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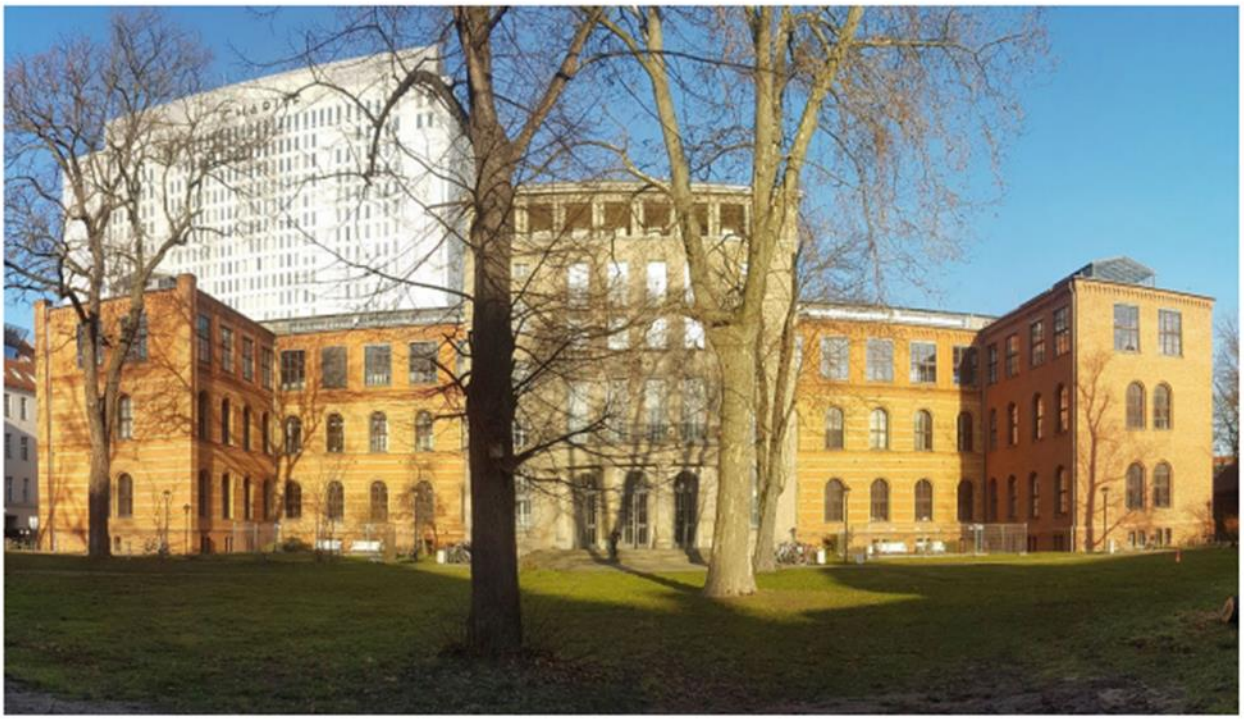

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Talk 1:

Title:
Inhibition of the enzyme autotaxin reduces critical excitability and ameliorates the outcome in stroke

Authors:
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Abstract:
Stroke penumbra injury caused by excess glutamate is an important factor in stroke outcome; however, several therapeutic approaches aiming to rescue the penumbra have failed. This was related to a mistargeted glutamatergic signaling inducing a vicious circle and subsequent excitotoxicity, which continued far beyond the primary stroke event. Synaptic lipid signaling, previously reported to modulate glutamatergic transmission via presynaptic lysophosphatidic acid (LPA)2-receptors, acts via the LPA-synthesizing molecule autotaxin (ATX) present in astrocytic perisynaptic processes. Here we set out to analyze the effect of deregulated synaptic lipid signaling on stroke outcome.

Analyses were performed using the MCAO model applied in transgenic mouse models displaying genetic modifications at specific checkpoints of synaptic lipid signaling. Human stroke analyses and stroke outcome data was used to confirm the translational significance.

we detected long-lasting increases in brain ATX-concentrations after experimental stroke, as well as high cerebrospinal fluid ATX-concentrations up to 14 days following stroke in humans. Using astrocyte-specific deletion and pharmacological inhibition of ATX at different time points after experimental stroke, we discovered that inhibition of LPA-related cortical excitability significantly improved stroke outcome. In transgenic mice and stroke patients expressing a single nucleotide polymorphism that leads to increased LPA-related glutamatergic transmission, we found an adversarial role of dysregulated synaptic LPA-signaling in stroke outcome.

ATX-inhibition in stroke may be a potent translational therapy to treat stroke.

Talk 2:

Title:
MicroRNAs determine structure and function of a central synapse

Authors:
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Abstract:
MicroRNAs (miRs) play a crucial role in posttranscriptional gene regulation via sequence-specific binding to mRNA. A point mutation in miR-96, which is part of the highly conserved miR-183 cluster (also including miR-183 and miR-182), has been shown to cause non-syndromic progressive hearing loss in men and mice and to change the morphology and function of central auditory synapses. To gain further insights into the roles of miRs in the central auditory system, we investigated structure and function of the calyx of Held synapse in the auditory brainstem, in the absence of miR-96.

We used a miR-183-96 double ko mouse model to determine the gross anatomy of auditory brainstem nuclei. Furthermore, we employed immunohistochemistry, electron microscopy and electrophysiology to determine synaptic structure, synaptic protein distributions and synaptic transmission at the calyx of Held synapse.

We observed reduced volumes specifically for auditory brainstem nuclei. Moreover, the calyx of Held showed alterations in the molecular composition of active zones, synaptic vesicle distribution, synaptic AMPA receptor content and synaptic transmission.

Our data identify a genetic regulatory mechanism that plays an important role for the establishment of proper synaptic transmission at the calyx of Held synapse both, pre- and postsynaptically. Furthermore, we show that miR-183/96 plays a role in regulation of auditory hindbrain development.

Talk 3:

Title:
To translate or not to translate - unique post-transcriptional requirements for the formation of upper cortical layers

Authors:
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Abstract:
Evolutionary expansion of the neocortex is associated with the increase in upper layer neurons.

Using mouse genetics, state-of-the-art protein translation techniques, and high throughput mass spectrometry, we show unique sensitivity of upper layer fate to cellular translation rates. We present Inositol-Requiring Enzyme 1a, IRE1A, as an essential determinant of upper layer fate, neuronal polarization and cortical lamination. We demonstrate a non-canonical function of IRE1A in the regulation of global translation rates in the developing neocortex through its dynamic interaction with the ribosome and regulation of eIF4A1 and eEF-2 expression.

Inactivation of IRE1A engenders lower protein synthesis rates associated with stalled ribosomes and decreased number of translation start sites. Whereas eEF-2 is required for cortical lamination, eIF4A1 regulates acquisition of upper layer fate downstream of IRE1A in a mechanism of translational control dependent on 5'UTR-embedded structural elements in fate determinant genes.

Our data unveil developmental regulation of ribosome dynamics as post-transcriptional mechanisms orchestrating neuronal diversity establishment and assembly of cortical layers and brain circuitry.

Talk 4:

Title:
Rotating field tracer electrophoresis: a novel method to increase neuronal tracing distance and speed in the postmortem human brain

Authors:
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Abstract:
In humans, the precise wiring between brain areas at axonal level is mainly extrapolated from animal models as tract tracing in postmortem human brains relies on passive diffusion of lipophilic tracers within the plasma membrane of neurons, resulting in long incubation time and short tracing distance. With a rotating electric field setup for accelerated tracer diffusion, we propose a new approach to overcome these constraints.

We constructed a novel electrophoresis chamber to allow fast tracer distribution along different fiber orientations. The cationic tracer fast-DiI was injected in the formaldehyde-fixed human occipital lobe (at depth of the calcarine sulcus). The injection site was aligned centrally to the anode, the location of the cathode rotated in relation to the tissue. Acrylamide embedding and cooling to 4°C prevented heat-induced tissue damage. Histological sections were prepared using a vibratome or cryostat.

Tracer reached as far as the lingual, inferior and superior occipital and fusiform gyrus, and cuneus. Labeling of axons, boutons en passant, perikarya, and dendrites could be determined. Tracing distance was approximately 4.5 times longer and diffusion speed 20 times faster than previously described. By optimizing temperature, hydration, calcium content, mounting medium, and storing conditions, we delayed signal deterioration typical for lipophilic tracers, enabling large-scale analysis.

With the gain in time and distance, our rotating field tracer electrophoresis setup could push tract-tracing with lipophilic tracers in the human brain towards routine application. It will complement existing approaches for studying fiber architecture and will enable validation of animal and diffusion imaging data in humans.

Talk 5:

Title:
Multi-modal functions of FOXG1 in the mouse hippocampus

Authors:
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Abstract:
Mutations in the FOXG1 gene, one key instructor of the developing telencephalon, cause a rare and severe neurodevelopmental disorder called “FOXG1 syndrome”. Patients present with a spectrum of phenotypes including microcephaly, seizures, and varying degrees of cognitive dysfunction. However, the pleiotropy of FOXG1 functions and molecular changes underlying the functional abnormalities remain largely unexplored. Here, we provide the first multi-omics data set exploring functions of FOXG1 at the chromatin level and characterize the transcriptional and epigenetic landscape upon reduced FOXG1 expression in mouse hippocampal neurons.

We studied mouse hippocampal neurons with FOXG1 levels reduced through shRNA-mediated knockdown and validated our findings in a mouse model in which one allele of Foxg1 was replaced by the cre recombinase (Foxg1cre/+). We used a multi-omics approach to unravel FOXG1 functions at the chromatin level.

On a genome-wide level, FOXG1 (i) both represses and activates transcription, (ii) binds mainly to enhancer regions, (iii) reconfigures the epigenetic landscape through bidirectional alteration of H3K27ac, H3K4me3, and chromatin accessibility, and (iv) operates synergistically with NEUROD1. Here, we provide the first evidence that they act in a highly cooperative manner to control neuronal maturation. Genes affected by the chromatin alterations impact synaptogenesis and axonogenesis. Moreover, inhibition of histone deacetylases partially rescues transcriptional alterations upon FOXG1 reduction.

This integrated multi-omics view of changes upon FOXG1 reduction reveals an unprecedented multi-modality of FOXG1 functions converging on neuronal maturation. It fuels novel therapeutic options based on epigenetic drugs to alleviate, at least in part, neuronal dysfunction.

Talk 6:

Title:
Adult neurogenesis in the telencephalon of the pigeon (Columba livia f.d.) is influenced by spatial experience

Authors:
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Abstract:
Adult neurogenesis (AN) encompasses the generation, maturation and proliferation of new neurons in the brain over lifespan. In birds, adult neurogenesis is more widespread compared to mammals and was reported in most of telencephalic structures, but their functional significance is also still ambiguous here.

Here, 29 homing pigeons (Columba livia f.d.) were raised together with 10 of them (Group I) remaining permanently in the loft while the other 19 were allowed to fly around the loft. After reaching sexual maturity, all pigeons were treated with 5-bromo-deoxyuridine (BrdU) to label dividing cells. Then, pigeons of Group I had to absolve a learning task in a standard operant chamber. Pigeons of Group II (n=10) got an individual training with several releases from unknown places. Remaining 9 animals served as a control group (Group K). After three months, all pigeons were sacrificed, brains were dissected and immunohistochemically processed with several markers to examine newly generated cells of the hyper- and mesopallium.

The number of newly generated immature neurons, mature neurons and glial cells differs between the groups. Group II and K pigeons showed significantly more immature cells than Group I pigeons. Highest numbers of new mature neurons were found in pigeons of Groups I and II. Hyperpallial structures showed more AN than the mesopallium.

Our findings indicate that spatial learning processes have a positive effect on AN. Moreover, individual life history has an influence on AN. It seems to be that there is a link between brain-structure and function, species-specific requirements and AN.

Talk 7:

Title:
HNF1B alters an evolutionary conserved nephrogenic program of target genes in congenital kidney disease

Authors:
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Abstract:
Hepatocyte nuclear factor 1-beta (HNF1B) is a transcription factor involved in various stages of nephrogenesis and maintenance of renal tubular functions. Mutations in HNF1B are the most common monogenic causes for developmental renal disease, yet the underlying pathways affected are not fully understood. By comparative analysis in Xenopus and directly reprogrammed mammalian cells (iRECs) we investigated a patient-specific mutation (R295C) associated with cystic-dysplastic kidneys.

We used HNF1B to form renal-like organoids from Xenopus explants. In parallel, we analyzed how HNF1B R295C effects nephrogenesis in iRECs. Transcriptional changes were comparatively analyzed in two different species. We confirmed HNF1B target candidates in vivo using CRISPR/Cas0 editing of Xenopus embryos.

HNF1B is not only an essential component in direct reprogramming but can also induce ectopic pronephric tissue in Xenopus ectodermal explants. Changes in the transcriptomic profile demonstrated alterations in specific transcriptional modules and identified novel direct and indirect targets of the transcription factor HNF1B, which are linked to signaling pathways associated with renal morphogenesis, cilia and organic anion transport.

The combined use of directly reprogrammed mammalian cells and Xenopus renal organoid experiments allow us to gain a unique perspective into evolutionary conserved mechanisms of renal development and HNF1B associated kidney disease.

Talk 8:

Title:
Zyxin is important for cell adhesion of cultured podocytes under mechanical stress

Authors:
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Abstract:
Glomerular hypertension induces mechanical load to podocytes, often resulting in podocyte detachment and the development of glomerulosclerosis. Although it is well known that podocytes are mechanosensitive, the mechanosensory mechanism is still unknown. Since zyxin which is localized at focal adhesions as well as along the actin cytoskeleton, is known to be a key player in the mechanotransduction, we hypothesized that zyxin could be important for the outside-in signaling of mechanical stressed podocytes.

Mouse podocytes were cultured on silicone membranes that were connected to the stretch apparatus (“Stretchy”, NIPOKA GmbH, Greifswald) for three days at 0.5 Hz and 5% extension. To study the role of zyxin in cultured podocytes under mechanical stretch, zyxin was knocked down by siRNAs. Additionally, we established a zyxin knockout podocyte cell line by CRISPR/Cas9. Cell lysates of control and zyxin KD/KO podocytes were analyzed by LC-MS/MS, qRT-PCR, Western blot and immunostaining.

We found that zyxin is highly expressed in cultured podocytes and co-localized with F actin and focal adhesion proteins. The knockdown of zyxin changed the F-actin organization and reduced the expression of paxillin. LC-MS/MS and Western blot analysis of zyxin KO podocytes revealed that the loss of zyxin significantly reduced the expression of extracellular matrix proteins like nidogen and fibronectin. Interestingly, the zyxin interaction partner VASP was also significantly downregulated in zyxin KO podocytes. Furthermore, the loss of zyxin also affect cell mobility and filopodia formation. In addition, zyxin knockdown as well as zyxin KO podocytes showed an increased cell detachment after mechanical stress compared to control podocytes.

Zyxin plays an important role in the adhesion of cultured podocytes under mechanical stress due to altered expression of extracellular matrix and focal adhesion proteins.

Talk 9:

Title:
HP1 deficiency results in De-Repression of Endogenous Retroviruses and Induction of Neurodegeneration via Complement

Authors:
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Abstract:
In aging cells and animal models of premature aging, heterochromatin loss coincides with the transcriptional activation of normally silenced endogenous retroviruses (ERVs). Here we show that loss of heterochromatin maintenance and de-repression of ERVs results in neurodegeneration via the Complement cascade in an age dependent manner. We discovered differential contributions of HP1 proteins to ERV silencing where HP1γ is necessary and sufficient for H4K20me3 deposition and HP1β deficiency is detrimental to DNA maintenance methylation. Progressive ERV de-repression in HP1β/γ DKO mice was followed by stimulation of the integrated stress response, the induction of Complement 3+ reactive astrocytes and increased infiltration and activation of microglia. This chronic inflammatory state coincided with age-dependent reductions in dendrite complexity and cognition. Our results demonstrate the importance of preventing loss of epigenetic maintenance, as this will be the only way postmitotic neuronal genomes can be protected and/or renewed.

Talk 10:

Title:
Cytoskeletal anchorage of different Dsg3 pools revealed by combination of hybrid STED/SMFS-AFM

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Abstract:
Desmoglein 3 (Dsg3) is a desmosomal cadherin mediating cell adhesion within desmosomes and is the antigen of the autoimmune blistering skin disease pemphigus vulgaris. Therefore, understanding of the complex desmosome turnover process is of high biomedical relevance. Recently, super resolution microscopy was used to characterize desmosome composition and turnover. However, studies were limited because adhesion measurements on living cells were not possible in parallel. Before desmosomal cadherins are incorporated into nascent desmosomes, they are not bound to intermediate filaments but were suggested to be associated with the actin cytoskeleton. However, direct proof that adhesion of a pool of desmosomal cadherins is dependent on actin is missing.

Here, we applied single-molecule force spectroscopy (SMFS) measurements with the novel single molecule hybrid-technique STED/SMFS-AFM to investigate the cytoskeletal anchorage of Dsg3 on living keratinocytes for the first time. AFM = atomic force microscopy and STED = stimulated emission depletion

By application of pharmacological agents we discriminated two different Dsg3 pools, only one of which is anchored to actin filaments. We applied the actin polymerization inhibitor Latrunculin B in order to modify the actin cytoskeleton and the PKCα activator PMA to modulate the anchorage between desmoplakin and intermediate filaments. At the cellular surface Dsg3 adhesion was actin-dependent. In contrast, at cell-cell contacts, Dsg3 adhesion is independent from actin but rather is regulated by PKC which is well established to control desmosome turn-over via intermediate filament anchorage.

Taken together, using the novel STED/SMFS-AFM technique, we demonstrated the existence of two Dsg3 pools with different cytoskeletal anchorage mechanisms.

Talk 11:

Title:
Cysteinyl leukotrienes and acetylcholine are biliary tuft cell cotransmitters

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Abstract:
The gall bladder stores bile between meals and empties into the duodenum upon demand, thereby being exposed to the intestinal microbiome. This raises the need for antimicrobial factors, among them mucins produced by gall bladder epithelial cells. The role of the much less frequent biliary tuft cells in this scenario is still unknown.

Gall bladder contraction and mucin granule exocytosis were measured by force recording and electron microscopy, respectively, in wildtype and genetically modified mice. Stimuli were blue light in an appropriate optogenetic model, expressing channelrhodopsin-2 selectively in tuft cells, and short chain fatty acids. Acetylcholine, prostanoids and cysteinyl leukotrienes were directly assayed in supernatants of stimulated, explanted gall bladders. Reporter mice, in situ-hybridization and immunolabeling localized mediator synthesizing enzymes and receptors.

Selective optogenetic stimulation of gall bladder tuft cells revealed corelease of acetylcholine and cysteinyl leukotrienes. Acetylcholine triggers exocytosis of mucin granules from cholangiocytes through the muscarinic receptor M3, and cysteinyl leukotrienes cause bladder contraction through the receptor CysLTR1. We identify propionate, a major metabolite of intestinal bacteria, as a naturally occurring stimulus activating tuft cells via the short chain free fatty acid receptor 2 and downstream signalling involving the cation channel TRPM5.

Our results establish gall bladder tuft cells as sensors of a microbial product, initiating two independent innate defence mechanisms through cotransmission. Acetylcholine, best characterized as a neurotransmitter, serves here as a paracrine factor triggering epithelial defence, and cysteinyl leukotrienes, known from immune effector cells, target the muscular component, emptying and closing the bladder.

Talk 12:

Title:
Peritoneal carcinomatosis formation of human pancreatic cancer depends on integrin expression in vivo

Authors:
Alina Schiecke (Anatomy and Experimental Morphology, University Medical Center Hamburg-Eppendorf, Hamburg), Alexander Ewe (Rudolf-Boehm-Institute for Pharmacology and Toxicology, University of Leipzig, Leipzig), Achim Aigner (Rudolf-Boehm-Institute for Pharmacology and Toxicology, University of Leipzig, Leipzig), Kristoffer Riecken (Stem Cell Transplantation, University Medical Center Hamburg-Eppendorf, Hamburg), Udo Schumacher (Anatomy and Experimental Morphology, University Medical Center Hamburg-Eppendorf, Hamburg), Daniel Wicklein (Institute for Anatomy and Cell Biology, University of Marburg, Marburg);
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Abstract:
Peritoneal carcinomatosis is a frequent type of metastatic spread in pancreatic ductal adenocarcinoma, and the current therapeutic options are still very limited. During peritoneal carcinomatosis, tumor cells detach from the primary tumor and adhere to the mesothelial cell layer of the peritoneal cavity and to the submesothelial extracellular matrix through cell-cell and cell-matrix interactions, mediated by adhesion molecules such as integrins. In pancreatic cancer, many integrins are highly overexpressed compared to normal tissue. Consequently, a closer look at these molecules is urgently needed to develop novel therapeutic options to inhibit intraperitoneal spread.

In this study, stable shRNA-mediated integrin α3 (ITGA3) and integrin β4 (ITGB4) knockdowns (KDs) were separately established in three pancreatic cancer cell lines. In addition, control cell lines were generated showing no changes in integrin expression. Intraperitoneal dissemination of these tumor cells was analyzed using intraperitoneal xenograft models.

ITGB4 KD led to an improved overall survival rate in all three tested xenograft models, which was due to a delayed development of peritoneal metastases and malignant ascites. KD of ITGA3, which is known to be a clinical prognostic and diagnostic marker in pancreatic cancer, caused a survival benefit of the mice in two of the three tested models. In vitro experiments revealed reduced adhesion, proliferation, and colony formation (in an extracellular matrix-containing environment) upon both integrin KDs.

The adhesion molecules ITGA3 and ITGB4 are significantly involved in intraperitoneal metastasis formation of human pancreatic cancer cells and thus represent promising targets for future therapies.

Talk 13:

Title:
Inhibitory synapse diversity in health and disease

Authors:
Dilja Krueger-Burg (Institut für Anatomie, Universitätsmedizin Mainz, Mainz); dkruegerburg@uni-mainz.de

Abstract:
Abnormalities in the balance of excitatory to inhibitory neurotransmission have been proposed to play a key role in the etiology of psychiatric and neurodevelopmental disorders, and substantial evidence links mutations in the proteins that mediates excitatory synaptic transmission to these disorders. In contrast, the role of alterations in the molecular machinery at inhibitory synapses has received surprisingly little attention. In recent years, however, an increasing number of variants in GABAergic postsynaptic proteins has been identified in patients with autism spectrum disorder, schizophrenia and/or intellectual disability, highlighting the urgent need for a better understanding of the involvement of these proteins in health and disease.

Here I present recent studies on the molecular mechanisms by which the prototypical GABAergic synaptic adhesion protein Neuroligin-2 and its interaction partners regulate behavioral circuits in mouse models.

In particular, I focus on a key theme that emerges from these studies, i.e. the importance of GABAergic synapse diversity in understanding the consequences of mutations in these proteins on behavioral output. The GABAergic inhibitory system is highly heterogeneous, with a large number of neuronal subtypes contributing vastly different functions to neuronal information processing, and recent evidence indicates that this cellular diversity is accompanied by a corresponding molecular diversity at GABAergic synapses.

By identifying synapse- and circuit-specific functions of individual GABAergic postsynaptic proteins, it may not only be possible to better understand their role in the pathogenesis of psychiatric and neurodevelopmental disorders, but also to develop circuit-specific therapeutic approaches with improved selectivity for the targeted behavioral symptoms.

Talk 14:

Title:
Differential innervation of VIP neuron subtypes by basket cells in layer2/3 of mouse primary somatosensory cortex

Authors:
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Abstract:
In the cerebral cortex, individual interneuron types play unique roles. VIP interneurons contribute to sensory processing, sensorimotor integration, and behavioral control. Certain VIP cells are sensitive to depolarization-inducing neuromodulation from acetylcholine or serotonin which can effectively switch their firing patterns in a brain state-dependent manner. Here we hypothesize that GABAergic basket cells could play a major role in this regulation in a temporally precise manner. Since VIP neurons are not a uniform cell type, we aim to utilize intersectional targeting of VIP neurons to study the VIP/calretinin (CR) and VIP/cholecystokinin (CCK) expressing interneurons in the mouse cortex. Since basket cells also come in at least 2 flavors, fast-spiking parvalbumin-expressing (PV) expressing basket cells and non-fast-spiking cannabinoid receptor-1 (CB1-R) expressing basket cells, different VIP cells could be targeted by different types of basket cells

In the present study, we will morphologically analyze the number and distribution of basket cell boutons onto VIP+ neurons in L2/3 of the mouse primary somatosensory cortex barrel field (S1BF) by histological means. Three-channel CLSM-airy scanned images will be used for putative contact analyses by Neurolucida.

The preliminary results from VIP/CCK suggest that both types of basket cell boutons were more densely distributed on the somatic domains. The density of PV inputs to the somata was comparable to that of CB1R inputs, whilst PV inputs on dendrites were 13.5 fold denser than CB1R inputs.

Because of the “blanket inhibition” caused by PV basket cells, all VIP cells should be innervated in a comparable manner and because basket cells strongly innervated other basket cells, CB1-R basket cells target more strongly VIP/CCK than VIP/CR neurons.

Talk 15:

Title:
Parvalbumin interneurons are differentially connected to principal cells in inhibitory feedback microcircuits along the dorsoventral axis of the medial entorhinal cortex

Authors:
Sabine Grosser (Institute of Integrative Neuroneuroanatomy, Charité, Berlin), Federico J Barreda (Institute of Integrative Neuroneuroanatomy, Charité, Berlin), Pratep Beed (NWFZ, Charité, Berlin), Dietmar Schmitz (NWFZ, Charité, Berlin), Imre Vida (Institute of Integrative Neuroneuroanatomy, Charité, Berlin)

Abstract:
The medial entorhinal cortex (mEC) shows a rich repertoire of spatial-modulated neuronal activity patterns, including a prominent grid-cell activity of principal cells. Grid-cell activity is reliant on inhibition provided by local interneurons, in particular, fast-spiking parvalbumin (PV) basket cells (BCs) embedded in feedback inhibitory microcircuits. However, PV BC-mediated inhibition onto principal cells is not uniform, but shows a gradient along the dorsoventral axis with strong inhibition in the dorsal and weak in the ventral mEC. This is in good correlation with divergent grid field sizes observed along this axis, but the underlying morphological and physiological mechanisms remain unknown. In this study, we characterized the intrinsic physiology, morphology, and synaptic connectivity PV BCs in layer 2/3 of the mEC in the juvenile rat using whole-cell recordings combined with intracellular-filling and subsequent morphological analysis. We found that while intrinsic physiological properties and the morphology are broadly similar over the dorso-ventral axis, PV BCs form more synaptic connections onto local principal cells in the dorsal mEC. In turn, the two major principal cell subtypes of this region, pyramidal and stellate cells, form excitatory connections onto PV BCs with lower, but equal probability. Our results, thus, identify inhibitory connectivity as the source of the gradient of inhibition, explaining divergent grid field formation along the dorso-ventral axis of the mEC

Talk 16:

Title:
Unique Properties and Networks of Cajal-Retzius cells in the Entorhinal Cortex

Authors:
Max Anstötz (Institut for Anatomy II, Heinrich-Heine-Universität Düsseldorf, Düsseldorf), Gianmaria Maccaferri (Dept. of Neuroscience, Northwestern University, Chicago); max.anstoetz@med.uni-duesseldorf.de

Abstract:
Cajal-Retzius cells (CR-cells) are early born glutamatergic neurons, present in the most superficial layers of the brain cortex. Commonly regarded as a transient population, CR-cells die shortly after the postnatal brain development. Although this holds true for the neocortex, we show here that CR-cells are expressed in fully mature allocortical areas such as the entorhinal cortex (EC). This novel observation raises the question of their relevance and function.

We have taken advantage of transgenic reporter mouse lines, optogenetics and calcium imaging to study CR cells in the medial and lateral entorhinal cortex (EC). Using a combination of morphological and functional techniques we have studied the developmental profile, morphology, electrical properties, and synaptic connectivity of EC CR-cells.

Our results show that CR-cells in the EC are heterogeneously distributed along mediolateral axis and remain expressed lifelong. Single cell morphology reveals widespread axons that cover the molecular layers of multiple cortical regions, including the hippocampus and perirhinal cortex. Functionally, we show that EC CR-cells provide strong glutamatergic output onto local GABAergic-Interneurons and thereby GABAergic feed-forward inhibition onto adjacent principal neurons in deeper cortical layers. Local neurotransmitter application combined with Calcium Imaging as well as optogenetic stimulation Somatostatin-Interneurons reveal, that EC CR cells are stimulated and activated by GABA.

Taken together, our data show that EC CR-cells display peculiar morphological properties, are strongly integrated into the synaptic microcircuit, and can project into more distant cortical regions of the brain. These findings highlight the potential importance of CR cells for modulatingprocessing of local and long-range microcircuits.

Talk 17:

Title:
Homeostatic synaptic plasticity recruits coordinated structural and functional changes in the human brain

Authors:
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Abstract:
Homeostatic synaptic plasticity aims at compensating for perturbations in network activity, thereby keeping neurons in a functional dynamic range. Among the mechanisms that regulate synaptic plasticity, coordinated structural and functional changes at synaptic sites represent a hallmark in adaptive processes. Nevertheless, the precise regulatory mechanisms and the relevance of homeostatic plasticity in the human brain warrant further investigation.

In this study, we investigated the impact of neural network silencing through pharmacological inhibition of voltage-gated sodium channels or glutamatergic neurotransmission (i.e., common targets of anticonvulsant substances) on functional and structural properties of murine and human cortical tissue.

Using mouse organotypic tissue cultures and adult human neocortical slices, we demonstrated that network silencing promotes a compensatory functional and structural reorganization of excitatory synapses. This homeostatic synaptic adjustment was accompanied by characteristic (epi)transcriptomic changes.

Our findings provide first experimental evidence for homeostatic synaptic plasticity in the adult human neocortex. They suggest an important role for mRNA modifications and protein synthesis in the regulation of synaptic homeostasis in mice and humans.

Talk 18:

Title:
Mechanical properties of Achilles tendon and metabolites of exercise

Authors:
Alvin Curtis Lin (Institut für makroskopische und klinische Anatomie, Medizinische Universität Graz, Graz), Niels Hammer (Institut für makroskopische und klinische Anatomie, Medizinische Universität Graz, Graz); alvin.lin@medunigraz.at

Abstract:
The Achilles tendon is one of the most common tendons prone to rupture. During exercise, metabolites from contracting muscles are released into the interstitial space. Whether these metabolites play role in Achilles tendon rupture is unknown. HYPOTHESIS: The mechanical properties of Achilles tendon are altered by metabolites such as lactic acid that are generated during exercise.

Achilles tendon samples from bovine tissues were subjected to different lactic acid solutions for up to 24 hours. Mechanical testing of samples was performed, examining material properties of stiffness (Young's Modulus or YM) and ultimate tensile strength (UTS). The same experiments were repeated with human Achilles tendon samples from post-mortem tissues.

A significant difference ($p<0.05$) was observed in YM and UTS for Achilles tendon samples exposed to different lactic acid solutions at a range of physiologically-relevant parameters.

Achilles tendon rupture may be promoted by metabolites generated during exercise. Future investigations into the mechanical properties and ultrastructure of the Achilles tendon under different physiologically-relevant parameters will be performed.

Talk 19:

Title:
Ramification of the Dorsal Clitoral Nerve along its Course on the Human Clitoris

Authors:
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Abstract:
The human clitoris, a strongly erogenous organ of the female erectile apparatus, serves to form and support sexual arousal. It consists of erectile tissue segments and is sensitively innervated by a branch of the pudendal nerve: the dorsal clitoral nerve (DCN), which can be divided into five segments (I-V). Our study aimed for further insight into the ramification pattern of the DCN in its distal segments (II-V) and the revelation of important clinical-anatomical details.

These details were elaborated by dissecting n=12 human clitoris specimens with subsequent staining using the modified Sihler-technique, followed by micro-dissection of the stained DCN.

The microdissection of the stained fasciculi in the distal segments II-V showed that the DCN unites in its course several nerve branches that arise from different areas around the clitoris (e.g., branch for the suspensory ligament). The DCN has a strictly ipsilateral course with no branches crossing the median line. Due to interindividual different formation of anterolateral nerval trunks in segments III and IV supplying the clitoral glans, different ramification patterns can be distinguished. Furthermore, we show that dorsal and ventral areas of the clitoral glans are innervated by different fasciculi that enter the DCN, especially in its third and fourth segments. Finally, a ventral region of the glans clitoridis has been identified, where no bigger fascicle or branch of the DCN courses.

Results may serve as a basis for further improvement of reconstructive surgical techniques for patients that suffer from female genital mutilation or other post-traumatic or iatrogenic affection of the clitoral glans.

Talk 20:

Title:
Chances and Challenges in Provenance Research on Human Remains of Blumenbach‘s and the Anthropological Collection at Göttingen University

Authors:
Katharina Stötzel (Institute of Anatomy and Embryology, University Medical Center Göttingen, Göttingen), Viebahn Christoph (Institute of Anatomy and Embryology, University Medical Center Göttingen, Göttingen); katharina.stoetzel@med.uni-goettingen.de

Abstract:
Human remains from colonial contexts in two Göttingen University collections have recently become the focus of grant-funded provenance research, the aim being to examine the origin of the human remains, the circumstances of their acquisition and to re-individualise the remains only known by their inventory numbers.

Sets of human remains were examined using methods of physical anthropology to establish the sex, age-at-death, and health status of the individuals. The preservation of bones as well as post mortem changes and manipulations were recorded on standardised fact sheets. In accordance with the wishes of the representatives of the societies of origin only non-invasive techniques were carried out.

While no complete paleopathological examination was ethically approved for the human remains from 13 individuals from Hawai’i, a broader spectrum of methods including x-ray analysis and endoscopy of the brain case was possible to be used on a Tanzanian set of remains. This led to the discovery of the poor health of these individuals (including scurvy and malnourishment).

The anthropological-anatomical research was imperative for the establishment of the number of individuals from Hawai’i, because it enabled us to determine whether bones are likely to be derived from one and the same or from several different individuals. Detailed anatomical knowledge of the soft as well as the hard tissue, in contrast, aided in the identification of major diseases from which individuals suffered. We propose that a complete anthropological-anatomical examination of human remains is highly desirable when conducting provenance research on human remains from ethically sensitive contexts.

Talk 21:

Title:
Microbiological evaluation of selected historical anatomical specimens

Authors:
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Abstract:
During inventory work in the basement of the 19th-century building of the Department of Anatomy, interest was aroused in the neglected collection of anatomical specimens. The extent of conservation care for them remained unknown. The jars of slides had sat unattended for more than 80 years. The aim of the study was to assess its microbiological condition

Microbiological analyses were based on culture and isolation methods, analysis of microcopy slides and MALDI-TOF analysis.

In microbiological tests of swabs taken from the examined anatomical specimens both bacteria and fungi were isolated. The bacterial flora was less numerous than the fungal flora. Among the bacteria, environmental Gram positive Bacillus cereus, Bacillus thuringiensis and a rare bacterium of the Cupriavidus genus were isolated, whereas among the fungal organisms, the yeast-like fungi Candida boidinii and Geotrichum silvicola as well as mold fungi Penicillium sp. and Fusarium sp.

The primary causes of the changes in the anatomical specimens were probably leaky containers and the place where the collection was stored, i.e. a dark, cool, damp cellar with limited ventilation. Evaporation of the components of preservation mixtures and their oxidation by air reaching the surface of the fluid may have affected the volume and concentration of the fluids, but also their antiseptic properties. In the presented study, it was shown that in certain concentrations, substances traditionally used in preservation can become nutrients for microorganisms (e.g. ethanol for Candida boidini, heavy metal ions for Cupriavidus metallidurans).

Talk 25:

Title:
A new plakoglobin-phosphodeficient mouse models reveals that plakoglobin regulation is important for epidermal integrity via keratin anchorage of desmosomes

Authors:
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Abstract:
In the life-threatening disease pemphigus vulgaris (PV) autoantibodies directed against desmosomal cadherins cause loss of intercellular adhesion clinically manifested as flaccid blisters of the skin and mucous membranes. Current therapies focus on suppression of autoantibody formation. However, especially for the acute phase of the disease an additional treatment paradigm directly stabilizing keratinocyte adhesion would fulfill an unmet medical need. Thus, we here analyzed the mechanisms by which Apremilast, a phosphodiesterase 4 inhibitor already used to ameliorate other skin diseases such as psoriasis, for its therapeutic potential in PV and established a new plakoglobin-phosphodeficient mouse model.

Pg-S665 phosphodeficient mouse model, Atomic force microscopy, cAMP ELISA, electron microscopy, ex-vivo pemphigus skin model, immunostaining, keratinocyte dissociation assay, Western blot, Fluorescence Recovery after Photobleaching

Apremilast abrogated PV-IgG-induced loss of keratinocyte cohesion in vitro as well as epidermal blister formation ex-vivo in human epidermis. Morphologically, apremilast inhibited PV-IgG-induced keratin retraction and ameliorated desmosome splitting. Importantly, apremilast also induced phosphorylation of plakoglobin at serine 665 – a mechanisms which is known to stabilize cardiomyocyte cohesion. Interestingly, epidermis of mice expressing phospho-deficient plakoglobin (Pg-S655A) displayed altered distribution of desmosomal proteins and keratin filaments and were susceptible to mechanical stress. In contrast, Apremilast failed to ameliorate PV-IgG-induced loss of cell adhesion and to modulate Dsg3 turnover in Pg-S655A keratinocytes.

These data identify a novel mechanism of desmosome regulation and propose that apremilast restores keratinocyte adhesion via keratin anchorage in pemphigus, which involves Pg phosphorylation at serine 665. Thus, Apremilast may serve as treatment option during the acute phase in pemphigus.

Talk 26:

Title:
Nrf2 loss correlates with progression of age-related osteoporosis in women

Authors:
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Abstract:
Nuclear Factor Erythroid 2-Related Factor 2 (Nrf2) is an essential transcription factor for maintaining cellular redox homeostasis but also affects bone turnover. We aimed to elucidate the potential impact of Nrf2 on the development of age-related bone loss using a mouse model.

Female wild-type (WT) and Nrf2-knockout (KO) mice were sacrificed at 12 weeks and 90 weeks, morphological cortical and trabecular properties of femoral bone were analyzed by micro-computed tomography (µCT) and compared to histochemistry. We counted empty osteocyte lacunae in cortical bone and evaluated osteoclast-like cells, and aromatase expression by osteocytes immunohistochemically. Mechanical properties were derived from quasi-static compression tests.

When compared to old WT mice, old Nrf2-KO mice revealed a significantly reduced trabecular bone mineral density (BMD), cortical thickness (Ct.Th), cortical area (Ct.Ar), and cortical bone fraction (Ct.Ar/Tt.Ar). Surprisingly, these parameters were not different in skeletally mature young adult mice. Occurrence of empty osteocyte lacunae did not differ between both groups, but a significantly higher number of osteoclast-like cells and fewer aromatase-positive osteocytes were found in old KO mice.

Our results confirmed lower bone quantity and quality as well as an increased number of bone-resorbing cells in old female Nrf2-KO mice. The restriction of aromatase expression in Nrf2-deficient mice may indicate a chronic lack of estrogen in bone. Thus, chronic Nrf2 loss seems to contribute to the age-dependent progression of female osteoporosis.

Talk 27:

Title:
TNF-α-mediated CEACAM1 expression in endothelial cells

Authors:
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Abstract:
The carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1) is a central regulator of vascular physiology. Previously we have shown that progressive age leads to a mutual upregulation of vascular CEACAM1 and TNF-α expression that maintains a chronic pro-inflammatory milieu in the vasculature. This age-dependent CEACAM1 upregulation is crucial to vascular aging processes, e.g. oxidative stress and arterial collagen deposition.

In the current study, we analyzed the signaling pathways that are involved in the TNF-α-mediated CEACAM1 expression. Using endothelial cell cultures, we conducted pharmacological as well as biochemical experiments.

We found that the upregulation of endothelial CEACAM1 expression in response to TNF-α stimulation shows a biphasic pattern. Inhibitor studies point to an early response that is mediated by activation of NFκB, whereas at later time-points the persistent upregulation might depend on Catenin-driven expression.

Our data support a novel mechanism of CEACAM1 expression regulation. Especially in inflammatory settings with enhanced TNF-α signaling, i.e. atherosclerosis or cancer that regulation might significantly be involved in the pathology.

Talk 28:

Title:
Investigating the mechanisms of prostate cancer bone metastasis – optimization of current in vivo models and detection methods

Authors:
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Abstract:
Prostate cancer (PCa) mortality is primarily linked to metastatic disease, with bone being the most common site of recurrence. Detailed mechanisms regulating bone metastasis (BM) development remain to be uncovered, emphasizing the need to develop and optimize clinically relevant in vivo models with advanced detection methods for single disseminated tumor cells (DTCs).

To recapitulate the full metastatic cascade human PCa PC-3 (Luc2/RGB+ve) cells were subcutaneously injected into male immunodeficient mice. Metastatic load was determined using bioluminescence imaging (BLI), Alu-qPCR and histology. Spearman correlations were calculated to compare these methods for the detection of BM and lung metastases (LM). To facilitate the detection of DTCs in bone, advanced imaging methods (confocal, two-photon microscopy) were established.

BLI, Alu-qPCR and histological analysis showed the strongest correlation in spontaneous LM samples indicating that all three methods are equally precise to detect PCa LM. In bone, the correlation between the three different techniques was much weaker, highlighting the complexity of investigating PCa BM. Compared to standard histological analysis (5 µm sections), fluorescence staining, confocal- and two-photon microscopy have proven to be beneficial to achieve a high-resolution and more in depth (35-100 µm) three-dimensional analysis of DTCs in bone.

The partial discrepancy in BM quantification between the detection methods highlights the need for multiple detection methods in preclinical BM models. Combining clinically relevant in vivo models with advanced imaging methods will provide novel insights into the establishment of BM, particularly into the process of PCa dormancy and reactivation in bone.

Talk 29:

Title:
The subpopliteal fat body

Authors:
Christoph Hellmund (, Universität Leipzig, Leipzig), Pierre Hepp (Klinik und Poliklinik für Orthopädie, Unfallchirurgie und Plastische Chirurgie, Universitätsklinikum Leipzig, Leipzig), Hanno Steinke (Institut für Anatomie, Universität Leipzig, Leipzig); christoph.hellmund@icloud.com

Abstract:
The knee is surrounded by ligaments, connectives and muscles. These highly active structures are imbedded in fatty tissue, which was disregarded as unimportant for a long time. Similar to the ventral Hoffa fat pad, we investigated a dorsal fat body, ventral to the popliteus muscle.

11 fresh knees were investigated. All muscles but the popliteus muscle were removed. It was released from its tibial origin and dissected craniolaterally. Thereby, a subpopliteal fat body (SFB) was exposed. Relating nerves and vessels were evaluated. Examples of histological slices were stained with HE and immunostained against neurofilament.

The SFB lies at the concave posterior tibia and attaches to the tibial periost and the popliteus muscle. It is separated from the subpopliteal recess by a lamella deriving from the fibular head. Arterial and venous vessels, as well as a subbranch of the tibial nerve were seen to reach the SFB. The SFB could be identified in MRI scans and in plastinations.

The SFB provides a gliding space for the mobile part of the popliteus muscle. The SFB lies within the tibial concavity, where embryologically the popliteal artery passes through. Therefore, the SFB may contain perivascular autonomic nerves which encompass embryologically created arteries. The nerves and vessels form a neurovascular bundle which could be a source of pain. This may explain the autonomic component of pain in the deep lateral region of the knee. The SFB is functional fat, comparable to Hoffa’s fat pad in the ventral knee.

Talk 30:

Title:
An uncharacterized human bone marrow derived peptide positively modulates synaptic plasticity of primary neurons

Authors:
Vivien Nöth (Institute of Anatomy and Cell Biology, Ulm University, Ulm), Tobias M. Boeckers (Institute of Anatomy and Cell Biology, Ulm University, Ulm), Alberto Catanese (Institute of Anatomy and Cell Biology, Ulm University, Ulm); vivien.noeth@uni-ulm.de

Abstract:
The aim of this study was to identify novel endogenous modulators of synaptic plasticity. In fact, interventions aimed at manipulating neuronal activity have often proven neuroprotective in pathological contexts such as neurodegenerative diseases and aging.

We set out a screening workflow based on the anti-synaptotagmin antibody feeding assay to evaluate the effect of small human peptides on neuronal activity. Following a sequential sub-fractionation strategy, we tested the peptidome derived from the human bone marrow on synaptic activity and the most promising hits were confirmed using multi electrode array (MEA) measurements. Subsequently, we exploited pharmacological treatments and multi-omics approaches to dissect the molecular cascades triggered in primary neurons by the top candidate molecule.

Our unsupervised approach allowed the identification of an uncharacterized 14-amino acids long peptide able to increase neuronal firing in primary cortical cultures. Notably, we observed that its effect occurred within few minutes after treatment to a magnitude comparable to the one of known synaptic activators such as AMPA and Apamin. Mechanistically, explorative investigations suggested that the increased synaptic activity depends on a downstream activation of G-protein coupled receptors, clathrin-mediated endocytosis, as well as on the activation of the transcription factor CREB and the immediate early gene cFOS.

We discovered a novel and potent endogenous enhancer of neuronal firing, whose effect will be further dissected to identify the specific molecular targets involved in its modulation of synaptic activity and to test its potential neuroprotective effect in different models of neurodegeneration.

Talk 31:

Title:
Expression and putative role of inflammatory-related miRNAs during acute CNS injury

Authors:
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Abstract:
Acute injuries in the central nervous system (CNS) cause irreversible neural dysfunction and cell death and often result in life-long disabilities or death of patients. Brain damage entails a high degree of dysregulation of coding and non-coding genes, including regulatory microRNAs (miRNAs).

Two experimental models were included in the study, i.e. ischemic stroke induced by an occlusion of the middle cerebral artery (tMCAo) in rats and spinal cord injury (SCI) in mice caused by a moderate contusion of the thoracic SC. After 6, 12, 24, and 72 h, animals were sacrificed, blood and tissue samples from different organs and CNS regions were collected. Selected miRNAs and gene expression of suitable targets were analyzed.

miR-223-3p, miR-155-5p, miR-448-5p, miR-3473, and miR-124-3p displayed significant time- and model dependent alterations after injury. Importantly, expression levels also changed at the systemic level. Concerning putative target genes for the studied miRNAs, we found a good correlation with its regulatory miRNAs in the CNS and peripheral organs.

We describe here a time-dependent regulation of inflammatory miRNAs and partially of their target genes in two acute brain damage models. Interestingly, similar changes in miRNA levels appear outside the destructed neural tissue. Although we assume, it is not clear that the circulating miRNAs derive from the injured site. Our data allow concluding that the hampered tissue signals to other organs and other brain circuits to communicate the destruction possibly thereby attracting immune cells and switching physiological processes in support for tissue/organism protection.

Talk 32:

Title:
Chronic voluntary alcohol consumption alters promoter methylation and expression of Fgf-2 and Fgfr1 in a region-specific manner of the mouse brain

Authors:
Leonie Herburg (Institute of Neuroanatomy and Cell Biology, Hannover Medical School, Hannover), Mathias Rhein (Department of Psychiatry, Social Psychiatry and Psychotherapy, Hannover Medical School, Hannover), Sabrina Kubinski (Institute of Neuroanatomy and Cell Biology, Hannover Medical School, Hannover), Claudia Grothe (Institute of Neuroanatomy and Cell Biology, Hannover Medical School, Hannover); Herburg.Leonie@mh-hannover.de

Abstract:
Alcohol use disorder is a chronic and relapsing disorder, characterized by compulsive, heavy drinking and impaired ability to control alcohol intake. Recently, fibroblast growth factor 2 (FGF-2) and its main receptor FGFR1, have been reported to act as positive regulators of alcohol consumption as their inhibition leads to attenuation of alcohol intake.

We investigated whether alcohol alters DNA methylation of Fgf-2 and Fgfr1 and if such regulations correlate with alterations in mRNA transcription of these genes. To determine how chronic alcohol intake and its withdrawal affect promoter-specific DNA methylation of Fgf-2 and Fgfr1 direct bisulfite sequencing was used to analyze blood and brain tissues of wild-type mice receiving alcohol intermittently in a voluntary choice setup over six weeks.

We found for the Fgf-2 promotor, but nor for Fgfr1, a hypermethylation downstream of the ATG start codon in the alcohol-treated group compared to water-drinking control animals. However, both the Fgf-2 and Fgfr1 gene showed differentially methylated CpG positions, which are located within binding sites for transcription factors. On mRNA level, Fgf-2 and Fgfr1 were significantly decreased in alcohol-receiving mice compared to control animals and that this effect was specific to the dorsomedial striatum, a brain region that is associated with the reward system

Overall, our data showed alcohol-induced alterations in both, mRNA expression and methylation pattern of Fgf-2 and Fgfr1. Furthermore, those alterations are specific to certain regions of the reward system and might bear the potential for future pharmacological interventions.

Talk 33:

Title:
Regional heterogeneity in human meninges

Authors:
Elise Vanrkriekelsvenne (, Universitätsmedizin Rostock, Rostock), Markus Kipp (, Universitätsmedizin Rostock, Rostock), Sarah Joost (, Universitätsmedizin Rostock, Rostock); elise.vankriekelsvenne@med.uni-rostock.de

Abstract:
The central nervous system is surrounded by three layers of meninges: The outermost dura mater, the innermost pia mater, and the arachnoid mater The meninges are large and complex structures that are, according to recent studies, involved in the recruitment of immune cells, but regional heterogeneity of human meninges has never been systematically addressed. We hypothesize that the meninges, especially the arachnoid mater, show region-specific heterogeneity in morphology and gene expression.

We collected post-mortem meningeal material of 17 distinct regions of the central nervous system from 8 body donors. Overall meningeal morphology and spatial distribution of blood vessels within the meninges were assessed by (immuno-)histochemical analysis. Gene expression was analyzed by next-generation sequencing in an explorative approach for differential gene expression and by quantitative real-time PCR of specific marker genes of meningeal fibroblasts and blood vessels.

Histologic sections revealed distinct morphological differences in thickness and structure of meninges depending on the sampled region. Meningeal blood vessels showed a higher density and were of a larger caliber within the sulci than on top of the gyri. Preliminary results of next-generation sequencing analysis pointed toward a strong genetic divergence of the meningeal tissue spanning the cisterna basalis when compared to the other brain regions. Further results of gene expression analysis were still pending at the time of abstract submission.

Our findings point to a distinct regional heterogeneity of meninges on the histological and gene expression level. Further studies will concentrate on the relevance of regional heterogeneity on a functional level.

Talk 34:

Title:
Food restriction in early adolescent mice induces hyperactivity, amenorrhea and food-anticipatory activity

Authors:
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Abstract:
Anorexia Nervosa (AN) is a debilitating psychiatric disorder characterized by the relentless pursuit of thinness, leading to severe emaciation, hyperactivity and amenorrhea. Although the underlying pathophysiology of AN is unknown, recent results of our lab suggest cerebral involvement. To what extent AN-related symptoms are due to a primary neuronal dysfunction or secondary due to food restriction is currently unknown. In this project we aim to understand the relevance of severe food restriction on AN-related symptoms.

Starvation was induced by restricting food access of either early adolescent or adolescent (4/8 weeks old) mice to 40% of their baseline food intake until a 20% weight reduction was reached (acute starvation). To mimic chronic starvation, weight was maintained for another 2 weeks. Amenorrhea was determined by histological examination of vaginal smears. Locomotor activity was investigated using running wheel sensors, whereas a change in circadian rhythm-related activity was measured using a newly developed, infrared-sensory based home-cage tracking system (Goblotrop®).

All cohorts showed an increase in locomotor activity up to 4 hours before food presentation (i.e. food-anticipatory activity; FAA). Whereas amenorrhea was present in all groups except in early adolescent acutely starved animals, hyperactivity exclusively was found in early adolescent groups. Of note, adolescent chronically starved mice showed a decrease in circadian rhythm-related activity at night.

Chronic starvation in early adolescent mice most closely mimics AN-related behavioral changes. It appears that hyperactivity, amenorrhea and FAA are direct consequences of food restriction.

Talk 35:

Title:
Structural connectivity differences reflect microstructural heterogeneity of the human insular cortex

Authors:
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Abstract:
The human insula is a hub for multifunctional integration, which consists of 16 microstructural areas. This raises the question whether this diversity is reflected by specific structural connectivity patterns? Since microstructural parcellations could provide a framework for functional interpretation of connectome data, this study aims to disentangle functionally relevant network integration of different insular areas in terms of structural connectivity.

Probabilistic, anatomically constrained streamline tractography based on constrained spherical deconvolution was performed on diffusion images from 914 subjects from the 1000BRAINS cohort. Microstructurally defined areas from the Julich-Brain Atlas and a subcortical parcellation were used as regions of interest. The resulting connectome displayed the connectivity strength between insular areas and other areas/subcortical regions. Cluster analysis was performed to determine connectivity differences and similarities between insular areas.

Areas of the dorsal anterior insula are particularly connected to the ventrolateral prefrontal cortex, while areas of the ventral anterior insula are primarily linked to the orbitofrontal cortex. Areas of the dorsal posterior insula are mainly connected to the parietal lobe and the auditory cortex, whereas areas of the middle insula show broad connections to frontal, temporal and occipital regions.

Our results demonstrate systematic differences in connectivity between areas of the posterior, middle, ventral anterior, and dorsal anterior insula, reflecting functional differentiation, such as the anterior insula's involvement in higher cognitive functions and the posterior insula's involvement in sensory processing. Therefore, the microstructural parcellation provides a suitable mediator to integrate connectome data of the human insular cortex into a structural-functional framework.

Talk 36:

Title:
Lipocalin 2 attenuates oligodendrocyte loss and immune cell infiltration in mouse models for multiple sclerosis

Authors:
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Abstract:
Multiple sclerosis (MS) is a CNS disease characterized by degenerative and inflammatory processes. Various secreted mediators are involved in the complex interplay of degeneration and innate immunity on one hand and peripheral adaptive immunity on the other hand. The secreted protein Lipocalin 2 (LCN2) is involved in cellular stress responses and an inflammatory modulator in a variety of pathologies. Although elevated intrathecal levels of LCN2 have been reported in MS patients, their functional role in MS pathogenesis is widely unknown.

We identified a subpopulation of reactive astrocytes as a source of LCN2 in human MS lesions and in MS animal models. The functional role of LCN2 for autoimmune and degenerative aspects was investigated in three MS mouse models including wild type and Lcn2-/- mouse strains. The EAE model reflects primary autoimmunity, whereas the cuprizone model represents selective oligodendrocyte loss and demyelination. In addition, we included a combinatory Cup/EAE model in which primary cytodegeneration is followed by the formation of inflammatory lesions within the forebrain.

While in the EAE model, the clinical phenotype and histopathology was comparable in between the two mouse strains, we found an increased loss of oligodendrocytes in the brains of cuprizone intoxicated Lcn2-/- animals. In the Cup/EAE model, the number of inflammatory perivascular cuffs, IBA1-positve monocytes/microglia and T-cells were increased in Lcn2-/- animals.

Together, our results highlight LCN2 as a potentially protective molecule in MS lesion formation which might be able to limit loss of oligodendrocytes immune-cell invasion.

Talk 37:

Title:
Synaptic alterations drive motor neuron degeneration in ALS

Authors:
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Abstract:
Amyotrophic lateral sclerosis (ALS) is a progressive fatal neurodegenerative disease, which mainly affects neurons belonging to the corticospinal tract. To date, despite the extensive efforts in trying to dissect the specific pathomechanisms underlying the loss of this specific neuronal population, the specific contribution of the synaptic microenvironment to the neurodegenerative processes observed in this disease have still to be clarified.
In this project, we aimed at dissecting the synaptic aberrations occurring in ALS-related motor neurons with the final goal of identifying novel strategies for contrasting disease progression.

We isolated the synaptosomal fraction from post-mortem spinal cord samples and cultured hiPSC-derived motor neurons, both derived from ALS patients and healthy controls. These samples were analysed by MS proteomics and combined to phospho-proteomics data to highlight downstream aberrations for pharmacological targeting.

Our combinatorial approach based on the integration of human synaptic- and phosphoproteomics highlighted a deep impairment in the molecular machinery involved in the release of synaptic vesicles. These data were backed up by MEA data showing progressive loss of electrophysiological properties in ALS-related cultures. Notably, increasing the levels of synaptophysin proved neuroprotective in ALS by reducing the malactivation of the apoptosis-related transcription factor cJun.

In contrast to the hyperexcitability theory, our data clearly highlight that altered synaptic composition triggers activity abnormalities and drives disease progression in ALS. We conclude that restoration of the synaptic proteome and firing properties might represent a valid entry point to contrast motor neuron degeneration in ALS.

Talk 38:

Title:
Desmosomal hyper-adhesion protects keratinocytes from pemphigus autoantibody-induced loss of intercellular adhesion and partially blocks direct inhibition of desmoglein interactions

Authors:
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Abstract:
During differentiation, keratinocytes acquire a strong, hyper-adhesive state, in which desmosomal cadherins convert to a Ca2+-independent state. In the bullous autoimmune disease pemphigus vulgaris (PV), autoantibodies (PV-IgGs) against the desmosomal cadherins desmoglein (Dsg) 1 and 3 and desmocollin (Dsc) 3 modulate Dsg single molecule binding properties and cause loss of keratinocyte cohesion. As hyper-adhesion reduced effects of PV-IgGs, we here investigated the impact of hyper-adhesion on desmosomal cadherin single molecule binding properties in PV.

Keratinocyte dissociation assay, Immunofluorescence, Atomic force microscopy, Stimulated Emission Depletion (STED) microscopy

Indeed, hyper-adhesion reduced PV-IgG-induced loss of intercellular adhesion and Dsg depletion. AK23, a pathogenic Dsg3 antibody targeting the NH2-terminal extracellular domain (ECD) 1 induced direct inhibition under both adhesive states, although hyper-adhesive keratinocytes were protected from loss of intercellular adhesion. In contrast, both polyclonal PV-IgGs and a pathogenic antibody (2G4) targeting the membrane-proximal extracellular domain (ECD) 5 of Dsg3, induced direct inhibition under non-hyper-adhesive conditions only, suggesting that hyper-adhesion changes susceptibility to autoantibody-induced direct inhibition in an epitope dependent way. In accordance, STED experiments revealed reduced desmosomal cadherin (DC) clustering after treatment with PV-IgGs only in non-hyper-adhesive cells. Similarly, a membrane-proximal targeting Dsc3 antibody cause direct inhibition solely in non-hyper-adhesive keratinocytes. Importantly, no direct inhibition was observed for Dsg1 interaction under both adhesive states, although hyper-adhesion ameliorated PV-IgG-induced decrease in Dsg1 motility.

Taken together, the data show that hyper-adhesion reduces susceptibility to autoantibody-mediated direct inhibition of Dsg3 binding in an epitope-dependent way.

Talk 39:

Title:
CAP2-dependent actin dynamics controls myofibril differentiation and SRF activity during skeletal muscle development in mice

Authors:
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Abstract:
The highly structured complex of myosin and actin filaments is essential for the coordinated contraction of muscle fibers. In order to achieve this function, actin filaments have to build up and rebuilt dynamically during muscle development. The need to elucidate these mechanisms arises from the finding that a large number of human myopathies are associated with defects in the actin cytoskeleton. Previous studies identified the transcription factor serum response factor (SRF) as a major regulator of skeletal muscle development. In a feedback mechanism, SRF is activated actin-dependently and in turn controls the expression of actin and actin-regulatory proteins. Cyclase-associated proteins (CAPs) are a family of actin regulators with largely unknown physiological functions. We reported a function for CAP2 in regulating myofibril differentiation and SRF activity in mice.

CAP2 functioning was investigated in systemic KO mice on the level of animal behavior, morphology, molecular biology and cell culture.

CAP2 controls the remodeling of actin filaments in developing skeletal muscles and is therefore essential for the differentiation of muscle fibers. CAP2 KO mice developed structural changes in skeletal muscles, deficits in motor functions and muscle weakness, reflecting symptoms of human myopathies. Additionally, loss of CAP2 in mouse embryonic fibroblasts lead to disturbed SRF activity. Specifically, we found that CAP2 changed subcellular distribution of the SRF trans-activator myocardin-related transcription factor (MRTF) which lead to impaired SRF-mediated gene expression.

CAP2-dependent actin dynamics controls myofibril differentiation and SRF activity during skeletal muscle development. Given that skeletal muscle differentiation is similar in humans, we propose a crucial function for CAP2 in human myofibril differentiation.

Talk 41:

Title:
Microglia mediate synaptic plasticity induced by transcranial magnetic stimulation

Authors:
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Abstract:
Microglia are the resident immune cells of the brain. Their role in physiological processes such as the regulation of neural excitability and plasticity is well recognized. Here, we investigated the role of microglia in synaptic plasticity induced by 10 Hz repetitive magnetic stimulation (rMS), a clinically employed non-invasive brain stimulation technique.

Microglia were depleted from mouse organotypic tissue cultures with PLX3397 (Pexidartinib). Whole-cell patch-clamp recordings, confocal microscopy, immunohistochemistry, protein and transcriptome analyses were used to assess rMS-induced structural and functional plasticity of CA1 pyramidal neurons in the presence or absence of microglia.

The expression of excitatory synaptic plasticity in CA1 pyramidal neurons after 10 Hz rMS required the presence of microglia. Although rMS did not alter the morphology or the dynamics of microglia, an increased production and secretion of microglia-related cytokines were observed 3 h after stimulation. Concordantly, substitution of these cytokines in microglia-depleted tissue cultures rescued the expression of rMS-induced synaptic plasticity.

We conclude that clinically employed non-invasive electromagnetic brain stimulation affects synaptic plasticity by modulating the production and release of microglial cytokines.
Supported by DFG (SFB/TRR 167) and the MOTI-VATE Program, Faculty of Medicine, University of Freiburg.

Talk 42:

Title:
Cortactin is required for endothelial barrier enhancement through cAMP-mediated Rac1 activation

Authors:
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Abstract:
Endothelial barrier function is modulated via cell junction dynamics and actin cytoskeleton remodelling. Both are controlled by a number of molecules e.g., cAMP, small GTPases and actin-binding proteins, such as Cortactin (Cttn). The latter is involved in regulating endothelial permeability and cell contact integrity both in vivo and in vitro. However, little is known about the role of Cttn in cAMP-mediated endothelial barrier integrity.

Trans-Endothelial Resistance (TER), Western Blot, PCR, Immunostaining, G-LISA, cAMP ELISA

Using TER measurements, we confirmed that the loss of Cttn interferes with the basal integrity of the monolayer. The effect was associated with fragmented staining of VE-cadherin and β-catenin; however, claudin-5 protein level was significantly increased. In WT-cells, augmentation of cAMP by Forskolin (F) and Rolipram (R) increased TER and induced profound signal intensity for β-catenin, but not for VE-cadherin. In contrast, Cttn-KO cells did not respond to F/R with higher TER despite augmented intracellular levels of cAMP, indicating disturbed cAMP-mediated downstream signalling. Therefore, we analysed the activity of Rac1 and RhoA GTPases. As expected, WT-cells showed significantly enhanced Rac1 activation upon F/R treatment. Interestingly, this effect was abolished in Cttn-KO cells. In line with this, we observed that the cAMP-independent simultaneous direct activation of Rac1 and RhoA via CNO4 significantly increased TER in both cell lines.

Our data strongly suggest that Cttn is required for cAMP-mediated endothelial barrier tightening via Rac1 activation.

Talk 43:

Title:
Succinate enhances mucociliary clearance in the murine tracheal epithelium by triggering acetylcholine release from chemosensory cells

Authors:
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Abstract:
: Solitary cholinergic chemosensory cells (SCCC) are rare tracheal epithelial cells. They express a wide range of GPCR including taste receptors and their signaling machinery (TRPM5) and the acetylcholine (ACh) producing enzyme ChAT. We here investigated if tracheal SCCC are equipped with the succinate receptor SucnR1 and if succinate triggers innate defense mechanism through activation of tracheal SCCC.

Expression of SucnR1 was analyzed in isolated tracheal epithelial cells by RT-PCR and by analyzing existing single cell sequencing data sets. Particle transport speed (PTS) and ciliary beat frequency (CBF) were examined. Ussing chamber experiments were performed to investigate ion transport processes.

Within the tracheal epithelium, SucnR1 was exclusively expressed by SCCC. Succinate increased CBF and, consequently, PTS. This effect required Sucnr1 (lost in SucnR1-/--mice) and SCCC (lost in Pou2f3-/--mice). In mice with SCCC-specific deletion of ChAT and in the presence of the muscarinic antagonist atropine, the effect of succinate was nearly abolished. In Ussing chamber experiments, succinate induced a sharp increase in ion flux across the tracheal epithelium, which was lost in SucnR1-, and reduced in Pou2f3- and Trpm5-deficient mice. The succinate-induced increase could be abolished by blocking cholinergic signaling and by the general chloride channel inhibitor NPPB. Furthermore, the gap junction blocker Gap27 abolished both, the succinate-induced increase in PTS and ion secretion.

Succinate activates SCCC by binding to SucnR1, thereby triggering release of ACh. This increases ciliary activity and induces secretion of chloride ions into the periciliary fluid, likely trough further spread via gap junctions.

Talk 44:

Title:
Activation of tracheal epithelial brush cells leads to protective immune responses via stimulation of brush cell approaching sensory nerves

Authors:
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Abstract:
Tracheal epithelial brush cells (BC) play an important role in eliciting innate immune processes, e.g., an increase in mucociliary clearance. BC are equipped with a functional bitter taste signalling cascade, including the transient receptor potential melastatin 5 (Trpm5) channel. Here, we characterise the mechanisms by which BC-activation elicits neurogenic inflammation and combats Pseudomonas aeruginosa (PA) infections.

After tracheal in vivo BC-stimulation with 1 mM denatonium we measured neuropeptide release in tracheas by ELISA, quantified nerve fibre volumes and contacts between BC and nerves fibres in tracheal whole mount preparations, analysed Evans blue (EB) extravasation and neutrophil numbers and characterised immune cell and cytokine profiles after PA infection using FACS and ELISA.

BC-stimulation induced a Trpm5-dependent release of calcitonin gene-related peptide (CGRP) and substance P (SP) in mouse tracheas. Supportively, BC-stimulation decreased nerve fibre volumes and contacts between BC and CGRP+ or SP+ nerve fibres. Treatment of mice with CP96345 (neurokinin 1 receptor inhibitor), CGRP8-37 (CGRP receptor antagonist) or mecamylamine and atropine (cholinergic receptor blockers) inhibited the EB extravasation and neutrophil recruitment observed after BC-stimulation. Ablation of sensory nerves using a Trpa1-DTR mouse model abolished BC-mediated EB extravasation and neutrophil recruitment. Four hours after PA infection, we observed lower numbers of neutrophils, monocytes and alveolar macrophages in the respiratory tract of Trpm5-/- mice. Additionally, several cytokines, e.g., IL-1a, G-CSF and KC were increased in blood samples of wild-type but not Trpm5-/- mice.

BC-mediated activation of cholinergic Trpa1+ sensory nerve fibres induces protective neurogenic inflammation, essential to combat infections.

Talk 45:

Title:
Lipofibroblasts in structurally normal, fibrotic and emphysematous human lungs

Authors:
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Abstract:
Lipofibroblasts are characterized by a substantial amount of lipid bodies and the localization within the alveolar interstitium. They are thought to contribute to surfactant synthesis, play an important role in alveolar development and seem to be implicated in fibrotic remodeling of the lung, indicating a therapeutic potential of these cells. While lipofibroblasts are a common cell type in rodents, their occurrence in the human lung has been controversially discussed.

To investigate the presence of lipofibroblasts in the human lung, lung tissue from the periphery of tumor resections (structurally normal) as well as from explanted fibrotic and emphysematous lungs was analyzed with i) fluorescence microscopy and ii) a correlative approach combining antibody-based identification of cells at a low resolution (light microscopy) with subsequent ultrastructural characterization at a high resolution (electron microscopy).

Cells positive for the lipofibroblast marker ADFP were detectable in normal, fibrotic and emphysematous lungs. The ADFP+ cells also exhibited vimentin marking them as cells of mesenchymal origin, but showed no costaining with the macrophage marker CD68, the alveolar type 2 cell marker pro-SP-C or the myofibroblast marker ACTA2. At the ultrastructural level, ADFP+ cells were localized in the alveolar interstitium, were located in close connection with collagen fibrils as a univocal characteristic of fibroblasts, and possessed intracellular lipid droplets. Moreover, another staining approach with the lipid-marker Sudan Black also confirmed the presence of lipid-droplet-containing cells in the respective lung/lung compartments.

Thus, lipofibroblasts are present in the structurally normal as well as the fibrotic and emphysematous human lung.

Talk 46:

Title:
The ultrastructural heterogeneity of lung surfactant revealed by serial section electron tomography: Insights into the 3-D architecture of human tubular myelin

Authors:
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Abstract:
Tubular myelin (TM) is known as a unique “lattice-like” lung surfactant subtype found in the hypophase of the alveolar lining layer. Although initial descriptions by electron microscopy (EM) were already published in the 1950s, a uniform morphological differentiation from other intra-alveolar surfactant subtypes is missing and potential structure-function relationships remain enigmatic. Technical developments in volume EM methods now allow a detailed reinvestigation.

We examined ultrathin sections of humanized SP-A1/SP-A2 coexpressing mouse and human lung samples by conventional transmission EM und combined these obtained 2-D information with 3-D analyses of single- and dual-axis electron tomography of serial sections, providing high z-resolution in a range of a few nanometers and extended z-volumes of up to 1 µm.

Our investigation reveals that TM constitutes a heterogeneous surfactant organization mainly comprised of distorted parallel membrane planes with local intersections distributed all over the TM substructure. Besides various polygons, these intersecting membrane planes form the well-known 2-D "lattice", respectively 3-D quadratic tubules. In many analyzed spots of human TM, the tubules appear to be less abundant than also observed nonconcentric 3-D lamellae.

The additional application of serial section electron tomography to conventional transmission EM demonstrates a high heterogeneity of TM membrane networks, indicating dynamic transformations between its substructures. Our method provides an ideal basis for further in and ex vivo ultrastructural analyses of surfactant under various conditions at nanometer scale in all dimensions.

Talk 47:

Title:
Alveolarization and microvascular development in the preterm rabbit and term mouse hyperoxia model of bronchopulmonary dysplasia

Authors:
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Abstract:
Bronchopulmonary dysplasia (BPD) is a developmental disorder occurring mainly in infants born prematurely. Among others, it is characterized by disrupted alveolarization and microvascular maturation. According to the vascular hypothesis of BPD, the adverse changes of the vasculature precede the interruption of alveolarization. We used two established animal models making use of important sequelae of human BPD, namely preterm birth and hyperoxia, to test their effects on alveolar and vascular development, respectively.

Term born mice were exposed to hyperoxia (85% O2) or normoxia. At 7, 14 or 21 days of hyperoxia mouse lungs were fixed by vascular perfusion and prepared for stereological investigation. Rabbit pups were born by cesarean section three days before term birth and exposed for 7 days to hyperoxia (95% O2) or normoxia. Term born rabbits exposed to normoxia for 4 days were used as control. Rabbit lungs were fixed by vascular perfusion and prepared for stereological analysis.

In the mouse, hyperoxia caused a developmental impairment of the alveolar capillaries already at 7 days whereas the changes of alveolarization became significant at 14 days supporting that the vascular impairment precedes the disruption of the alveolarization. In the rabbit, preterm birth had a significant adverse effect on alveolarization but hyperoxia had a more pronounced effect on the development of capillaries.

In conclusion, the present data support the vascular hypothesis of BPD under hyperoxia but preterm birth seems to have a greater effect on alveolarization than hyperoxia.

Talk 48:

Title:
EGFR inhibition led ROCK activation enhances desmosome assembly and cohesion in cardiomyocytes

Authors:
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Abstract:
Arrhythmogenic cardiomyopathy (AC) is a familial heart disease partly caused by impaired desmosome turnover. Thus, stabilization of desmosome integrity may provide potential new treatment options. In addition, desmosomes, apart from cellular cohesion, provide the structural framework of a signaling hub. Here, we investigated the role of the epidermal growth factor receptor (EGFR) in cardiomyocyte cohesion.

Dissociation assays, Immunostaining, Western blot, siRNA knockdown of proteins, Calcium switch assays, Fluorescence recovery after photobleaching (FRAP), immunoprecipitations, PamGene kinase assay, STED, and Atomic force microscopy experiments (AFM) were applied in either HL-1 cells or cardiac slices from Wild-type(WT) and plakoglobin knockout (Jup-/-) mouse as AC model.

EGFR was upregulated in the hearts of Jup-/- mice. Dissociation assays in HL-1 cells and murine cardiac slice cultures showed that EGFR inhibition led to increased cardiomyocyte cohesion. Immunoprecipitation showed an interaction of EGFR, DSG2 and DP, indicating that altered EGFR signaling might affect desmosomes. Immunostaining and AFM revealed enhanced DSG2 localization and binding at cell borders upon EGFR inhibition. Enhanced area composita length and desmosome assembly were observed upon EGFR inhibition, confirmed by enhanced DSG2 and desmoplakin (DP) recruitment to cell borders. Erlotinib, an EGFR inhibitor, activated ROCK. Erlotinib-mediated desmosome assembly and cardiomyocyte cohesion were abolished upon ROCK inhibition.

We conclude that inhibiting EGFR, thereby enhancing desmosome assembly via ROCK stabilizes desmosome integrity and cardiomyocyte cohesion. We believe our study is a first step that paves the way for future studies targeting EGFR inhibition or ROCK activation by erlotinib as a treatment option for AC.

Talk 49:

Title:
Inhibition of the integrin-αVβ6/TGFβ cascade as novel treatment strategy for Arrhythmogenic Cardiomyopathy

Authors:
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Abstract:
Arrhythmogenic Cardiomyopathy (ACM) is characterized by progressive loss of cardiomyocytes with fibrosis, systolic dysfunction and life-threatening arrhythmias. Mutations in genes of the desmosomal adhesion complex are the major cause, but the underlying mechanisms are not well understood. Accordingly, only symptomatic treatment is available. Here, we investigate a new strategy to ameliorate fibrosis in an ACM model.

We applied the recently established DSG2-W2A mouse line, an ACM model with abrogated desmosomal adhesive interface. The disease progression in this model was evaluated by echocardiography, electrocardiogram and histological techniques. To address the mechanisms, RNA sequencing, structured illumination microscopy, immunostaining, and protein separation were performed. The relevance of the identified pathway was confirmed in vivo with the small molecule EMD527040.

Mutant mice develop a phenotype mimicking the characteristics of ACM, including cardiac fibrosis, impaired output function and arrhythmia. The mutation induces a disruption of the intercalated disc with deregulation of integrin-αVβ6. Subsequent TGF-β signaling was identified as driver of cardiac fibrosis. Accordingly, blocking integrin-αVβ6 activity via EMD527040 led to reduced expression of pro-fibrotic markers and reduced fibrosis formation in mutant animals in vivo.

We show that the DSG2-W2A model fulfils the clinical criteria to establish the diagnosis of ACM. Mechanistically, deregulation of integrin-αVβ6 and TGF-β signaling was identified as a central step towards fibrosis. A pilot in vivo drug test revealed this pathway as promising target to ameliorate fibrosis. This highlights the value of this model to discern mechanisms of cardiac fibrosis and to identify and test novel treatment options for ACM.

Talk 50:

Title:
Developmental cell death of cortical projection neurons is regulated by a Bcl11a/Bcl6/Foxo1-dependent pathway

Authors:
Christoph Wiegreffe (Institute of Molecular and Cellular Anatomy, Ulm University, Ulm), Tobias Wahl (Institute of Molecular and Cellular Anatomy, Ulm University, Ulm), Natalie Joos (Institute of Molecular and Cellular Anatomy, Ulm University, Ulm), Jerome Bonnefont (Institut de Recherche Interdisciplinaire en Biologie Humaine et Moléculaire (IRIBHM), and ULB Neuroscience Institute (UNI), Université Libre de Bruxelles (ULB), Brussels), Pentao Liu (School of Biomedical Sciences, The University of Hong Kong, Hong Kong), Stefan Britsch (Institute of Molecular and Cellular Anatomy, Ulm University, Ulm); christoph.wiegreffe@uni-ulm.de

Abstract:
Developmental neuron death plays a pivotal role in refining organization and wiring during neocortex formation. Aberrant regulation of this process results in neurodevelopmental disorders including impaired learning and memory. Underlying molecular pathways are incompletely determined.

Loss of Bcl11a in cortical projection neurons induces pronounced cell death in upper-layer cortical projection neurons during postnatal corticogenesis. We use this genetic model to explore genetic mechanisms by which developmental neuron death is controlled.

We find Bcl6, previously shown to be involved in the transition of cortical neurons from progenitor to postmitotic differentiation state to provide a major checkpoint regulating neuron survival during late cortical development. We show that Bcl11a is a direct transcriptional regulator of Bcl6. Deletion of Bcl6 exerts death of cortical projection neurons. In turn, reintroduction of Bcl6 into Bcl11a mutants prevents induction of cell death in these neurons. Finally, we show Foxo1 to be downregulated in both, Bcl6 and Bcl11a mutant cortical projection neurons. Normalization of Foxo1 expression is sufficient to suppress increased apoptosis in Bcl11a mutant cortical projection neurons suggesting Foxo1 to participate in the regulation of developmental cell death in cortical projection neurons during postnatal neocorticogenesis.

Together, our data identify a novel Bcl11a/Bcl6/Foxo1-dependent molecular pathway in regulation of developmental cell death during corticogenesis.

Talk 51:

Title:
Teashirt1 controls the differentiation and layering of embryonic olfactory bulb granule cell neurons

Authors:
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Abstract:
The olfactory bulbs (OBs) relay odor information to the olfactory cortex. Formed initially as evaginations of the rostral telencephalon, they become populated by neurons born locally within the OB ventricular zone, or more distally in the telencephalon. This latter population moves tangentially in a rostral direction before migrating radially within the OBs, where it matures into granule and periglomerular cell interneurons. One gene expressed in the developing and adult OB and rostral forebrain is Teashirt-1 (Tshz1), encoding a zinc-finger homeodomain factor, previously shown by us to be required for normal olfaction in mice and humans (Ragancokova et al., J. Clin. Invest. 2014), most likely through Tshz1-dependent expression of Prokr2, whose mutation causes human Kallmann syndrome.

We generated gene-targeted Tshz1-/- mice in order to characterize the expression and function of Tshz1 in OB development and maturation.

We assign to Tshz1 essential roles in the radial migration and molecular specification of early-born, distally generated granule cells. Such cells arrived within the OBs of Tshz1-/- mutant mice, but distributed aberrantly within the radial dimension, forming discrete aggregates in which mutant Tshz1GFP+ cells failed to express the zinc-finger transcription factors Sp8 and Sall3 and remained immature as defined by the loss of expression of the markers Rbfox3/NeuN, GAD67, GABA, tyrosine hydroxylase and a panel mRNA transcripts identified in microarrays.

Tshz1 is critical for OB development and function. Furthermore, our studies indicate that soluble and/or cell surface molecules produced by the Tshz1+ outer granule cells regulate the distribution of other neuronal populations within the OB.

Talk 52:

Title:
Weakly-supervised spatio-temporal learning and causal analysis reveals anatomical basis of neuropathic pain

Authors:
Hongwei Zheng (Heidelberg University, Institute of Anatomy and Cell Biology, Heidelberg), Vijayan Gangadharan (Heidelberg University, Institute of Pharmacology and Max Planck Institute for Brain Research Frankfurt am Main, Heidelberg), Moritz Helmstädter (Max Planck Institute Frankfurt am Main, Max Planck Institute for Brain Research Frankfurt am Main, Frankfurt am Main), Rohini Kuner (Heidelberg University, Institute of Pharmacology, Heidelberg), Thomas Kuner (Heidelberg University, Institute of Anatomy and Cell Biology, Heidelberg); hongwei.zheng@uni-heidelberg.de

Abstract:
Despite recent breakthroughs in understanding central nervous system-based mechanisms of neuropathic pain (NP), the anatomical mechanisms underlying NP of damaged peripheral nerve fibers remain unclear. We developed a novel machine learning and causal analysis approach using in-vivo two-photon imaging and serial-block-face-scanning-electron-microscopy (SBEM) multi-scale datasets. Their combination with behavioral analyses provides prime access to identify structural alterations in the context of behavior.

The approach consists of several steps: acquisition, preprocessing, registration, tracing and modeling of fibers in a blinded manner. First, we acquired genetically labelled populations of fibers that sense noxious stimuli (nociceptors) and gentle touch (low-threshold afferents) peripherally in the skin of entire distal phalanges (I-III-V) in parallel to behavioral analyses for up to 10-months using the spared-nerve-injury paradigm. Second, we traced fibers changes with high accuracy in a weakly-supervised Bayesian-ensemble-learning approach using 35TB raw data. The SBEM ultrastructure of YFP-positive A-fibers and mGFP-expressing nociceptor-free-nerve-endings were identified in fully reconstructed Meissner corpuscles (MC) showing nano-scale innervations.

We found that C-fibers sprouted from the uninjured sural territory into the tibial territory after a complete loss of tibial fibers. The C-fibers did not form the typical intraepidermal free nerve endings, but now were closely associated with MC and started to develop NP in parallel. Full EM reconstructions of MC revealed that C-fibers were meandering through the MC and contacting with the sheath cells directly.

Hence, C-fibers replace A-fibers after reinnervation, thereby transducing gentle touch into a sensation of pain, a novel category of pain that we refer to as reinnervation NP.

Talk 53:

Title:
R-Spondin1-LGR5/6 signaling - a new player in neuronal differentiation in the enteric nervous system?

Authors:
Peter Neckel (Institute of Clinical Anatomy and Cell Analysis, University of Tübingen, Tübingen), Melanie Scharr (Institute of Clinical Anatomy and Cell Analysis, University of Tübingen, Tübingen), Simon Scherer (Department of Pediatric Surgery, University Children's Hospital, Tübingen), Bernhard Hirt (Institute of Clinical Anatomy and Cell Analysis, University of Tübingen, Tübingen); peter.neckel@uni-tuebingen.de

Abstract:
Neural progenitor cells from the enteric nervous system (ENS) are a potential source for cell-replacement therapies. Yet, the regulation of this ENS-progenitor cell pool remains poorly characterized, especially its high proliferative capacity in vitro despite its quiescent state in vivo. Our previous studies indicate an extensive involvement of the Wnt-signaling cascade in ENS-progenitor proliferation. Here, we hypothesize that the Wnt-regulator R-Spondin1 drives enteric neuronal differentiation via LGR5/6-receptors, extending the functions of the Wnt-regulatory-network in the ENS.

We investigated the influence of R-Spondin1 on murine and human ENS-progenitors using BrdU-incorporation, immunohistochemistry, Western blot, qRT-PCR, and protein phosphorylation profiling. We used FACS analysis and single-nuclear-RNA-sequencing to stratify the human ENS-progenitor cell pool.

Here we present sound evidence that R-Spondin1 significantly increases the neurogenic potential of murine and human ENS-progenitors. Surprisingly, this was paralleled by a reduced rate of proliferation in ENS cells expressing the R-Spondin-receptor LGR5. Instead, we found that LGR5/6 was increasingly expressed over the course of neuronal differentiation in vitro and in vivo. This was underpinned by FACS-experiments in human ENS-progenitors. Mechanistically, R-Spondin1 stimulation led to a diverse activation of non-canonical Wnt pathways suggesting an elaborate regulatory network of ENS-progenitor differentiation.

Unlike in other adult stem- and progenitor cells niches, R-Spondins are likely to drive neuronal differentiation in ENS-progenitors. Thus, the Wnt-regulatory-network is deeply involved in the cellular homeostasis of the ENS, potentially contributing to the quiescent state of ENS-progenitors in vivo. Our results may therefore pave the way to unleash the regenerative capacity of the mature ENS for future therapies.

Talk 54:

Title:
Paradoxical effects of acute ethanol exposure on neuronal morphology

Authors:
Niklas Schneider (Functional Neuroanatomy, Heidelberg University, Heidelberg), Sophie Lugani (Functional Neuroanatomy, Heidelberg University, Heidelberg), Chris Strahle (Functional Neuroanatomy, Heidelberg University, Heidelberg), Sidney Cambridge (Dr. Senckenbergische Anatomie, Institut für Anatomie II, Goethe University Frankfurt, Frankfurt am Main); cambridge@med.uni-frankfurt.de

Abstract:
The effects of acute and chronic ethanol exposure on neurons are substantial including significant molecular, cellular, and morphological changes. We previously showed with longitudinal two-photon imaging that a single dose of ethanol to alcohol-naive mice increased spine turnover in cortical neurons in vivo, while not affecting the overall spine density.

Conversely, we report here that ethanol induced a net spine increase in dissociated hippocampal neurons in vitro and net spine decrease in acute ex vivo hippocampal slices.

Such paradoxical morphological responses have been reported also with other stimuli, but nevertheless call into question the validity of extrapolating in vitro/ex vivo results to the in vivo situation of living animals. Molecularly, we find that overexpression of MAP6 suppressed the net spine increase and overexpression of AnkyrinG produced a net decrease following ethanol exposure in vitro.

These data suggest that the molecular composition of spines is criticial as the synaptic proteome dictates the unexpected morphological plasticity towards the ethanol stimulus and possibly other stimuli.

Talk 55:

Title:
Neocortical microcircuits for information processing

Authors:
Henning Sprekeler, Prof. Dr. (Modellierung kognitiver Prozesse, Fakultät Elektrotechnik und Informatik, Technische Universität Berlin, MAR 5-3, Marchstraße 23, 10587 Berlin)

Abstract:
Neocortical circuits display a puzzling variety of cell types with complex wiring patterns. While much is known about their anatomy and physiology, the rich set of interactions in these circuits makes it hard to assign specific functions to their various elements. I will try to provide a modeller's perspective on neocortical microcircuits, by attempting to link different anatomical circuit motifs to potential computational functions.

Talk 56:

Title
Intraindividual Cellular and Synaptic Heterogeneity of Human Layer 2-3 Pyramidal Neurons

Authors:
Henrike Planert (Institute of Neurophysiology, Charité - Universitätsmedizin Berlin, Berlin), Franz. X. Mittermaier (Institute of Neurophysiology, Charité, Berlin), Sabine Grosser (Institute of Integrative Neuroneuroanatomy, Charité, Berlin), Pawel Fidzinski (Neurology, Charité, Berlin), Ulf C. Schneider (Neurosurgery, Charité, Berlin), Helena Radbruch (Neuropatholgy, Charité, Berlin), Julia Onken (Neurosurgery, Charité, Berlin), Martin Holtkamp (Neurology, Charité, Berlin), Dietmar Schmitz (NWFZ, Charité, Berlin), Henrik Alle, (Institute of Neurophysiology, Charité, Berlin) Imre Vida (Institute of Integrative Neuroneuroanatomy, Charité, Berlin), Jörg R.P. Geiger (Institute of Neurophysiology, Charité, Berlin), Yangfan Peng (Institute of Neurophysiology, Charité, Berlin)

Abstract:
Neurons exhibit great heterogeneity in their physiological, anatomical and molecular properties even within established cell types. Detailed neuron taxonomies based on gene expression in human cortical layer 2-3 identified several pyramidal neuron subtypes. We investigated to what extent this diversity is reflected on the physiological level, how intrinsic heterogeneity of pyramidal neurons is associated with anatomical and synaptic diversity, and to what extent intrinsic and synaptic heterogeneity arise from interindividual difference.
We performed patch-clamp recordings of more than 1000 layer 2-3 pyramidal neurons and more than 1300 monosynaptic connections from the human temporal cortex as well as biocytin filling and post-hoc anatomical reconstruction of stained neurons. In resected tissue from 23 patients undergoing temporal pole surgery, up to ten neurons were recorded simultaneously, and we analyzed cellular electrophysiological and synaptic properties from up to 130 pyramidal neurons per individual patient.
We found large heterogeneity of pyramidal neuron cellular physiology. Hierarchical clustering of the high dimensional parameter space revealed distinct clusters of functionally similar neurons (e-types) which were present across individual patients. Scholl analysis of fully reconstructed neurons revealed differences in dendritic length between some e-types, and recurrent connectivity as well as synaptic properties were partially related to pre- or postsynaptic e-type. Linear Mixed Models further revealed severalfold larger intrapatient variability of intrinsic and synaptic properties as opposed to variability between patients.
Our results suggest large functional heterogeneity of pyramidal neuron cellular and synaptic properties, encompassing a generic organizational principle of cellular properties that is shared among individuals and associated with synaptic microcircuit organization and computations. Large intraindividual heterogeneity has important implications for computational capacity and for the design of studies relating intrinsic or synaptic

Talk 57:

Title
Inflammatory Bowel Disease caused by desmoglein 2 cytoplasmic truncation

Authors:
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Abstract:
Desmoglein (Dsg) 2 is together with Desmocollin (Dsc) 2 the principal desmosomal adhesion protein in enterocytes and is a crucial regulator of the intestinal epithelial barrier. Whether it‘s dysfunction contributes to pathogenicity in inflammatory bowel diseases (IBD), which rely on the integrity of intestinal barrier, has been suggested but remains elusive. Here, we investigate the role of a novel human Dsg2 nonsense mutation, recently identified in an IBD patient, in the regulation of intestinal barrier.

We generated an enterocyte-specific knock-in mouse model where Cre recombinase is driven by a villin promotor to induce a premature stop codon in the Dsg2 gene, leading to a truncation in the cytoplasmic domain of the protein (Dsg2MUT). Besides evaluating growth and survival of the Dsg2MUT mice and their littermates, we examined intestinal barrier properties by analyzing histological and immunofluorescent stainings and Western blots of intestinal tissue as well as an ex vivo FITC-Dextran intestinal permeability assay

While homozygous Dsg2MUT mice were phenotypically indistinguishable from their littermates at birth, they showed reduced growth and weight gain during postnatal development and died within two weeks after birth. Several gut segments in Dsg2MUT mice displayed severe dilatation and gas bloat when compared to wild-type mice. In addition, Dsg2MUT mice showed intestinal barrier defects and an upregulation of claudin 2.

Our results indicate that a truncation in the cytoplasmic domain of Dsg2 causes a severe intestinal barrier defect, which is lethal in mice, indicating that the mutation is pathogenic and contributes to IBD in humans.

Talk 58:

Title:
Gender Diversity in anatomical teaching

Authors:
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Abstract:
The worldview of most cultures is strongly heteronormative. This continues in medicine - we differentiate between men and women and often exclude non-binary or intergender people - likewise we rarely address trans people. The classic anatomy textbooks that we use to teach our students categorically exclude this topic, although it has been enshrined in German law since 2017 that there are other gender identities in addition to male and female.

In order to be able to continue to teach in accordance with the constitution, it is necessary that we expand our teaching to include these categories and deal with the topic appropriately. Under certain circumstances, this means that we have to review our own image of gender identity, precisely because many of our students naturally assume more than two genders due to their school education and contemporary influences.

In addition, medicine has been very guilty of non-binary people in the past and immeasurable damage has been caused by mistreatment and discrimination.

In order to prevent the continuation of the same, a fundamentally revised training of future doctors is necessary, and we can make a significant contribution to this.

Talk 59:

Title:
A comparison on the suitability of Ethanol-glycerin and Thiel fixation for undergraduate medical training

Authors:
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Abstract:
Anatomical dissection is known to serve as a beneficial tool in teaching gross anatomy, including postgraduate training. Different embalming techniques exist, resulting in different tissue properties. This study aimed at objectifying learning outcomes and student perceptions related to the use of two embalming techniques, namely ethanol-glycerin and Thiel-embalming.

First- and second-year students in medicine in courses on topographic anatomy between 2020 and 2022 participated in this study. Tag flag examinations were conducted on a voluntary basis following dissection of the respective anatomical region prior to student oral examinations. Six to ten numbered tags were marked in prosections of each region in ethanol- and Thiel-embalmed specimens. Following the examinations, the students were surveyed on their opinion regarding the suitability of the two embalming techniques with respect to preservation, color, fastness, tissue pliability and the suitability in preparing for their anatomy examinations.

Students consistently achieved higher scores in the tag exams for the thorax and abdomen regions with ethanol- when compared to Thiel-embalmed specimens. No benefit was found in Thiel-embalmed musculoskeletal tissues. For ethanol-embalmed tissues, preservation and suitability of tissues was rated higher, tissue pliability was rated higher for Thiel-embalmed tissues.

Ethanol-embalmed tissues seem to provide certain advantages for undergraduate students in recognizing viscera structures in line with student perceptions on tissue suitability. In consequence, the advantages reported for Thiel embalming for postgraduate study do likely not reflect best practice for novices.

Talk 60:

Title:
Evaluation of the (clinical) relevance of gross anatomical education for dental students and practicing oral surgeons in Berlin

Authors:
Anna Steinborn, Gerald Buhlheller, Irene Brunk (Institute of Integrative Neuroanatomy, Charité, Berlin)

Abstract:
Anatomy as a cornerstone of the preclinical dental curriculum is important for dental practitioners to perform invasive procedures and patience examination. This study investigates both, dental students' and oral surgeons' perceptions of the (clinical) relevance of the anatomical curriculum. The oral surgeons were chosen as the reference group due to their more invasive procedures in their daily practice in comparison to general dental practitioners. Therefore, we assumed the oral surgeons are best qualified to assess the clinical relevance of their anatomical education in retrospective.

Dental students from all semesters at the Charité-Universitätsmedizin Berlin (n=379) have been asked to participate in the designed survey, of which 322 completed the questionnaire (response rate 84,96 %). From the 128 oral surgeons working in Berlin who had been invited to participate in the survey 78 completed the questionnaire (response rate 60,9 %).

Dental students and oral surgeons expressed highly significant the great relevance of learning anatomy. In comparison to the dental students the oral surgeons stated retrospectively an even higher motivation to learn anatomy. The oral surgeons declared a stronger disapproval to the statement of learning anatomy just to pass the anatomical examination. Both surveyed groups stated highly significant the great relevance of learning neuroanatomy and disapproved to the reduction of the anatomical education to the head and neck anatomy. In comparison the oral surgeons disapproved stronger to the reduction of the demanded anatomical knowledge. The results of this survey display a broad consent regarding the dissection course and learning with body donors among students of dentistry and even significantly higher approval among the oral surgeons. Dentistry requires a lot of haptic skills. The group of dental students estimated the manual training effect of dissecting indifferent, while the oral surgeons appreciated the training effect significantly higher. Both dental students and oral surgeons confirmed the benefits of integrating medical imaging into the dissection course, whereas the students' approval decreased with increasing semester.
In summary, the results of the study show a great approval of the anatomical education, the teaching of anatomical regions beyond the head and neck anatomy and the dissection course by oral surgeons and students of dentistry. The higher motivational levels declared by the oral surgeons in retrospective can also be seen as a chance to elicit higher motivation in students by referring to clinical relations during their preclinical anatomical education.

Talk 61:

Title:
Body painting, ultrasound, clinical investigation and peer-teaching: a student-centered combined approach to enhance musculoskeletal anatomy learning integrated in a reformed medical curriculum

Authors:
Alessandro Bilella (Anatomy, Department of Medicine, University of Fribourg, Switzerland, Fribourg), Karl Link (Anatomy, Department of Medicine, University of Fribourg, Switzerland, Fribourg), Luis Filgueira (Anatomy, Department of Medicine, University of Fribourg, Switzerland, Fribourg), Elisabeth Eppler (University of Bern, Institute of Anatomy, Bern)

Abstract:
We present an optional course offered to 20 students/year to enhance learning of clinical-orientated anatomy of the musculoskeletal system of upper and lower limbs. Course aims were to incease knowledge in anatomy and physiology, and to offer a maximum of student-focused and hands-on learning by combining clinical investigation and ultrasound examination of the joints and studying physiological/anatomical conditions and pathological alterations of the musculoskeletal system.

The first three courses (2016-2018) were attended by 69 (41 females) 2nd-year medical students. The students followed an introduction by the teacher and then prepared two team-based presentations related to clinical anatomy and pathological condition. The students rotated through three activities: body painting, ultrasound, and clinical investigation under supervision of a faculty member or an experienced medical doctor. At the end of each session, the students reported on their own learning experience through a reflective diary. The course was finished with a written examination. The course was evaluated by a voluntary anonymous online questionnaire with special emphasis on the impact of the different learning compounds on the improvement of students' knowledge and understanding of the musculoskeletal system.

The analysis of the journal reports and answers in the questionnaire revealed that the students highly appreciated the course. The different course tasks and learning tools achieved a differential feedback reflecting students' preferences. All students returning the questionnaire recommended the course to their younger peers.

The voluntary course has been integrated into the reformed curriculum and is highly valued by male and female students.

Talk 62:

Title:
The Summer School of Anatomy-Based Sonography in Heidelberg (SASH): Opportunities and challenges in partnering with European universities

Authors:
Fabian Bauer (Anatomy, University of Heidelberg, Heidelberg), Leonie Hennigsen (Anatomy, University of Heidelberg, Heidelberg), Leo Nonnenbroich (Anatomy, University of Heidelberg, Heidelberg), Ansh Tandon (Physiology, Development and Neuroscience, University of Cambridge, Cambridge), Edward Wakefield (Physiology, Development and Neuroscience, University of Cambridge, Cambridge), Maurizio Vertemati (Biomedical and Clinical Sciences, Università degli Studi di Milano, Milan), David Kachlik (Anatomy, Charles University, II. Faculty of Medicine, Prague), Cecilia Brassett (Physiology, Development and Neuroscience, University of Cambridge, Cambridge), Ralph Nawrotzki (Anatomy, University of Heidelberg, Heidelberg); nawrotzki@uni-heidelberg.de

Abstract:
In 2016 SASH was established as a week-long hands-on ultrasound workshop for international medical students. The idea was to enable students to acquire ultrasound skills early in their training, and to improve their anatomical knowledge. This study examines the opportunities and challenges of implementing SASH at various European universities.

Since 2016, international students have learned techniques of abdominal ultrasound and FAST (Focused Assessment with Sonography in Trauma) in a 5-day summer school at Heidelberg. This course is unique in teaching students relevant skills and equipping them to be trainers for their peers. Over the years, participants returning to their home countries sought to launch their summer schools based on SASH. Here, we share our experiences learned through these collaborations.

Around 60 students from multiple countries have attended SASH over the past years, and 12 students have subsequently received further tutor training at Heidelberg. “Sister” summer schools include CamSASH, which will take place in August 2022 at the University of Cambridge, and SASH at the Universities of Prague and Milan. These collaborations offer opportunities for transnational sharing and exchanging ideas, which have resulted in the improvement of the course handbook. Challenges – in implementation and continuation – include linguistic and logistical issues, necessitating good translation and editing, selecting participants, and acquiring devices which are not always accessible in preclinical departments.

As ultrasound becomes increasingly important for immediate patient-care decisions, the SASH model is successful in enabling preclinical students to acquire this essential skill early on in their medical education.

Talk 63:

Title:
On the added benefit of virtual anatomy for dissection-based skills

Authors:
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Abstract:
Methods deploying 3D visualization and integrating virtual anatomy are increasingly used to provide medical students with state-of-the-art teaching. To maintain the quality and hands-on experience in anatomy teaching, strategic partnerships between Austrian universities have evolved in recent years, joining forces by bilateral student exchange in undergraduate teaching. This given study aimed at substantiating student benefit achieved from a merged approach, comprising of dissection course-based anatomy teaching combined with virtual 3D anatomy courses.

One-hundred and twenty second year medical students at Johannes Kepler University Linz were enrolled in this study. Following a full scale four-week dissection course, the students took a 23-item tag exam conducted on human specimens prior to and following a course on virtual anatomy, using the advanced digital volume rendering technique Cinematic Rendering. Likert-based surveys were conducted to assess student experience on the benefit of both courses.

Exposure to virtual anatomy teaching was unrelated to significant improvements in student performance on cadaveric specimens, as seen in the tag exams (1.5% increase). While the students rated the dissection course as being important and impactful, the virtual anatomy course helped display the learning content in a more comprehensible and clinically applicable way.

Cinematic Rendering based virtual anatomy seems to affect knowledge gain in other domains than in anatomical specimens-based recognition of anatomical structures. The findings underline the need of dissection and provides evidence on the added benefit of blending this classical approach in undergraduate medical training with novel developments, thus preparing future doctors for their clinical work.

Talk 64:

Title:
3D-printing a skull for teaching. Is it feasible?

Authors:
Fabian Eggimann (AddMan Factory, University of Zurich, Zurich), Dominic Gascho (Institute of Forensic Medicine, University of Zurich, Zurich), Victor Mergen (Department of Radiology, University Hospital Zurich, Zurich), Hatem Alkadhi (Department of Radiology, University Hospital Zurich, Zurich), Natascha Lier (Institute of Anatomy, University of Zurich, Zurich), Soeren Lienkamp (Institute of Anatomy, University of Zurich, Zurich)

Abstract:
The use of human skulls for teaching in hands-on human anatomy classes is limited by their fragility. Plastic cast models exit but lack sufficient detail and resolution to be a viable alternative. We aimed to develop a workflow to generate high-resolution digital models and 3D-prints of human skull that are suitable for use in the classroom.

Three different scanning methods were used to generate a segmentation of the skull, including conventional CT (body donor and macerated skull) and photon-counting CT (macerated skull). Digital models were created using commercial software and 3D-printed using selective laser sintering. The preservation of anatomical structures and details was compared.

CT-scans of macerated skulls allowed fine structures (orbital walls, ethmoidal cells) to be contrasted much better than in scans of body donors. However, accurate segmentation was limited by the resolution of the CT-scanner rather than the 3D-printer. In addition, low signal intensities of intricate structures posed challenges to complete segmentation. The use of higher resolution photon-counting CT scans and medical grade segmentation software overcame most of these issues.

High resolution CT scanning and 3D-printing is necessary to generate skull models with sufficient detail for use in the classroom.

Talk 65:

Title:
Lactic acid as a non-toxic and non-carcinogenic substitute for tissue fixation in biomedicine – promising experimental results for histochemistry and immunohistochemistry

Authors:
Jonas Keiler (Institute of Anatomy, Rostock University Medical Center, Rostock), Julia Uzdil (Institute of Anatomy, Rostock University Medical Center, Rostock), Philipp Christoph Warnke (Institute for Medical Microbiology, Virology and Hygiene, Rostock University Medical Center, Rostock), Markus Kipp (Institute of Anatomy, Rostock University Medical Center, Rostock); jonas.keiler@med.uni-rostock.de

Abstract:
As an effective tissue fixative, formaldehyde is widely used for the preservation of biological specimens. However, due to the well-known cancerogenic and embryotoxic effects, the use of formaldehyde is highly restricted. Having already only limited approval as a biocide, further restrictions of formaldehyde in biomedicine must be expected anytime soon, without adequate substitutes being available so far. As an alternative, food preservatives might be promising as these are well known to inhibit bacteria and mold growth. We, thus, ask in this project whether food preservatives are suitable to fix biological specimens for subsequent histological evaluations.

Various food preservatives were initially tested for its preservative effects, with lactic acid (LA) demonstrating the most promising results. To further systematically investigate the fixative properties of LA, different murine organs were LA-fixed, and macroscopic and microscopic preservations were compared to the current gold standard (i.e., formaldehyde). Immunohistochemical antigen detection based on chromophore precipitation was studied by qualitative and quantitative measurements. Antimicrobial effects were assessed quantitatively and by mass spectroscopy-based species identification.

In comparison to formaldehyde, LA-fixation resulted in excellent tissue integrity and structural retention, with only minor shrinkage in some tissues. Antigen detection in LA-fixed tissues was largely superior to those fixed with formaldehyde due to a better signal-to-noise ratio. Bacterial load after FA-fixation was low and limited to Bacillus species.

Due to its good preservation effects, excellent antigen retention and its low costs, LA has the outstanding potential to substitute the harmful FA as a tissue fixative for histochemistry and immunohistochemistry.

Talk 66:

Title:
A novel glial barrier structure of the choroid plexus: the glia limitans perichoroidalis

Authors:
Sarah Joost (Institute of Anatomy, Rostock University Medical Center, Rostock), Katerina Manzhula (Institute of Anatomy, Rostock University Medical Center, Rostock), Louise Baumann (Institute of Anatomy, Rostock University Medical Center, Rostock), Theresa Greiner (Institute of Anatomy, Rostock University Medical Center, Rostock), Jens Runge (Institute of Anatomy, Rostock University Medical Center, Rostock), Jonas Keiler (Institute of Anatomy, Rostock University Medical Center, Rostock), Markus Kipp (Institute of Anatomy, Rostock University Medical Center, Rostock); sarah.joost@med.uni-rostock.de

Abstract:
Peripheral immune cells invade the central nervous system (CNS) under neuroinflammatory conditions via different anatomical migration routes: crossing of the blood-brain-barrier at the level of postcapillary venules, egressing from meningeal blood vessels or crossing of the choroid plexus (CP) epithelium. We hypothesize that an additional migratory route exists at the attachment region of the CP that connects the CP stroma and the CNS parenchyma and that this potential migration route is protected by a highly specialized glial barrier structure.

Fixated murine brains were scanned by micro-computed tomography after immersion contrastation. Glial, basal laminal and T cell marker proteins were labelled by immunohistochemistry in control and neuroinflammatory (CupEAE model) murine and human paraffin-embedded sections. The ultrastructure and gene expression of the CP attachment point was analyzed in murine brains by transmission electron microscopy and laser-assisted microdissection.

The attachment regions of the murine CP with close spatial relationship to the subarachnoid space were localized in the third and fourth ventricle in three-dimensional reconstructions. Immunohistochemical analysis of the CP attachment region in the third ventricle revealed a local increase of anti-GFAP, anti-IBA1 and anti-laminin immunoreactivity. Ultrastructural analysis demonstrated highly intertwined astrocytic processes surrounded by a continuous basal lamina. In neuroinflammation, T cell densities were increased at the attachment region. Results from gene expression analysis and characterization of human sections were still pending at the time of abstract submission.

We conclude that the CP attachment region comprises a glial structure with glial barrier properties and therefore refer to this structure as glia limitans perichoroidalis.

Talk 67:

Title:
Opposite regulation of Homer signal at the NMJ postsynaptic micro domain between slow- and fast-twitch muscles in an experimentally induced autoimmune myasthenia gravis (EAMG) mouse model

Authors:
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Abstract:
The objective of the study was to further investigate molecular mechanism of postsynaptic transmission in autoimmune neurodegenerative disease. We therefore explored Homer protein isoforms expression, crosslinking activity, and neuromuscular subcellular localization in mouse hind limb muscles of an experimentally induced autoimmune model of myasthenia gravis (EAMG) and correlate it to motor end plate integrity.

Mice were immunized by subcutaneous injection of Torpedo AChR-antigens (EAMG); an age-matched cohort of mice was used as a reference control group (CTR). Mice were sacrificed at 20- and at 68-weeks after first immunization. Soleus (SOL), extensor digitorum longus (EDL) and gastrocnemius (GAS) muscles were used to investigate Homer mRNA, protein expression and neuromuscular subcellular localization. nAChRs membrane clusters were studied to monitor neuromuscular junction (NMJ) integrity.

Homer short isoform (Homer 1a) transcript was upregulated in hindlimb muscle of EAMG mice. A discrepancy for Homer expression between slow- and fast-type muscle was present. Slow-type muscles showed an upregulation in mRNA transcription of short/Homer 1a and long/Homer 2 isoform, while simultaneously displaying a higher overall decrease of Homer dimers in muscle than fast-type muscles. Densitometry analysis showed increase in Homer protein expression in EDL, and decrease in SOL of EAMG mice. In contrast, nAChRs fluorescence pixel intensity decreased in endplates of EAMG mice, more distinct in type-I dominant SOL muscle.

Postsynaptic Homer signaling is impaired in slow-type SOL muscle of EAMG mice which correlate with a decrease in AChR pixel intensity at NMJ, suggesting a functional coupling between Homer and nAChR, thus highlighting the importance of Homer in muscle cell neurophysiology.
Funding: Grant no. 50WB2116

Talk 67:

Title:
Introduction to normal enthesis anatomy & dedication to Mike Benjamin

Author:
Hannah Shaw (Cardiff)

Abstract:
The point at which a tendon, ligament or joint capsule attach to bone is known as an enthesis, because it is a junction between a hard and soft tissue it is a region of high stress concentration. Entheses have several normal adaptations which aim to reduce this stress, but despite this they are still prone to overuse injuries. Entheses are also the primary target for a group of rheumatic diseases known collectively as seronegative spondyloarthropathies. The complex normal structure of entheses also make them a challenge to reconstruct through engineering approached and reconstitute following the surgical reattachment. This talk will introduce the normal anatomy of the enthesis and enthesis organ to provide a framework for the subsequent talks on enthesopathies and their engineering during the Anatomical Society Symposium and include a short dedication to Professor Mike Benjamin.

Talk 68:

Title:
Development of Therapeutic Platforms for Osteoporotic Impaired Vertebral Bone Regeneration

Authors:
Ciara Murphy (Organisation: Royal College of Surgeons in Ireland,Department: Anatomy and Regenerative Medicine)

Abstract:
Osteoporosis is the most prevalent metabolic bone disease in the world, causing fractures worldwide at a rate of 1 every 3 seconds1. Osteoporotic vertebral fractures (OVFs) are the most common complication of osteoporosis and patients with OVFs are 5 times more likely to suffer secondary vertebral fragility fractures, resulting in pain and decreased quality and span of life2. Clinical gold standards of care for OVFs are vertebroplasty or kyphoplasty, whereby cement is injected into the damaged vertebrae to stabilise the bone and reduce pain. These cements are permanent and do not repair ailing bone, often leading to complications such as cement leakage and secondary fractures in adjacent diseased vertebrae3. There is no reparative treatment. Our research focuses on developing injectable natural polymer based biomaterials, using a unique combination of nano-materials, to achieve mechanical properties that reach stiffness levels close to that of vertebral trabecular bone. An important consideration in treating osteoporotic bone is targeting disease impaired bone remodelling, whereby bone formation is outweighed by bone resorption. As such, a key component in our biomaterial development is the delivery of therapeutic cargos that can promote bone formation and impede pathological bone resorption. We have a particular interest in the use of metal ions such as lanthanides and strontium to induce this response. This talk will provide an overview on our research to date on the development of mechanically robust collagen and chitosan injectable biomaterials, functionalised with therapeutic ions, as innovative treatment platforms for OVFs.

Talk 69:

Title:
Microstructure and mechanics of the periodontal ligament

Authors:
Flora Gröning, School of Medicine, Medical Sciences and Nutrition, University of Aberdeen, Scotland

Abstract:
Objective: The periodontal ligament (PDL) is the connective tissue that surrounds the tooth roots and attaches them to the alveolar bone. It plays an important role in load-bearing and bone remodelling during orthodontic treatment. The mechanical functions of the PDL are determined primarily by its fibrous collagen network and fluid components. This talk aims to provide an overview of recent work on this collagenous network and how its fibre arrangement regulates the mechanical behaviour of the PDL.

Methods: Reviewed studies have used a wide range of imaging techniques to visualise the collagen network in PDL samples (e.g., conventional histology, confocal microscopy, scanning electron microscopy and micro-computed tomography). Some studies have applied loads to samples and measured the resulting changes in fibre arrangement.

Results: These studies have shown variations in the organisation of the collagen network between different regions within PDL sample as well as between samples. Imaging of PDL fibres under mechanical loading has shown how the arrangement of the fibres is adapted to bear tensile loads. However, studies in this area are typically limited by small sample sizes and there is a scarcity of work on human PDL samples.

Conclusions: Advances in high-resolution imaging techniques have provided new insights into the PDL fibre arrangement of the PDL. These data can inform the development of more accurate computational models to investigate the relationships between fibre organisation and mechanical behaviour of the PDL and could also guide tissue engineering approaches for PDL regeneration.

Talk 70:

Title:
Studying healthy tendons using single cell technologies

Authors:
Sarah Snelling (NDORMS, University of Oxford, Oxford), The Tendon Seed Network CZI (, , N/A)

Abstract:
Tendons are essential for locomotion and fine motor control yet are prone to painful tears. They are extracellular matrix rich tissues that are traditionally considered to be mainly composed of fibroblast-like cells. Tendon tears are characterised by inflamamation and fibrosis, with infiltration of immune cells seen histologically. The reparative capacity of tendons is limited in adults and many patients require surgery. Unfortunately, surgery fails in up to 40% of cases. To develop more effective treatments for tendon tears it is essential to understand the cellular composition of healthy tendons – as this provides meaningful metrics for identifying and assessing new therapeutics. Given the diversity of tendons in terms of their anatomical location, function and tear propensity it is also important to understand whether healthy tendons across anatomy have a similar cellular composition or if anatomical-site specific treatments might be needed.

Healthy Achilles, Supraspinatus, Hamstring and Long Head of Biceps tendons were collected from patients undergoing surgery for other pathologies. Samples were snap frozen before being utilised for single nuclei RNAseq, Spatial Transcriptomics or imaging.

Fibroblast, immune and endothelial cell subsets are found in healthy tendons from across anatomy. The relative abundance of these subsets varies between tendon types.

Tendons have a diverse cellular composition, with an immune compartment existing within healthy tendons. The differing cellular composition of distinct tendon types suggests that tendon-type specific controls and metrics should be used in future studies of disease mechanism or in the identification and testing of therapeutics.

Talk 71:

Title:
Engineered matrices for musculoskeletal tissue regeneration

Authors:
Dr. Shane Browne, Lecturer Department of Anatomy and Regenerative Medicine, 123 St. Stephens Green, Dublin 2, Ireland.

Abstract:
Objective
Tissue regeneration involves an orchestrated series of events to restore tissue function. Disruption to this process leads to imperfect healing, or the formation of non-functional scar tissue. The extracellular matrix (ECM) is a highly dynamic structure that plays a critical role in disease and regeneration.1 Synthetic ECM (sECM), mimic features of the native ECM offering promise for the regeneration of injured tissue.2,3. This work focuses on the development of sECM-based treatments for tissue regeneration.

Methods
Key biophysical and biochemical properties of sECM can be tailored to promote tissue regeneration. With this in mind, a tunable sECM composed of acrylated Hyaluronic acid (HyA), matrix metalloproteinase (MMP)-cleavable crosslinking peptides, adhesion peptides (bsp-RGD(15)) and high molecular-weight heparin was developed and assessed in vitro and in pre-clinical models of volumetric muscle loss (VML).4,5

Results
In a rat tibialis anterior model of VML, this HyA-based sECM resulted in robust recovery, accompanied by volume reconstitution, muscle regeneration and native-like vascularisation.. This HyA-based sECM also shows great promise for the delivery of therapeutic cells.6,7 Delivery of Fibro-Adipogenic Progenitors (FAPs), a population of muscle residential progenitor cells, within the HyA-hydrogel promotes cell survival, differentiation and muscle regeneration in vivo.

Conclusions
These observations bode well for the potential of this sECM for the treatment of VML. In addition, key properties of this sECM can be tailored for alternative tissues. This can include modification of biophysical properties including storage modulus, degradation kinetics or the adhesion peptide sequences, or the delivery of tissue-specific cell populations and/or therapeutic molecules to promote regeneration and functional recovery.

Talk 73:

Title:
Making Connections: Anatomical design for musculoskeletal tissue engineering

Authors:
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Abstract:
Objectives: The tissues of the musculoskeletal system join together through a series of important interfacial regions. Our work is focussed on the enthesis, the specialised junction between tendon/ligament and bone, and exploring options for repair/regeneration of this vital connection1. While many attempts to engineer musculoskeletal tissues focus on a specific tissue type, we employ the use of a variety of anatomically and clinically relevant co-culture designs to establish options for tissue engineering the enthesis in vitro.
Methods: Co-culture models of specific anatomical sites are designed and constructed using a combination of anatomical morphometric analysis and 3D printing to produce bespoke anatomical culture wells. For example, a region of interest the flexor digitorum profundus (FDP) tendon onto the distal phalanx. Anatomically–relevant bone-tendon constructs were produced using a calcium phosphate cement (brushite; bone) and a fibroblast-seeded fibrin hydrogel (tendon).
Results: Our previous work has demonstrated that morphometric analysis of human fingers revealed the size and shape of the FDP tendon insertion site, as well as the angle of FDP tendon fibre insertion angle to the distal phalanx2. Based on these specific measurements, bespoke anatomical culture wells were manufactured to form bone-tendon constructs with anatomically and clinically relevant dimensions.
Conclusions: This novel approach in tissue engineering produces artificial structures with dimensions that will increase the likelihood of future translation. We are currently exploring this approach for other body regions to produce potential replacements for implantation after injury or disease.
1. Loukopoulou et al., (2022) Eur Cells Mater 43:179-201
2. Mortimer et al., (2021) BMC Musculoskelet Disord 1032

Talk 74:

Title:
A Change of Perspective: Soft Biomaterials as Bone Anchors for Investigating the Regeneration at the Bone-Tendon Interface

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Abstract:
Objective
Marine collagen is considered an evolutionary ancient stem collagen, so called collagen type O, which makes it universal and a safer and a disease-free alternative to the traditional mammalian collagen. This study aims to investigate the 3-dimensional jellyfish collagen sponges (3D-JCS) as prospective bone anchors for enthesis regeneration primarily focusing on the potential of 3D-JCS to support bone-like extracellular matrix (ECM) production.

Methods
A comparative study was conducted and both qualitative and quantitative analyses were performed in order to evaluate the bone-like ECM production of osteoblastic cells on 3D-JCS (manufactured by Jellagen Ltd). Samples were analysed using histological and fluorescent dyes (i.e. Alizarin Red S (ARS), Von Kossa, Tetracycline hydrochloride) and the compressive strength of the 3D constructs was investigated using the CellScale MicroTester LT.

Results
Successful bone-like ECM production was observed both on the inner and outer surface of 3D-JCS. Furthermore, quantitative analysis of the mineralized ECM suggested the augmented osteogenic potential of osteoblastic cells when cultured on 3D-JCS. Nevertheless, the cell-seeded 3D-JCS cultured in osteogenic conditions exhibited an 82.3-fold increase in compressive modulus compared to acellular 3D-JCS, with recorded values of 0.4 ± 0.1 kPa.

Conclusions
The unique features of jellyfish collagen sponges promoted the bone-like ECM production of osteoblastic cells. Furthermore, the compressive strength of 3D-JCS was significantly enhanced by the cell-mediated mineral deposition. These findings highly recommend the jellyfish collagen sponges as superior scaffolds for bone tissue regeneration. We predict that the 3D-JCS could be successfully used as potential bone anchors for studying bone-tendon interface regeneration.

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