potential TRPV4-dependent mechanisms affecting cyst enlargement.

mCCDcl1 cells (mouse cortical collecting duct cells) form cysts within a collagen/matrigel matrix and show forskolin-dependent cyst growth. We tested the effects of activation/inhibition of TRPV4 on cyst growth. In addition, ADPKD kidneys as well as mCCDcl1 cysts were stained for TRPV4 using anti-TRPV4 antibody (ab94868).

TRPV4 is localized in the apical membrane of proximal tubule cells and in the basolateral membrane of distal/collecting duct cells (i.e. cyst-lining cells) in mouse kidney. This TRPV4 staining is absent in TRPV4 knockout mice. Polarized mCCDcl1 cells form cilia by serum-deprivation with TRPV4 being localized in the cilium. In contrast, in the presence of serum cilia formation was largely reduced and TRPV4 localization was basolateral. In mCCDcl1 cysts, TRPV4 was also localized in the basolateral membrane. Importantly, activation of TRPV4 resulted in decreased cyst growth, whereas inhibition of TRPV4 led to increased cyst expansion.

Changes in TRPV4 activity may modify cyst growth in ADPKD, which makes TRPV4 a potential therapeutic target.

Vortrag 35

Titel:
Establishment of a new NASH-derived murine hepatocellular carcinoma cell line

Autoren/Adressen:
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Abstract:
Adipositas and associated liver diseases evolve into a worldwide advancing pandemic. Up to 22% of all hepatocellular carcinoma (HCC) develop on the basis of a non-alcoholic fatty liver disease (NAFLD) and associated non-alcoholic steatohepatitis (NASH). Primary HCCs are characterized by limited treatment options and patients with inoperable HCCs die within a few months. Hence, a fundamental understanding of HCC biology is necessary for future therapeutic strategies. In this study, we isolated and characterized a novel NASH-HCC mouse model-derived cell line that allows mechanistic investigations of NASH-HCC biology.

NASH-HCC was induced in mice by combining western diet with a single postnatal treatment with 7,12-Dimethylbenz[a]anthracen (DMBA). Cells were isolated via intra-hepatic collagenase-based perfusion as frequently described for primary hepatocytes. Tumor cells were obtained by sub-culturing since primary hepatocytes died within days. The established cell line was characterized regarding cell-specific markers by conventional PCR. Chromosomal aberrations were analyzed by cytogenetic analyses. Flow cytometry and the real-time cell analysis device xCELLigence were used to investigate cell cycle and proliferation.

The isolated cells expressed typical tumor markers as well as hepatocellular markers proving their hepatocytic origin. Genomic alterations such as increased polyploidy, chromosomal translocations and the presence of marker- and double-minute chromosomes confirmed the cancerous nature of these cells. Cell cycle, as well as proliferation experiments, revealed increased mitotic activity.

In contrast to existing HCC cell lines, our novel cell line originates from a real NASH-induced HCC mouse model. These cells represent a powerful tool to investigate HCC biology and thereby discover new therapeutic approaches.

Vortrag 36

Titel:
The Impact of Non-Lethal Single-Dose Radiation on Tumor Invasion and Cytoskeletal Properties

Autoren/Adressen:
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Abstract:
Irradiation is the standard therapy for glioblastoma multiforme. Glioblastoma are highly resistant to radiotherapy and the underlying mechanisms remain unclear. To better understand the biological effects of irradiation on glioblastoma cells, we tested whether nonlethal irradiation influences the invasiveness, cell stiffness, and actin cytoskeleton properties.

Two different glioblastoma cell lines were irradiated with 2 Gy and changes in mechanical and migratory properties and alterations in the actin structure were measured. The invasiveness of cell lines was determined using a co-culture model with organotypic hippocampal slice cultures.

Irradiation led to changes in motility and a less invasive phenotype in both investigated cell lines that were associated with an increase in a "generalized stiffness" and changes in the actin structure.

In this study we demonstrate that irradiation can induce changes in the actin cytoskeleton and motility, which probably results in reduced invasiveness of glioblastoma cell lines. Furthermore, "generalized stiffness" was shown to be a profound marker of the invasiveness of a tumor cell population in our model.

Vortrag 37

Titel:
Interaction of gut microbiota and the brain in Anorexia nervosa

Autoren/Adressen:
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Abstract:
It is well established that variations in the bacterial composition in the intestinal tract influence the physiology of the host and are implicated in several gut associated diseases. However, the role of the microbiome in the pathogenesis of psychiatric disorders was underestimated in the past. Recently, changes in microbiota were shown to be causally related to psychiatric disorders such as depression.
Anorexia nervosa (AN) is an eating disorder characterized by a combination of physiological and psychological symptoms: next to reduced food intake and fear of gaining weight, patients with AN show a distorted body self-perception as well as neuropsychological deficits such as reduced logical thinking and visual-spatial skills and impairments in learning and memory tasks. Current research supports that changes in the intestinal microbiota composition and a reduction in diversity are involved in the pathophysiology of AN.

We used a rodent activity-based anorexia (ABA) model for AN with female 4 week old Wistar rats. Fecal samples were analyzed by 16S rRNA sequencing with an Illumina MiSeq.

We could show that rats that underwent a starvation period with access to running wheels display an altered microbiota diversity compared to normally fed animals. Specifically, we found an increase in Verrucomicrobia which are a mucus-degrading species. These results are in line with current research suggesting the development of a gut hyperpermeability in AN and ABA rats.

Furthermore, we are analyzing gut-brain interactions by combining behavioral test and microbiota sequencing measurements in our AN animal model. We expect to find correlations between changes in bacterial composition and behavior as for example altered depressive- or anxiety-like behavior and memory impairments.

Vortrag 38

Titel:
Pathological connectivity of the hippocampal CA2 region in temporal lobe epilepsy

Autoren/Adressen:
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Abstract:
Temporal lobe epilepsy (TLE) is frequently characterized by neuronal loss in the hippocampal CA3 and CA1 regions and a dispersed granule cell layer. Some aspects of the associated structural plasticity, in particular mossy fiber (MF) sprouting in the dentate gyrus, are well-investigated. Using the intrahippocampal kainate mouse model for TLE, we have recently shown sprouting of the MF–CA2 projection resulting in aberrant somatic synapses on CA2 pyramidal cells which we aim to characterize in more detail here.

The intrahippocampal kainate injection was followed by (I) implantation of plantinum-iridium wires or silicon probes to measure activity in CA2, or (II) immunocytochemistry with synaptic markers in Thy1-eGFP mice expressing eGFP mainly in granule cells and MF or (III) fluorescent tracing with Cre-dependent adeno-associated viruses in a granule cell-specific Cre-line (RBP4-Cre) followed by immunocytochemistry.

We show that epileptic population discharges and multi-unit firing are measureable in CA2. Identified MF synapses in the CA2 region express the synaptic proteins synaptoporin and bassoon and the vesicular glutamate transporter 1 indicating glutamatergic transmission. Interestingly, the aberrant MF synapses strongly express glutamic acid decarboxylase 65 (GAD65), the key enzyme for GABA production. Yet, no overlap with the vesicular GABA transporter (vGAT) was found.

Our data indicate that CA2 actively contributes to epileptic activity. MF synapses retain a release machinery and glutamatergic transmission and aberrantly express GAD65. Yet, lacking vGAT expression renders a dual phenotype of aberrant MF synapses unlikely. Together, our results highlight CA2 as an important part of the epileptic network.

Support: DFG (grant HA7597), Excellence Cluster ‘BrainLinks-BrainTools’ (DFG-grant EXC1086)

Vortrag 39

Titel:
Ammonia-induced changes in synaptic excitation/inhibition-balance: cellular and molecular mechanisms of hepatic encephalopathy

Autoren/Adressen:
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Abstract:
Hepatic encephalopathy is a neuropsychiatric disorder that accompanies acute or chronic liver failure. In this context, high blood and liquor levels of ammonia have been identified to play a role in the pathophysiology of hepatic encephalopathy. However, the cellular and molecular mechanisms of ammonia-induced alterations in neural function and plasticity remain not well understood.

In the present study we employed mouse organotypic entorhino-hippocampal slice cultures to study the effects of ammonia on excitatory and inhibitory neurotransmission. We assessed functional and structural features of excitatory and inhibitory synapses using single and paired whole-cell patch-clamp recordings, optogenetic stimulation, immunostainings and ultrastructural morphological analysis.

We here report that ammonia treatment (5 mM) of slice cultures rapidly suppresses excitatory synaptic transmission onto CA1 pyramidal neurons while not affecting inhibitory neurotransmission. Consistent with a homeostatic adjustment of excitatory synaptic strength at a later time point, i.e., 3 days in presence of 5 mM ammonia, a compensatory increase in synaptic strength and restoration of excitatory neurotransmission is observed. In search of the cellular and molecular mechanisms we describe glial activation and increased expression of inflammatory cytokines which accompany the characteristic changes in synaptic excitation/inhibition-balance.

Taken together, these results reveal an ammonia-induced synaptic phenotype that may explain some of the alterations in neural function seen in the context of hepatic encephalopathy. (supported by Deutsche Forschungsgemeinschaft, SFB974)

Vortrag 40

Titel:
Subcortical modulation of hippocampal Cajal-Retzius cells

Autoren/Adressen:
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Abstract:
Cajal-Retzius Cells (CRc) are among the early born neurons of the mammalian brain, which are known to play essential roles in cortical development. A developmental significance arise from their secretion of the signal protein Reelin and their excitatory synaptic integration in the network. In past years it was shown that CRc receive GABAergic input from local interneurons. To this end, a general, excitatory input was not described yet. However, serotonergic (5-HT) signals from subcorital nuclei were suggested to excite CRc.

We have taken advantage of transgenic reporter mice to specifically label CRc in the murine hippocampus. Next, using a combination of in-situ hybridization and immunohistochemistry we checked for 5-HT-receptor expression in CRc. We further combined patch-clamp electrophysiology, calcium-imaging and optogenetics to evaluate serotonergic signals in CRc.

Using in-situ hybridization we were able to demonstrate the expression of the ionotropic 5-HT-receptor-3a mRNA in CRc. By local application of 5-HT we captured big calcium responses in CRc, which were concordant with excitatory currents evaluated by patch-clamp electrophysiology. A transgenic mouse-line allowed the specific optogenetic activation of serotonergic fibers, thus enabling the evaluation of synaptic serotonergic input in CRc.

I previous studies we were able to link CRc to adult hippocampal neurogenesis. Intriguingly, the pharmacological modulation of 5-HT-reuptake was shown to increase hippocampal neurogenesis - a suggested mechanism for the effect of anti-depressant drugs. Our data provide first evidence that subcortical nuclei can excite and synchronize CRc, with the ability to support hippocampal neurogenesis and development.

Vortrag 41

Titel:
Efficient targeting of CD44 by hyaluronan coated-nanoparticles in outflow tissues – new therapeutic approach for glaucoma

Autoren/Adressen:
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Abstract:
Current glaucoma medications rely on decreasing IOP by topical eye drops, which have a long list of drawbacks such as poor compliance and inadequate application. Consequently, there is a strong need for new therapeutic concepts to prevent vision loss. For that purpose, we developed hyaluronan (HA) coated LBL-nanoparticles (NP) targeting the CD44-receptor to deliver siRNAs to the outflow pathway.

CD44 expression was analyzed in hTM cells after treatment with glaucomatous growth factors (TGF-beta 2; CTGF), in eyes of a glaucoma mouse model and in glaucomatous and healthy donor tissues. Porcine and human eye perfusion models were used to deliver HA-NPs or polyethylenimine- (PEI) NPs and NP distribution was investigated. The transfection efficiency of NPs was analyzed in TM cells by silencing CTGF with specific siRNA.

CD44 expression was significantly increased after treatment with TGF-beta 2 and CTGF in hTM cells and in the outflow pathway of the glaucoma mouse model. Elevated CD44 expression was detected in the outflow pathway tissues of eyes from glaucomatous donors. In porcine and human perfusion models HA-coated-NPs were found in a higher concentration within the outflow pathway tissue than PEI-coated-NPs. The analysis showed a distribution of HA-coated-NPs throughout the entire outflow pathway. The siRNA silencing of CTGF was significantly more efficient with HA-coated-NP than with PEI-coated-NPs.

We have identified CD44 as a promising target receptor to deliver NPs to the outflow pathway tissue. We suggest that the HA-decorated-NPs are an excellent therapeutic approach to deliver specific agents to the outflow pathway.

Vortrag 42

Titel:
Chemotherapy resistant retinoblastoma cells – potential risk to develop aggressive local relapse?

Autoren/Adressen:
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Abstract:
Retinoblastoma (RB) is the most common malignant intraocular tumor in early childhood. Chemotherapy resistance diminishes the clinical-therapeutic options and emphasizes the necessity for new therapeutic approaches. The present study compared etoposide and cisplatin-resistant human RB cell lines with regard to changes in proliferation and apoptosis levels, anchorage independent growth behavior in vitro as well as tumor formation capacity in vivo.

Effects of chemotherapy resistance on RB cell growth and viability were revealed by WST-1 and TUNEL assays as well as BrdU and DAPI cell counts. Anchorage independent growth and effects on RB cell tumorigenicity were analysed using soft agarose assays and in ovo chicken chorioallantoic membrane (CAM).

The proliferation rates were increased in the etoposide-resistant RB cell lines Y-79, WERI-Rb1 and RB-355 reflecting significantly higher growth kinetics compared to the parental controls. In line with these findings etoposide-resistant cell lines generated significantly increased numbers of tumors with higher tumor weights compared to their parental counterparts. In contrast, the cisplatin-resistant RB cell lines Y-79, WERI-Rb1 and RB-355 displayed increased apoptosis rates and reduced proliferation rates resulting in significantly decreased growth kinetics. Tumor formation capacity of cisplatin-resistant cell lines did not change, and in comparison with parental controls cisplatin-resistant Y-79 cells displayed significantly reduced tumor weight. Anchorage-independent growth of all chemotherapy-resistant cell lines analyzed was significantly decreased.

Summarizing, one can state that etoposide-resistant RB cells behave more aggressively than the tumor cells of origin and potentially represent a risk factor for local relapse, while cisplatin-resistant cells show a significantly decreased tumorigenic potential.

Vortrag 43

Titel:
A new ex-vivo human mucosa model reveals that p38MAPK inhibition in contrast to epidermis is not effective to prevent autoantibody-induced mucosal blistering in pemphigus

Autoren/Adressen:
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Abstract:
Introduction: Pemphigus vulgaris (PV) is an autoimmune disease characterized by blister formation in the epidermis and the oral mucosa due to loss of keratinocyte cohesion. Autoantibodies present in PV patients are known to primarily target desmoglein (Dsg) 1 and 3, in desmosomes. The mucosal-dominant subtype of PV is caused by autoantibodies (PV-IgG) against the cadherin-type adhesion molecule Dsg 3 whereas the mucocutaneous type is caused by autoantibodies targeting both Dsg1 and 3.
p38MAPK signaling has been characterized as an important pathway downstream of PV-IgG antibody binding and its inhibition is protective in vitro as well as in vivo and human skin ex vivo. However, the role of p38MAPK in mucosal-dominant PV is unknown since no experimental model was available.
Objective: To test the p38 MAPK dependency of blister formation as well as of ultrastructural alterations of desmosomes induced by PV autoantibodies in human mucosa compared to epidermis.

Ex vivo human skin and mucosa model, transmission electron microscopy, cryosectioning, H&E, immunostaining. Patients' antibodies were compared to the mouse monoclonal pemphigus Dsg3 autoantibody AK23.

In a newly established human ex-vivo mucosa model both AK23 and mucosal PV-IgG induced blisters and caused reduction of desmosome size and number which is different to ex-vivo skin where mucocutaneous PV-IgG caused blisters only. However, inhibition of p38 MAPK was not effective to prevent these alterations.

In contrast to human epidermis, PV patients' autoantibodies and AK23 induce blisters and associated ultrastructural alterations of desmosomes in labial mucosa via a mechanism not dependent on p38 MAPK.

Vortrag 44

Titel:
Plakophilins regulate desmosomal hyper-adhesion and modulate Dsg3 oligomerization and binding properties

Autoren/Adressen:
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Abstract:
Under mechanical stress, intercellular adhesion of keratinocytes is critically dependent on desmosomes which, during maturation, acquire a hyper-adhesive, i.e. Ca2+-independent state. The desmosomal plaque proteins plakophilins (Pkps) are known to be involved in desmosomal turnover and hyper-adhesion. However, the underlying molecular mechanism is not elucidated yet. Thus, we here used murine keratinocytes to dissect the molecular mechanism by which Pkps regulate desmosomal hyper-adhesion.

AFM, chemical crosslinking, dissociation assay, FRAP

We found that desmoglein (Dsg) 1 and 3 binding properties are differentially regulated by Pkps. For Dsg3, Pkp loss led to decreased binding frequency and strength which was accompanied with reduced intercellular adhesion and compromised oligomerization of Dsg3 but not E-cadherin. Further, loss of Pkp1 but not Pkp3 increased lateral mobility of Dsg3. Since changes in Dsg clustering and mobility were proposed as possible mechanisms for hyper-adhesion we investigated intercellular adhesion and Dsg3 binding properties in wt and Pkp1 and 3 k.o. keratinocytes, respectively. Wt keratinocytes become hyper-adhesive during 72h maturation in presence of Ca2+ whereas Pkp-deficiency abolished aquisition of Ca2+-independency. Inhibition of PKC improved desmosomal maturation in Pkp-deficient keratinocytes. Further, Pkp-deficiency reduced formation of Ca2+-independent Dsg3 oligomers. In contrast to wt keratinocytes, AFM in Pkp-deficient keratinocytes revealed that increase in Dsg3 binding strength and decrease in distance of bond rupture during maturation did not occur which may explain decreased hyper-adhesion in these cells.

In summary, Pkp-dependent changes in Dsg3 oligomerization and binding properties may provide a new concept for the molecular mechanism of hyper-adhesion.

Vortrag 45

Titel:
Role of Src and cortactin in pemphigus skin blistering

Autoren/Adressen:
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Abstract:
Autoantibodies against desmoglein (dsg) 1 and Dsg3 primarily cause blister formation in the autoimmune disease pemphigus vulgaris (PV). Herewith, Src was proposed to contribute to loss of keratinocyte cohesion. However, the role and underlying mechanisms are unclear.

Immunostaining, protein fractionation, Western blotting, dissociation assay, neonatal mouse model, ex-vivo human skin model, electron microscopy analyses

In keratinocytes, cell cohesion in response to autoantibodies was reduced in Src-dependent manner by two PV-IgG fractions as well as by AK23 but not by a third PV-IgG fraction, although Src was activated by all autoantibodies. Loss of cell cohesion was progredient and AK23 similar to PV-IgG interfered with reconstitution of cell cohesion after Ca2+-switch indicating that autoantibodies also interfered with desmosome assembly. Since Dsg3 co-localized along cell contacts and formed a complex with cortactin, a target of Src, we prepared cell lines from wildtype (wt) and cortactin-deficient mice. Cell adhesion was impaired in cortactin-deficient keratinocytes. Moreover, Src-mediated and AK23-induced loss of cell cohesion was cortactin-dependent after 24h but not after 2h. Similarly, AK23 impaired reconstitution of desmosomal adhesion in Src-dependent manner only when cortactin was expressed. Furthermore, AK23-induced skin blistering was abolished by Src inhibition in wt but not in cortactin-deficient mice.

The data suggest that the long-term effects of Src for loss of cell cohesion and skin blistering are dependent on cortactin-mediated desmosome assembly. However, in human epidermis PV-IgG-induced skin blistering and ultrastructural alterations of desmosomes were not affected by Src inhibition indicating that the contribution of Src for skin blistering is variable.

Vortrag 46

Titel:
Cardiac telocytes or cardiac lymphatics?

Autoren/Adressen:
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Abstract:
The study of cardiac interstitial Cajal-like cells (ICLCs) began in 2005 and continued until 2010 when they were renamed as telocytes (TCs). Since then, more than 80 papers, dealing with cardiac TCs/ICLCs were published until now. However, in the initial descriptions, upon which were based further researches, the authors failed to consider, identify and exclude by differential diagnosis lymphatic endothelial cells (LECs) and lymphatic capillaries. We therefore aimed at performing a critical review to oppose the diagnostic of cardiac TCs vs.cardiac LECs.

Existing evidence on cardiac TCs was documented from the specific database that was gathered by use of the available databases.

No specific antibodies for LECs (such as podoplanin or LYVE-1) were used in cardiac ICLCs/TCs studies although ultrastructurally, LECs and ICLCs/TCs have similar morphology traits, including the lack of a basal lamina. Migrating LECs involved in adult lymphangiogenesis have an ICLC/TC morphology, when the tissues are cut longitudinally within the lymphatic capillaries both in light and transmission electron microscopy. Proofs on cardiac TCs suggest that at least some cardiac TCs are actually LECs.

Therefore, a clear-cut distinction should be made between TCs and LECs, at molecular as well as ultrastructural levels, to avoid getting invalid data.

Vortrag 47

Titel:
Stimulation of chemosensory brush cells triggers cholinergic contraction in the mouse gall bladder

Autoren/Adressen:
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Abstract:
Cholinergic chemosensory (brush) cells in various mucosal epithelia are considered to monitor the chemical composition of the lining fluid through the bitter taste transduction cascade and, reflexively, initiate avoidance reflexes. In case of the gall bladder, this might be voiding of content.

Isolated mouse gall bladder contraction was studied in organ bath recordings. Stimuli were bitter compounds and, in appropriate strains expressing channelrhodopsin (ChR2) either in cholinergic nerve fibres or in chemosensory cells, LED stimulation (optogenetics). Acetylcholine (ACh) release was quantified by HPLC. Intracellular calcium concentration ([Ca2+]i) was recorded in isolated cells.

Muscarine (100 µM) evoked bladder contraction, reaching about 60% of that resulting from stimulation with cholecystokinin (0.1 µM). Dextromethorphan (100 µM), a bitter tastant stimulated brush cells (increase in ([Ca2+]i) and caused gall bladder contraction that was cholinergic (atropine sensitive) and required the taste transduction cascade (abolished in TRPM5-/- mice). In initial experiments (n=3) this was lost in brush cell deficient mice (Pou2f3-/-). Signalling to nerve fibres was not involved (neural action potential generation blockers TTX, 1 µM, and A-803467, 5 µM). Optogenetic stimulation of explanted gall bladders from ChAT-ChR2(H134R)-EYFP mice (ChR2 expression restricted to brush cells), but not from control strains, resulted in an about 6-fold increase in ACh content (from 1.75 to 10.20 nM; n=10) in the medium and also evoked atropine-sensitive bladder contraction.

Gall bladder contraction is one of the effector mechanisms triggered by cholinergic brush cell stimulation.

Vortrag 48

Titel:
Digitoxin enhances cardiomyocyte cohesion which is crucial for positive inotropy

Autoren/Adressen:
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Abstract:
The aim of the present study was to investigate the dependence of the positive inotropy caused by the heartglycoside digitoxin on intercellular adhesion of cardiomyocytes, which are connected via intercalated discs.

Dissociation assay, immunostaining, Western Blot, Triton extraction, siRNA, AFM, Langendorff

We investigated the intercellular adhesion of both HL-1 cardiomyocytes and murine cardiac slices with and without digitoxin. Dissociation assay in both HL-1 cardiomyocytes and murine cardiac slices revealed that digitoxin increases cardiomyocyte cohesion, which we refer to as positive adhesiotropy. Atomic force microscopy demonstrated that digitoxin enhances desmoglein 2 (Dsg2) unbinding force without affecting Dsg2 distribution along cell borders. This was accompanied by accumulation of Dsg2, desmoplakin (DP) and desmin to cell borders as revealed by immunostaining. siRNA-mediated depletion of either Dsg2 or DP abrogated positive adhesiotropy in response to digitoxin. However, no changes in protein levels and cytoskeletal anchorage of these proteins were observed after digitoxin treatment. Since increased phosphorylation of ERK was observed following treatment with digitoxin we inhibited ERK with UO126, which abrogated positive adhesiotropy. In Langendorff- perfused Plakoglobin-deficient mouse hearts digitoxin failed to induce a positive inotropic response indicating that intact desmosomal cell cohesion is required.

These results indicate that digitoxin enhances cardiomyocyte cohesion in an ERK-dependent manner, which correlated with and might depend on enhanced desmosomal adhesion.

Vortrag 49

Titel:
Towards the identification of effector molecules which are driving optic fissure fusion

Autoren/Adressen:
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Abstract:
The optic fissure is a transient gap in the optic cup. A persisting optic fissure, coloboma, is a major cause for blindness in children. The process of optic fissure fusion is still little appreciated, especially on a molecular level.

Me made use of Knock Out mice, transcriptomics, gene knock down and targeted genome editing using zebrafish, zebrafish transgenesis, expression and phenotype analyses to identify the effector molecules, which are driving optic fissure fusion.

We identified a coloboma in TGF beta 2 Knock out mice. Here, the optic fissure margins got in touch, however, failed to fuse. Transcriptomic analyses suggest that the extracellular matrix (ECM) composition was affected. TGF beta, well known for its ECM remodelling capacity, is here often inhibited by BMP. We found two BMP antagonists down-regulated. For functional analyses, we made use of zebrafish. We found TGF beta ligands expressed in the developing eye, the ligand binding receptor in the optic fissure margins, where we also found active TGF beta signalling and notably, homologs of the regulated BMP antagonists. We furthermore found that induced BMP expression is sufficient to inhibit optic fissure fusion. Currently we address the role of specific effector molecules which are driving fissure fusion.

Our findings can likely be applied also to other fusion processes especially when TGF beta signalling or BMP antagonism are involved, as in fusion processes during orofacial development.
We want to further study the interaction in between TGF beta and BMP signalling to get a better understanding of the process of optic fissure fusion.

Vortrag 50

Titel:
Transcriptional control of developmental cell death in the neocortex by Bcl11a/Ctip1

Autoren/Adressen:
Svenja Feldmann (Ulm University), Tobias Wahl (Ulm University), Natalie Joos (Ulm University), Christoph Wiegreffe (Ulm University), Stefan Britsch (Ulm University); stefan.britsch@uni-ulm.de

Abstract:
During development, progenitor cells in the dorsal telencephalon give rise to glutamatergic projection neurons of the neocortex. In mice, up to 40% of neocortical projection neurons die with a peak during the first postnatal week. The molecular mechanisms regulating developmental cell death in the neocortex are incompletely understood. Electrical activity of neurons modulating apoptosis pathways were suggested to be involved. We recently demonstrated that loss of the transcription factor Bcl11a/Ctip1 results in massively increased death of upper-layer projection neurons during the first postnatal week raising the possibility that this factor participates in control of developmental neuron death.

Using mice with forebrain-specific deletion of Bcl11a/Ctip1 we carried out microarray analyses on laser microdissected neocortical tissue to identify differentially expressed genes. Candidate genes were characterized by qRT-PCR, in situ hybridization, immunohistochemistry, and ChIP. Verified target genes down-regulated in Bcl11a/Ctip1 mutants were re-introduced into mutant brains by in utero electroporation and analyzed for their ability to rescue the mutant phenotype.

Forebrain-specific deletion of Bcl11a/Ctip1 releases pro-apoptotic pathways as identified by increased cleaved caspase 3 expression in upper-layer cortical projection neurons. Furthermore, Bcl6, which is involved in activity-dependent survival through inhibiting apoptosis pathways, is down-regulated in mutants. Reintroduction of Bcl6 cDNA into Bcl11a/Ctip1 deficient upper-layer neurons rescues apoptosis.

Our data suggest Bcl11a/Ctip1 to control developmental cell death in cortical projection neurons through regulation of Bcl6 expression.

Vortrag 51

Titel:
Anatomically defined neuronal ensembles in the infralimbic prefrontal cortex engaged in drug or natural reward seeking

Autoren/Adressen:
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Abstract:
Cue-reward associations form distinct memories that can drive appetitive behaviors and are involved in craving for both drugs and natural rewards. Distinct sets of neurons, so called neuronal ensembles, in the infralimbic area (IL) of the medial prefrontal cortex play a key role in alcohol seeking. Whether these ensembles are specific for distinct rewards or reward seeking is controlled in a generalized way remains unclear.

Here, we compared anatomically defined IL ensembles formed upon recall of drug (alcohol) or natural reward (saccharin) memories in rats. Therefore, we used an experimental framework that allows identification of the neurons belonging to two distinct reward-associated ensembles within the same animal.

We found that cue-induced seeking of either alcohol or saccharin activated ensembles of similar size and organization, whereby these ensembles consist of largely overlapping neuronal populations.

Thus, the IL seems to act as a general integration hub for reward seeking behavior, but also contains subsets of neurons that encode for the different rewards.

Vortrag 52

Titel:
The KEOPS complex in the developing brain: Implications for neurodevelopmental disorders?

Autoren/Adressen:
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Abstract:
The Galloway Mowat syndrome is a rare neurodevelopmental disorder, consisting of a variety of symptomes including microcephaly, gyral abnormalities and nephrotic syndrome. Although the underlying pathological mechanisms are not yet fully understood, there is evidence that mutations in the KEOPS (Kinase, Endopeptidase and Other Proteins of small Size) complex, a regulator of gene transcription, telomere maintenance and chromosome segregation can cause Galloway Mowat syndrome.

Here, we examined the regional distribution and the developmental dynamics of KEOPS complex proteins both in murine and human brain.

Our results indicate a distinct spatiotemporal distribution of KEOPS complex proteins in the brain. Functional investigations are on the way to unravel the mechanisms of KEOPS complex function in the developing brain.

Our efforts should serve as a neurobiological framework to better understand KEOPS complex function in both health and disease.

Vortrag 53

Titel:
Transgenic mice in Neuroanatomy – Blessing or Curse?

Autoren/Adressen:
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Abstract:
Transgenic mice provide an important experimental system for studying neocortical circuitry and the effect of population of neurons on it. GABAergic interneurons (IN) in the cerebral cortex have a profound impact on proper information processing, learning and memory or goal-directed behavior. Since cortical inhibitory neurons can be distinguished by molecular markers, such as parvalbumin (PV), somatostatin (SST) and vasoactive intestinal polypeptide (VIP), they are an exciting and up-to-date object of investigation. Most of these studies used transgenic mice to explore the remarkable diversity in morphology, electrophysiology and connectivity of IN. Therefore it is necessary to elucidate in detail the sensitivity and specificity of these transgenic mice to precisely investigate the role of individual subtypes of IN in the cortical circuitry.

Currently three different transgenic procedures are available to target IN: (i) Knock-in mice with bacterial artificial chromosome vectors, (ii) Cre-mice with the Cre-lox system and (iii) since recently Flp-mice with the FLP-FRT system, which allows intersectional investigations by combining the Flp- and the Cre-system. In the current study we quantitatively compared endogenous fluorescence with fluorescence-in-situ-hybridization and immunohistochemistry for labeling molecular markers in the barrel cortex of several knock-in mice (GIN-eGFP, PV-eGFP, VIP-eGFP), Cre-mice (SST-cre, PV-cre, VIP-cre) and Flp/Cre-mice (PV-Flp/Vgat-cre) in a layer-specific manner.

Additionally, we show morphological and electrophysiological characteristics of individual neurons during patch-clamp recordings and after biocytin-filling in the previously mentioned mice.

Our findings indicate that transgenic mice rarely represent a high specificity for a subpopulation or for a specific electrophysiological subtype of IN, which could lead to severe misinterpretation of scientific results.

Vortrag 54

Titel:
How to examine a human gene that is absent in mouse? – Studying epithelial sodium channels (ENaC) in guinea pigs

Autoren/Adressen:
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Abstract:
Epithelial sodium channels, ENaC, have caught a lot of attention due to their patho-/physiological role in health and disease. ‘Salt’ taste perception, kidney and lung function rely on ENaC channels, representing the rate-limiting step for transepithelial absorption of sodium ions. Mutations in its subunits cause hypertension, Liddle Syndrome or cystic fibrosis-like disease. Three subunits comprise one ENaC channel and four different subunit isoforms (ALPHA, BETA, GAMMA, and DELTA-ENaC) have been identified, which are encoded by the respective SCNN1A, B, G and D genes. DELTA-ENaC-containing channels are the least characterized in a biological context since the SCNN1D gene is absent in mice and rats escaping genetic manipulation expression analysis and immunological characterization. We aimed at finding a suitable rodent model to study DELTA-ENaC physiology.

We applied sequence alignment and phylogenetic tree analysis (>40 species), molecular cloning and RT-PCR from guinea pig DNA/RNA and two-electrode voltage-clamp recordings in a Xenopus oocyte expression system.

Phylogenetic analysis revealed dramatic changes specifically to SCNN1D resulting in its loss from certain mammalian branches including whales and the rodent infra-order myomorpha (i.e. mice/rats/hamsters). Its presence in guinea pigs, however, prompted us to clone all SCNN1 isoforms and fully characterize the SCNN1D gene in guinea pig. Subsequent ENaC channel function analysis in Xenopus oocytes revealed that combining BETA/GAMMA/DELTA subunits resulted in robust amiloride-sensitive transmembrane currents with kinetics similar to its human orthologue.

Our current data strongly support the idea that studying DELTA-ENaC in guinea pig is the rodent model of choice to assess its functional role in vivo.

Vortrag 55

Titel:
Palladin plays an important role for proper podocyte morphology and function

Autoren/Adressen:
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Abstract:
The size selectivity of the filtration barrier of the kidney is highly dependent on the actin cytoskeleton as well as on actin-associated proteins in podocytes. Here we studied the influence of the actin-associated protein palladin on podocyte morphology and function.

Palladin was knocked down in cultured mouse podocytes by siRNA and the effects were studied by immunofluorescence staining. Podocyte-specific palladin knockout mice (PodoPalld-/-) with a C57BL/6 genetic background were generated and finally investigated by immunofluorescence staining, electron and super-resolution microscopy. Mice were challenged by nephrotoxic serum (NTS). Human kidney biopsies were stained for palladin.

The knockdown of palladin (PalldKD) in cultured podocytes resulted in a strong reduction of actin filaments, a significant downregulation of the actin-associated proteins synaptopodin and actinin-4. A smaller average area of focal contacts was observed in PalldKD cells. Furthermore, PalldKD podocytes were more susceptible to disruption of the actin cytoskeleton after cytochalasin D treatment. Since palladin knockout mice die intra uterine, we generated a podocyte-specific palladin knockout to study the role of palladin in this specific cell type. We found glomeruli with a disturbed podocyte morphology associated with a mild effacement as well as a significant reduction of the slit diaphragm protein nephrin. After the injection of NTS, PodoPalld-/- mice developed significant higher proteinuria than their littermates. Kidney biopsies of patients suffering from diabetic nephropathy and focal segmental glomerulosclerosis showed a downregulation of palladin especially in podocytes.

Our results demonstrate an important role of palladin for podocyte function in renal physiopathology.

Vortrag 56

Titel:
Studying the role of fibronectin in mechanically stressed podocytes

Autoren/Adressen:
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Abstract:
Glomerular hypertension causes glomerulosclerosis via the loss of podocytes, which are challenged by an increased mechanical load. We and others have demonstrated that podocytes are mechanosensitive. However, the response of podocytes to stretch remains incompletely understood. The objective was to clarify the potential significance of the extracellular matrix protein fibronectin as a potential mechanosensor for podocytes and the role of fibronectin in diabetic nephropathy.

Mouse podocytes were cultured on stretch membranes that were connected to a stretching apparatus (3 days, 0.5 Hz and 5% extension). To study the role of fibronectin in cultured podocytes under mechanical stretch, fibronectin was knocked down (FN-KD) by specific siRNA. Additionally, we established a fibronectin knockout podocyte cell line (FN-KO) by CRISPR/Cas9.

In this study, we demonstrate that the extracellular matrix protein fibronectin is essential for the attachment of podocytes during mechanical stress. By qRT-PCR as well as by LC-MS we found a significant upregulation of fibronectin due to mechanical stress. During mechanical stress, we observed a significant loss of FN-KD as well as FN-KO podocytes (> 80%) compared to the control cells. Furthermore, FN KO podocytes showed a significant downregulation of the focal adhesion proteins talin, vinculin and paxillin and a reduced cell spreading indicating an important role of fibronectin for the adhesion of cultured podocytes. Analyzing kidney sections of patients suffering from diabetic nephropathy, we found a significant upregulation of fibronectin in contrast to control biopsies.

Fibronectin plays an important role in the adaptation of podocytes to mechanical stretch.

Vortrag 57

Titel:
Knockout of palladin in podocytes of mice with a 129S1 background disturbs glomerular morphology

Autoren/Adressen:
Alina M. Ritter (University Medicine Greifswald), Nadine Artelt (University Medicine Greifswald), Linda Leitermann (University Medicine Greifswald), Rabea Schlüter (University of Greifswald), Jens van den Brandt (University Medicine Greifswald), Karlhans Endlich (University Medicine Greifswald), Nicole Endlich (University Medicine Greifswald); ar143917@uni-greifswald.de

Abstract:
Effacement of interdigitating podocyte foot processes is the major cause for a leaky filtration barrier in the glomerulus resulting in proteinuria followed by the development of chronic kidney diseases. Since the function of the filtration barrier is depending on a proper actin cytoskeleton, we studied the role of palladin, an actin-binding protein, in podocytes.

Podocyte-specific palladin knockout mice on a C57BL/6 genetic background were back crossed to a 129S1 genetic background (PodoPalld129-KO) which is more susceptible for kidney disease. Mice at 6 months and 1 year were investigated by histological, immunofluorescence staining, electron and super-resolution microscopy as well as qRT-PCR.

PodoPalld129-KO mice at 6 months and 1 year showed glomeruli with significant larger capillaries than littermates. Furthermore, we found a significant loss of mesangial cells accompanied by a reduced expression of α8 integrin, a mesangial marker. Ultrastructural analysis of PodoPalld129-KO at 1 year showed more podocytes with an enlarged sub-podocyte space and a significant stronger effacement of podocyte foot processes compared to 6 month and control mice measured by PEMP (podocyte exact morphology measurement procedure). Moreover, PodoPalld129-KO of both age showed a reduced expression of pLASP-1 and Pdlim2, two palladin-interacting proteins.

Taken together, the results show that palladin is essential for a proper podocyte morphology in mice with a 129S1 background.

Vortrag 58

Titel:
Time-lapse imaging unveils the superposition of distinctive contractile patterns in rat and human seminiferous tubules

Autoren/Adressen:
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Abstract:
Peritubular smooth muscle cells surrounding the seminiferous tubules in the testis show contractile activity thereby ensuring transport of spermatozoa towards the rete testis. To assess and characterize potential differences in contractility and sperm transport in rat and man we combined time-lapse imaging with Fourier analysis.

Wall movements were tracked over time to yield an irregular curve reflecting the contractile activity of peritubular smooth muscle cells. Subsequent Fourier analysis allowed to decompose these irregular curves into characteristic frequency spectra of sine curves that represent the contractile pattern.

Rat seminiferous tubules showed spontaneous, irregular and undulating wall movements. Fourier analysis revealed several specific contraction patterns in different regions along the rat seminiferous tubule corresponding to different stages of spermatogenesis. In human seminiferous tubules where spermatogenic stages are rather arranged in a spiral manner, corresponding undulating wall movements could also be visualized albeit more subtle. Long-term observation by time-lapse imaging over several hours unveiled very slow contractions in human and rat seminiferous tubules that were superimposed onto the undulating wall movements. This very slow contraction pattern was characterized by its spontaneous occurrence, a rhythmic pattern and clear diameter changes of the seminiferous tubule which propelled intraluminal spermatozoa through the lumen.

Time-lapse imaging, combined with Fourier analysis where applicable, unveiled two differing, superimposed contractile patterns in seminiferous tubules of rat and man. While extremely slow contractions with diameter changes serve to transport spermatozoa, the faster undulating wall movements show distinct contractile patterns associated to certain spermatogenic stages and may contribute to germ cell development.

Vortrag 59

Titel:
Urinary exosomal miRNA expression in CKD: miR-21 as a potential biomarker

Autoren/Adressen:
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Abstract:
With a continuously rising incidence, chronic kidney disease (CKD) displays a major public health burden. Biomarkers from non-invasive sources like urinary exosomes are powerful tools for an early detection to prevent its progression and comorbidities. Especially microRNAs appear to be promising potential biomarkers, but only little is known about their expression levels in urinary exosomes of patients suffering from CKD.

In this study, we analyzed the expression levels of miR-21, miR-30a-5p and miR-92a in urinary exosomes of 41 CKD patients and 5 healthy controls by RT-qPCR. Since podocyte dedifferentiation is a mediator of glomerulopathies, we additionally measured the expression levels of miR-21 in differentiated and dedifferentiated podocytes of our podocyte dedifferentiation model (GlomAssay) by RT-qPCR. Cultured podocytes were transfected with miR-21 mimics and migration assays were performed. We also measured the miR-21 expression levels in nephrotoxic serum (NTS)-treated mice and control mice as a kidney injury model.

Our results revealed that miR-21 was significantly upregulated 3.3 times in CKD patients compared to healthy controls. MiR-21 was also negatively correlated with eGFR. In our podocyte dedifferentiation model miR-21 was 108.8 times upregulated in dedifferentiated glomeruli compared to differentiated glomeruli. Cultured podocytes transfected with miR-21 mimics showed a lowered cell count and enhanced migration. MiR-21 was also upregulated in NTS-treated mice (7.8 times).

MiR-21 from urinary exosomes could serve as a potential non-invasive biomarker for CKD and seems to have a functional role in podocyte dedifferentiation and injury.

Vortrag 60

Titel:
Adriamycin does not induce podocyte damage per se in zebrafish larvae

Autoren/Adressen:
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Abstract:
Podocyte-associated kidney diseases have an increasing prevalence worldwide making animal models necessary to study signaling pathways and for the screening of drugs. The aim of the study was to induce glomerulopathy in zebrafish larvae by adriamycin (ADR) in order to have a quick and reliable high-throughput animal model to study podocytopathies.

Adriamycin (ADR) was added to the tank water of zebrafish larvae in three different concentrations (20 µM, 40 µM, 60 µM) 7 days post fertilization (dpf). Our translucent ET strain that expresses eGFP under the wt1a promoter was used to visualize podocytes (Endlich et al. 2014). After 48 h of treatment, the larvae were either fixed for histological studies or used for RT-PCR.

ADR-treated larvae barely developed edema, a hallmark of kidney failure in zebrafish larvae. Histological stainings revealed no differences of the glomerular morphology of ADR-treated larvae compared to the controls. Immunohistological stainings with antibodies against nephrin and podocin showed no alterations after ADR treatment. These results were supported by mRNA expression analysis of nephrin and podocin. Ultrastructural analysis by electron microscopy showed no differences of the morphology of podocyte foot processes, endothelial cells and the glomerular basement membrane even in larvae treated with 60 µM ADR.

ADR is not a podocyte damaging drug per se in zebrafish larvae. The impairment of the glomerular function of ADR-treated zebrafish larvae observed in the past is most likely caused by developmental side effects and not by a direct effect on podocytes.

Vortrag 61

Titel:
The ancestral architecture of primate gastrulation

Autoren/Adressen:
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Abstract:
The notochord defines the anterior-posterior axis in chordates and originates from the dorsal organizer tissue which in lower vertebrates is represented by the dorsal lip of the blastopore. Birds and mammals develop the primitive node instead of the dorsal blastopore and a straight primitive streak instead of the lateral and ventral blastopore lips. The primitive streak evolves due to the elongation of the mesoderm forming domain. However, how the dorsal blastopore is modified into the primitive node remains unanswered. Reptiles display a flat embryonic disc like other amniotes but do not form the primitive streak and the node. Instead they form posteriorly so called primitive or blastoporal plate and a bona fide blastopore anterior to it. While the primitive plate tissue displays signs of ingression, the midline mesoderm derives from the dorsal lip of the reptilian blastopore by means of involution where involuting cells keep their epithelial polarity.

The phenomenon of the chordo-neural hinge was applied to the amphibian dorsal, ventral and lateral blastopore lips and to the primitive node in different amniotes and its cellular composition was formally compared.

Whereas the primitive node of most mammals and birds displays a dense structure and signs of ingression, the node equivalent in reptiles and primates shows the formation of a blastoporal or a neurenteric canal, respectively. The continuity of polarized, columnar epithelium between ectoderm and notochord is an intriguing sign of the ancestral mode of gastrulation in primates.

Our data indicates a divergent mode of dorsal organizer morphogenesis and notochord formation in higher amniotes.

Vortrag 62

Titel:
Morphology and topology of the cranial nerves of genetically normal and modified mouse embryos harvested at E14.5

Autoren/Adressen:
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Abstract:
Detailed knowledge of the normal morphology, topology and prevalence of individual variation of anatomical structures is essential for identifying abnormal phenotypes. This is especially important in mice serving as models for researching human diseases and malformations. In this study, we aim to provide a detailed description of the topology, morphology and dimensions of the cranial nerves of normal mouse embryos and selected mutants bred on the C57BL/6N background and harvested at embryonic day (E) 14.5.

Using the standardized methods of the Deciphering the Mechanisms of Developmental Disorders (DMDD) program, 60 genetically normal E14.5 embryos and 18 stage matching embryos with gene deletions were embedded in eosin dyed JB-4 resin and subjected to digital volume data generation with the High Resolution Episcopic Microscopy (HREM) method. Out of the HREM data the cranial nerves and ganglia were reconstructed and examined using the 2D and 3D data visualisation and analysis tools of the Amira Software.

We provide a highly detailed anatomical atlas describing the morphology and topology of the cranial nerves and their ganglia as they appear in E14.5 mouse embryos. In addition, we describe important anatomical variations and provide statistics of their prevalence. We also demonstrate the usefulness of our data to serve as a reference for diagnosing cranial nerve abnormalities in genetically engineered mouse embryos.

Our data permit distinction between malformations and variations. They fit excellently as a standard for diagnosing abnormalities in genetically engineered mouse embryos.

Vortrag 63

Titel:
Lateral line placodes of aquatic vertebrates are evolutionary conserved in mammals

Autoren/Adressen:
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Abstract:
In amniotes, neurogenic epibranchial and otic placodes spring from the multiplacodal posterior placodal area (PPA). This study aims to clarify whether and how spatiotemporally regulated apoptosis, predominantly found between the otic and epibranchial placodes, contributes to placode morphogenesis in the PPA.

To this end, C57BL/6N mouse embryos were cultured in the presence of pan-caspase inhibitors (Q-VD-OPh, Z-VAD-fmk) for up to 36 h. Additionally, dilution series of Q-VD-OPh were carried out. For negative controls, Q-VD-OPh was replaced by Q-VE-OPh. Alternatively, culture medium was applied with or without DMSO. Cultured embryos were analyzed using histological, immunohistochemical and 3D reconstruction techniques.

Pharmacological inhibition of apoptosis constantly reveals rudiments of anterodorsal, middle and/or posterior lateral line placodes that contain various maturation stages of neuromast primordia, including hair cells with kinocilia. Lateral line placodes of Q-VD-OPh-treated mice also exhibit the molecular signature of PPA derivatives (Six1, Pax2, Pax8, Sox10, Tbx3, Sox2, Isl1, Ngn1), and appear to produce neuroblasts destined to populate rudimentary lateralis ganglia.

Our discovery refutes the long-held evolutionary theory that the whole lateral line sensory system was completely lost in amniotes. It further substantiates the hypothesis that lateral line placodes may be considered the default fate of the PPA. Since mice, chicks and the primate-related Tupaia belangeri share almost identical patterns of PPA apoptosis, apoptotic elimination of vestigial lateral line placodes may prove a widespread phenomenon among amniotes. Modified versions of our experimental setting may provide an innovative possibility to further explore hitherto unknown developmental links between lateral line, otic and epibranchial placodes.

Vortrag 64

Titel:
Impact of shear stress on corneal epithelial cells using an in vitro flow culture model

Autoren/Adressen:
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Abstract:
The aim of this study was to establish and to evaluate an in vitro model for mimicry intermittent shear stress to epithelial cells of the ocular surface. For this, we analyze human telomerase-immortalized corneal epithelial (hTCEpi) cells under adjustable medium flow representing mechanical forces of eyelid closure.

We used a computer-controlled IBIDI pump system for our study. Confluent hTCEpi cell monolayers were cultured under unidirectional, continuous or oscillating, discontinuous medium flow. Cell surface and cytoskeletal architecture were investigated by scanning and transmission electron microscopy as well as immunofluorescence (IF). Additionally, expressions of cell junction molecules were analyzed by qPCR and western blot. Membrane-bound mucins were localized by IF and mucin barrier integrity was assessed by rose bengal staining.

Medium flow-induced shear stress dramatically changed cellular morphology of hTCEpi. SEM and TEM results showed an increase of desmosomal cell-cell contacts and intracellular cytokeratin filaments. Gene expression of E-cadherin, occludin and TJP were increased under oscillatory medium flow. Desmoplakin and occludin protein were upregulated under oscillatory shear stress. MUC1, -4, and -16 proteins were localized under all culture conditions, but did not change among the different shear stress conditions. Rose bengal uptake was diminished under unidirectional conditions.

Our results clearly show that flow-induced shear stress as it occurs at the ocular surface during eyelid closure exerts marked effects on hTCEpi. Our in vitro model may be useful for further investigations of ocular surface epithelia as it represents a much more physiologic state compared to commonly applied static culture conditions.

Vortrag 65

Titel:
50B11 cells as a suitable component of a pain-o-meter

Autoren/Adressen:
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Abstract:
Stimulation and peripheral sensitization of nociceptive neurons with cell bodies in dorsal root ganglia (DRG) play an important role in the development of chronic pain. The aim of this study was to establish whether an immortalized DRG cell line (50B11), can be selectively differentiated into functional basic nociceptor subtypes, nerve growth factor (NGF)-dependent peptidergic and glial derived growth factor (GDNF)-dependent non-peptidergic cells.
We used RNA-seq, quantitative real time-PCR (q-RT-PCR) and Western blotting to characterise the transcriptional and translational changes in 50B11 cells in response to NGF and GDNF. We examined the response to ATP, capsaicin, and PGE2, using calcium imaging and flow cytometry.
Stimulation of 50B11 cells with forskolin (10 μM) and NGF (50 ng/ml) or GDNF (50 ng/ml) induced differentiation within 24h, indicated by neuritogenesis (n = 10). RNA-seq and q-RT-PCR validation, showed growth factor-specific RNA expression patterns with ~500 genes up- or down-regulated (n = 3). Differentiated cells showed labelling for markers of nociceptive neurons, isolectin B4, TRPV1, P2X3, c-RET, GFRα1, TrkA and p75NTR. Differentiation with forskolin /NGF increased calcium responses to capsaicin and reduced response to ATP; this response profile was inversed in cells differentiated with forskolin /GDNF (n = 9). Differentiated 50B11 cells were sensitized to capsaicin following acute treatment of PGE2 (n = 4) and responded to serum from CCI animals but not controls (n = 6).
Our data indicate that 50B11 cells differentiate into functionally distinct peptidergic- and non-peptidergic-like nociceptors. This provides a valuable high-throughput model system for the study of nociceptive signaling.

Vortrag 66

Titel:
Effects of N-Arachidonoyl Glycine after Neuronal Injury

Autoren/Adressen:
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Abstract:
N-arachidonoyl glycine (NAGly) is postulated to be an immunomodulatory endocannabinoid involved in regulation of multiple immune cell functions. NAGly was shown to activate the GPR18 receptor, which is believed to be a switch between cytotoxic and protective macrophages. In this study, we investigated the role of targeting NAGly-GPR18 signaling in excitotoxically lesioned organotypic hippocampal slice cultures.

Wild type und CB2-/- murine organotypic hippocampal slice cultures (OHSC) were lesioned with N–methyl–D–aspartate (NMDA, 10 µM) followed by incubation with abn-CBD antagonist, O-1918 or CB2 antagonist, AM630 in combination with NAGly or NAGly alone. Primary microglial cells from BL6J or CB2-/- mice were analyzed by using staining, live cell imaging and molecular methods.

NAGly protected the dentate gyrus granule cells in wild type and CB2-/- mice after excitotoxical lesion. Incubation with O-1918 and AM630 abolished the protective effects of NAGly. NAGly affected the motility and ramification index of microglia. Those effects were absent if co-incubated with antagonist. Gpr18 mRNA was significantly decreased (6 h) after excitotoxic treatment in wild type OHSC, suggesting a role of GPR18 during the early phase of the neuronal injury.

Given the reduced expression of Gpr18 in OHSC after NMDA treatment and NAGly mediated actions on glial cells we speculate that GPR18 and its endogenous ligand NAGly plays a role during the neuronal injury and might be a target for therapeutical applications.

Vortrag 67

Titel:
Dysregulated Signaling in SMA: from isolated pathway approaches to a clustered network representation

Autoren/Adressen:
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Abstract:
Spinal Muscular Atrophy (SMA) is caused by low levels of functional SMN protein. However, downstream disease mechanisms remain elusive. In recent years, several studies suggested a number of signaling pathways which mediate pathological changes in SMA. We identified molecular mechanisms linking SMN with altered profilin/ROCK signaling as well as with an enhanced ERK-activity. Moreover, we showed a connection between both pathways since ROCK inhibits ERK and vice versa. Co-inhibition experiments in SMA-mice demonstrated that this lateral connection between both signaling axes is relevant for the SMA pathophysiology and emphasizes the network character of dysregulated signaling in SMA. Identification of key-signaling nodes within this network is an important milestone for a rescue of SMA-like phenotypes alone or in combined drug approaches.

Here, we employed a screening for phospho- and corresponding non-phospho-proteins in pre-symptomatic and symptomatic SMA-mice which allowed us to identify several dysregulated targets simultaneously. A bioinformatic analysis revealed a clustered network topology.

Network clusters were interconnected by a limited number of expressionally regulated central hubs. Among those, we identified and validated the downregulation of B-Raf – an important hub for neurotrophic factor signaling. A neuronal rescue of B-Raf in different SMA-model systems had positive effects on SMA-relevant neurodegenerative phenotypes.

Here, we demonstrate the strength of a systems biology approach towards novel SMN-independent treatment approaches for Spinal Muscular Atrophy: expanding complexity by an unbiased screening and reducing complexity by a bioinformatics-based selection of promising candidates for rescue approaches.

Vortrag 68

Titel:
Compact Myelin Detachment after metabolic Oligodendrocyte injury

Autoren/Adressen:
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Abstract:
Multiple sclerosis is a multifactorial disease of the central nervous system characterized by an inflammatory process and demyelination. Mechanisms leading to oligodendrocyte and myelin degeneration are poorly understood, however oxidative injury and mitochondrial dysfunction are likely to be involved. Our primary goal is to understand how metabolic stress can lead to oligodendrocyte dysfunction and demyelination. Here we hypothesize that metabolic injury of oligodendrocytes results in centrifugal oligodendrocyte degeneration, leading to demyelination and the breakdown of action potential propagation.

Oligodendrocyte cell-cultures were treated with the respiratory chain inhibitor sodium azide for 24 hours and morphological changes were analyzed using live-cell imaging microscopy. In vivo, we analyzed structural and ultrastructural changes in cuprizone-treated mice by immunohistochemistry and serial block-face scanning electron microscopy (3D-EM). Electrophysiological studies were performed to reveal axon conduction changes.

Dysfunction of the respiratory chain in vitro resulted in process retraction of cultured oligodendrocytes. Dysfunction of the respiratory chain in vivo, triggered by cuprizone intoxication, resulted in early oligodendrocyte stress followed by histopathological changes at the myelin-axon interface. As shown by 3D-EM, detachment of the myelin sheath from the axolemma was the predominant myelin pathology prior to overt demyelination (called compact myelin detachment; CoMyD). Eventually, rupture of the axolemma was found at sites of myelin pathology. Compound action potential measurements revealed a severe impairment of action potential propagation at this early time point.

Our study clearly shows that metabolic injury to oligodendrocytes results in centrifugal oligodendrocyte degeneration. Furthermore, we describe a novel mechanism of early myelin pathology.

Vortrag 69

Titel:
Temporal glyoxalase 1 changes after perforant pathway transection, excitotoxicity and controlled cortical impact injury

Autoren/Adressen:
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Abstract:
Acute neuronal lesions lead to metabolic imbalance enhancing the rate of glycolysis and thus the amount of methylglyoxal (MGO) which contributes to metabolic dysfunction and inflammation. The glyoxalase system represents the main detoxification system of MGO but is impeded in excitotoxicity and stroke. But so far, variations of the glyoxalase system in other neuronal damage models are still unknown.

Organotypic hippocampal slice cultures were utilized for perforant pathway transection (PPT; 5 minutes to 72 hours) and excitotoxic insult (N-methyl-D-aspartate [NMDA]; 50 µM for 4 hours). In addition, rats were subjected to controlled cortical impact injury (CCI; 2 hours to 14 days). Glyoxalase I (GLO1) was investigated by Western blot analyses and immunohistochemistry over time.

GLO1 protein content did not changed significantly following PPT. As described previously excitotoxic lesion led to an elevation in GLO1 immunoreactivity at 24 and 48 hours, PPT increased neuronal GLO1 immunoreactivity at 48 hours post injury. CCI led to positive GLO1 immunoreactions in neurons and astrocytes at 1 and 3 days after injury whereas at two hours and fourteen days after CCI no GLO1 immunoreactivity was detected.

GLO1 protein content variations are observed after excitotoxic and traumatic insults but not after fiber transection. Cell specific differences of GLO1 immunoreactivity seem to be common in severed injured neurons.

Vortrag 70

Titel:
The role of Lipocalin 2 in multiple sclerosis lesion development

Autoren/Adressen:
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Abstract:
Reactive astrocytes play a pivotal role in lesion development in multiple sclerosis (MS). Once activated, they undergo severe morphological and functional changes. Lipocalin 2 (LCN2) has been described to be a major regulator of astrocyte reactivity, chemokine expression and neuroinflammation. LCN2 expression is increased in MS lesions, and higher LCN2 levels have been found in the serum and cerebrospinal fluid of MS patients.

Here, we investigated the role of LCN2 in MS-related animal and in vitro models for the regulation of astrocyte responses and the development of inflammatory lesions. Cuprizone (Cup), experimental autoimmune encephalomyelitis (EAE) and a combination of both (Cup/EAE) were investigated for quantitative and cellular LCN2 expression.

The Cup/EAE model is characterized by immune cell invasion into the telencephalon. In Cup/EAE animals, we identified a unique LCN2-positive astrocyte subpopulation in close vicinity to perivascular immune cell infiltrates. Expression of the CXCR4 ligands CXCL9, CXCL10 and CXCL11 was significantly increased in the brains of Cup/EAE-animals in comparison to control, Cup and EAE. This indicates a functional role of these chemokines for the invasion of immune cells. In vitro stimulation of primary astrocytes with LCN2 induced the expression of these chemokines and supports a decisive function for astrocytes. Scratch assays and cerebral slice cultures from wild type and LCN2-deficient animals further confirmed that LCN2 synthesized by astrocytes is necessary to coordinate morphological and functional changes of surrounding astrocytes.

In summary, astroglial LCN2 appears to represent an important checkpoint factor for the inflammatory processes in the vicinity of developing MS lesions.

Vortrag 71

Titel:
High speed ventral plane videography as a method to measure motor dysfunction in mice

Autoren/Adressen:
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Abstract:
Experimental autoimmune encephalomyelitis (EAE) is the most commonly used animal model to study the pathogenesis of multiple sclerosis (MS) and to develop new therapeutic strategies. EAE mice typically develop predominantly motoric symptoms in a caudal to rostral pattern, which are rated following different scoring systems. Currently used methods to assess treatment efficacy in EAE are subjective and insensitive. Evaluation methods with a higher degree of accuracy and reliability for EAE assessment are urgently needed. We assume that complex gait analyses are superior to currently applied EAE evaluation protocols

EAE was induced in female mice by immunization with MOG35-55 peptide. The DigiGait™imaging system, consisting of a motorized treadmill with a digital camera positioned below a transparent belt, was used to record the location and timing of each paw contact on the belt. Measurements were performed at a belt speed of 15 cm/s for 5 s. Gait parameters were calculated by the accompanying software

Five out of five control mice, and six out of eight EAE mice could be evaluated using the DigiGait™imaging system. EAE severity was not influenced by the imaging procedure. During the preclinical phase, when conventional EAE evaluation protocols failed to detect any functional impairment, EAE mice had higher paw angles of hind limbs, and lower stride width and stand width variability compared to control mice

High speed ventral plane videography, using the DigiGait™imaging system is a valuable tool to study motor function deficits in EAE and probably other neurodegenerative mice models

Vortrag 72

Titel:
EGFL7 enhances surface expression of integrin ALPHA5BETA1 to promote angiogenesis in malignant brain tumors

Autoren/Adressen:
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Abstract:
Glioblastoma is a typically lethal type of brain tumor with a median survival of 15 months post diagnosis. This negative prognosis prompted the exploration of alternative treatment options. In an attempt to improve the efficacy of anti-VEGF treatment we explored the role of the egfl7 gene in malignant glioma.

Glioma formation has been studied in vivo using syngeneic and xenograft models of brain tumor formation. SCharacteristics of blood vessels were analyzed by immunohistochemistry and MRI. Molecular studies were applied to understand the regulation of integrin ALPHA5BETA1 by EGFL7. The clinical relevance of our findings was determined in human specimens.

We found that the extracellular matrix protein EGFL7 was secreted by glioma blood vessels but not glioma cells themselves, while no major role could be assigned to the parasitic miRNAs miR 126/126*. EGFL7 expression promoted glioma growth in experimental glioma models in vivo and stimulated tumor vascularization. Mechanistically, this was mediated by an upregulation of integrin ALPHA5BETA1 on the cellular surface of endothelial cells, which enhanced fibronectin-induced angiogenic sprouting. Glioma blood vessels that formed in vivo were more mature as determined by pericyte and smooth muscle cell coverage. Furthermore, these vessels were less leaky as measured by magnetic resonance imaging of extravasating contrast agent. EGFL7-inhibition using a specific blocking antibody reduced the vasculari¬zation of experimental gliomas and increased the life span of treated animals, in particular in combi-nation with anti-VEGF and the chemotherapeutic agent temozolomide.

Data allow for the conclusion that a combinatorial regimen of temozolomide, anti-VEGF and anti-EGFL7 antibodies may serve as a novel treatment option for glioblastoma multiforme.