Abstract Book

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Accumulation of aggregates leads to synaptic dysregulation in ALS related mutation

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Amyotrophic lateral sclerosis (ALS) is an incurable neurodegenerative disease mainly affecting the neurons of the corticospinal tract. Accumulation of cytotoxic protein aggregates and loss of synaptic contacts have been shown to be central pathomechanisms driving ALS progression. However, the interconnection between these two pathological manifestations is still unclear. Based on these considerations, we aim to investigate whether manipulation of synaptic physiology might restore autophagy-dependent catabolism and confer neuroprotection against ALS.

Here we combine primary rat neurons overexpressing poly(GA) inclusions (the most abundant toxic product generated by ALS-related mutations within the C9orf72 gene) and hiPSC-derived neurons from ALS patients to investigate the interplay between synaptic and autophagic alterations. Using high-resolution microscopy, multi-electrode array (MEA), and pharmacological approaches we aim to uncover and rescue the neurophysiological abnormalities affecting the neurons in ALS.

Overexpression of poly(GA) aggregates triggered a dramatic dysregulation of the autophagic machinery. This, as a consequence, led to a progressive loss of synaptic contacts and, eventually, neuronal death. Longitudinal MEA recordings backed up these findings by highlighting altered neuronal activity in poly(GA)-expressing cultures. Interestingly, neurons derived from ALS patients harboring C9orf72 mutations closely recapitulated the results obtained in our overexpression model, confirming that autophagy blockade and aggregates accumulation contribute to disease progression by affecting the synaptic microenvironment.

In this work, we show that the accumulation of aggregates causes synaptic dysfunction, which actively contributes to neuronal loss in ALS. In our study's next steps, we will investigate whether restoration of synaptic composition can rescue the catabolic impairments caused by toxic inclusions and contrast ALS progression.

Multi-modal functions of FOXG1 in the mouse hippocampus

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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.

Synaptic alterations drive motor neuron degeneration in ALS

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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.

Application of artificial intelligence for quantification of angiogenesis

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Angiogenesis is the process of new blood vessel formation. Manual methods, which are most commonly used to evaluate angiogenesis in vitro, are time-consuming and demand considerable effort. This study aims to implement a new artificial intelligence method for quantifying angiogenesis reliably and fast.

Human skin endothelial cells were cultured in nutrient medium and labelled with the endothelial marker anti-CD31. The cells proliferated and formed a 3D tubular network.
Number and diameter of the capillary-like structures (endothelial tubes), as well as their crossing points (knots) were manually counted as control.
Applying the AI-module (Segment.ai), a small set of hand-traced microscopic images of angiogenesis was used to train the software in the first phase (train phase). Training results were applied on similar images in the second phase (application phase) to identify various parameters.

Number and diameter of endothelial tubes were similar in both methods. For the manual method, mean of numbers and diameter of endothelial tubes were 835.17 ± 52.37 SEM and 9.91 µm ± 0.21 SEM, respectively; for the AI method 865 ± 103.58 SEM and 10.02 µm ± 0.80 SEM, respectively. Number of knots was 323 ± 26 for manual and 610 ± 159 for AI. Training the AI took about 50 hours. Time used for quantification per image was about 40 minutes for the manual and 5 minutes for AI method.

The artificial intelligence software is a suitable method for quantifying angiogenesis in vitro. Compared to the manual method, it saves time and is more efficient for experienced users.

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

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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.

Interaction of megalin and FcRn during endocytosis

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Endocytosis of filtered proteins is one of the main tasks of the renal proximal tubule. The neonatal Fc receptor (FcRn) is known to transcytose albumin, but ligand binding depends on acidic pH (pH 6). It is unclear where FcRn binds to its ligands. Megalin endocytose various filtered proteins, including albumin, and may be a prerequisite for albumin transcytosis. Currently it remains unknown whether megalin and FcRn interact to ensure proper endo-transcytosis function.

We analyzed the interaction of megalin/FcRn using bimolecular fluorescence complementation (BiFC) and co-immunoprecipitation. To evaluate dynamic interactions, we used endocytosis assays and super-resolution microscopy.

Using BiFC and co-IP analysis, we observed that megalin and FcRn interact. 8h post transfection of both proteins, no interaction was found in endoplasmatic reticulum, but interaction in Golgi apparatus and later on in different endosomal compartments. Interactions were specific, since no interaction was found between FcRn/amnionless – 'membrane anchor' of cubilin – another albumin receptor. Using truncated proteins, we identified an interaction of FcRn with the extracellular domain of megalin. When inhibiting the ligand-binding domain of megalin using RAP or shifting the pH into acidic pH (5.5), the interaction was not abrogated. Applying albumin (ligand of megalin and FcRn) or lactoglobulin (specific for megalin) enhanced the co-localization of megalin/FcRn in clathrin-vesicles and early endosomes (latter only for albumin). No change for recycling endosomes and a reduction in late endosomes/lysosomes was encountered.

In conclusion, megalin and FcRn interact specifically and may express similar sorting signals since both proteins traffic together. Co-localization is enhanced in early endosomal compartments during endocytosis, implying functional similarity.

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

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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.

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

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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.

Organs Weight in NPC1 Mutant Mice Partly Normalized by Miglustat, Cyclodextrin, and Combination Treatment

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Niemann-Pick Type C1 (NPC1) is a rare, progressive lethal, inherited autosomal-recessive endolysosomal storage disease caused by mutations in the NPC1 leading to intracellular disturbances. We analyzed alterations of weights of 14 different organs in the BALB/cNctr-Npc1m1N/-J Jackson NPC1 mouse strain in larger cohorts of female and male NPC1+/+ and NPC1-/- mice under various treatment strategies.

Male and female NPC1-/- mutant mice were treated with i) no therapy (None), ii) vehicle injection (Sham), iii) combination of miglustat (MIGLU), allopregnanolone and 2-hydroxypropyl-ß-cyclodextrin (HPßCD) (= COMBI), iv) MIGLU, v) HPßCD alone starting at P7 and repeated weekly throughout life, and vi) HPßCD alone given only once at P7. The 12 respective NPC1+/+ mice (male and female wild type mice) groups were evaluated in parallel. In total, 351 mice (176 NPC1+/+, 175 NPC1-/-) were dissected at P65.

In both sexes, body weights of None and Sham NPC1-/- mice were lower than of respective NPC1+/+ mice. The influence of the NPC1 mutation and/or sex on weights of various organs, however, differently considerably. In males, NPC1+/+ and NPC1-/- mice had comparable weights of lungs, spleen, and adrenal glands. In NPC1-/- mice, smaller hearts, livers, kidneys, testes, vesicular and scent glands were found. In female NPC1-/- mice, ovaries and uteri were significantly smaller. Treatment with especially with COMBI increased organ weights in NPC1-/- mice of both sexes.

In NPC1-/- mice, organs were to different extends altered by the therapy; the combination of the iminosugar miglustat, the neurosteroid allopregnanolone and the sterol chelator 2-hydroxypropyl-ß-cyclodextrin normalized the weights best.

Interrelations between the transverse facial artery and the parotid duct

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To study the interrelations between the transverse facial artery (TA) and parotid duct (PD).

Layer by layer cadaver dissection (n=60, years 35-90). Both gender. All cadavers were fixed by freezing method.

Four types of the interrelations between the TA and the PD were found. We offer new classification of it. 1st type: when the TA goes parallel to the zygomatic arch and parotid duct between them (45%, 23/51). 2nd type: when the TA goes on the surface of the PD (25,5%, 13/51). 3rd type: when the TA cross the PD from up to down in its middle and distal third (17,7%, 9/51). 4th type: when the TA goes near inferior margin of the PD (11,8%, 6/51).

Reconstructive and aesthetic operations in the plastic, maxillofacial surgery and cosmetology somehow affect TA. Therefore, in our opinion, it is extremely important to accurately understand the variant anatomy of the transverse facial artery. Our study revealed several types of the interrelations of the parotid duct and the transverse facial artery. In our opinion, this knowledge should be taken into account at the preoperative stage when planning the operations for this anatomical zone.

B

Study of the pterygomandibular space as a target point for inferior alveolar nerve block

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Determine the size and volume of the pterygomandibular space (PS).

The study was carried out on the cadaveric material (Institute of Anatomy, Skolkovo). The sample included 19 corpses. On the cadaveric material, was performed IANB according to the Halstead technique with silicone. After the silicone had solidified, the PS was opened, the silicone was removed and measured.
Statistical processing of the data was performed using IBM SPSS Statistics 23. Arithmetic mean and standard deviation were determined. Pearson's correlation coefficient was also used.

It has been noted the PS has the shape of a triangular depression that can be seen between the insertion of the temporalis muscle medially and the anterior edge of the mandibular ramus laterally. The temporal crest formed the longest border.
The mean length of the PS on the right was 3.04±0.29 mm, on the left 3.41±0.29 mm. The mean PS width on the right was 1.73±0.17 mm, on the left 2.16±0.16 mm. We did not find similar data for comparison in the available literature.
The thickness from the nerve entry point on the right was 1.02±0.03 mm, on the left 0.97±0.04 mm.
A correlation analysis was also carried out, according to the results of which we noted a medium and high positive relationship between the width and length of the PS on the left ($r=0.679$, $p=0.001$) and on the right ($r=0.808$, $p<0.001$).

The data obtained on the size and volume of the PS will help doctors more accurately calculate the dose of the anesthetic to be injected.

Binding of small heat shock protein HspB5/alphaB-crystallin to tubulin is dependent on phosphorylation status

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Many neurodegenerative diseases present with a rarefaction of the dendritic tree of neurons, leading to neuronal dysfunction. We have previously shown that the small heat shock protein HspB5/alphaB-crystallin is able to enhance and protect dendritic complexity in hippocampal neurons in dependency of its phosphorylation at serine 45 and 59. To elucidate the mechanism behind this effect, we now investigated the influence of phosphorylation on the binding of HspB5 to the cytoskeletal protein tubulin, a component of microtubules which is essential for dendritic stabilization and dynamics.

HspB5 wildtype as well as phosphomimics simulating or preventing phosphorylation at serine 19, 45 or 59 by exchange to glutamic acid or alanine, respectively, were recombinantly expressed in E.coli. Binding studies between purified HspB5/phosphomimics and porcine brain tubulin were then performed by pull-down assays followed by western blotting to determine the amount of bound HspB5.

The binding of the non-phosphorylatable HspB5 mutant to tubulin was comparable to that of wildtype HspB5. HspB5 phosphomimics simulating phosphorylation at one site showed a slightly better binding. HspB5 phosphomimics with two or all three serine residues replaced by glutamic acid revealed statistically significant stronger binding to tubulin than HspB5 wildtype.

Our data show that the phosphorylation status of HspB5 influences its binding to the microtubule component tubulin. The more serine residues were replaced by glutamic acid, the more tubulin was bound to HspB5. Thus, phosphorylation-dependent binding of HspB5 to tubulin might be one molecular mechanism by which HspB5 exerts its neuroprotective effect.

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

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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.

Loss of Desmoglein 3 does not profoundly alter epidermal differentiation in reconstructed human epidermis

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Desmosomes are not only static structures facilitating cell-cell-adhesion but are also proposed to act as sensors and mediators for tissue-specific cell behaviour and signalling. The isoform-specific patterning of desmosomal cadherins, desmoglein (DSG) 1-4 and desmocollin 1-3, within the differentiated layers of the human epidermis suggests a contribution of desmosomal cadherins to epidermal differentiation which is only partially understood.

We studied the influence of DSG3 on epidermal differentiation by silencing DSG3 in normal human epidermal keratinocytes (NHEK) by lentiviral transduction. No significant loss of cell-cell adhesion was detected in dispase-based dissociation assays of DSG3 knock-down cells. To investigate the differentiation behaviour of human epidermal keratinocytes, 3D-reconstructed human epidermis (3D-RHE) was cultivated at the liquid-air interface for 12 days. In Western blot analysis, the knock-down of DSG3 in 3D-RHEs was confirmed and expression of differentiation markers and other desmosomal cadherins was analyzed, showing a trend for increased DSG2 levels under knock-down conditions. 3D-RHE sections revealed fully differentiated epidermal equivalents with slightly reduced thickness upon loss of DSG3. Immunostainings for the proliferation marker Ki67 did not show reduced proliferation in knock-down 3D-RHEs.

Our data indicate that knock-down of DSG3 does not have a striking effect on cell cohesion, proliferation or differentiation behaviour of keratinocytes within reconstructed human epidermis while the upregulation of DSG2 supports the theory of a compensation mechanism.

3D-in vivo-model to study human renal cystic tissue and mouse kidney slices

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Autosomal dominant polycystic kidney disease (ADPKD) is a frequent monogenic disorder that leads to progressive renal cyst growth and renal failure. Strategies to inhibit cyst growth in non-human cyst models have often failed in clinical trials. There is a significant need for models that enable studies of human cyst growth and drug trials.

Renal tissue from ADPKD patients who received a nephrectomy as well as adult mouse kidney slices were cultured on the chorioallantoic membrane (CAM) for one week. Cyst volume was monitored by microscopic and CT-based applications. Weight and angiogenesis were quantified. Morphometric and histological analyses were performed after removal of the tissues from the CAM.

Mouse and human renal tissue mostly remained vital for about one week on the CAM. Growth of cystic tissue was evaluated using microscopic and CT-based volume measurements which correlated with weight, an increase of angiogenesis and was accompanied by cyst cell proliferation.

The CAM model might bridge the gap between animal studies and clinical trials of human cyst growth and provide a drug testing platform for the inhibition of cyst enlargement. Real-time analyses of mouse kidney tissue may provide insights into renal physiology and reduce the need for animal experiments.

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

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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.

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

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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.

Night shift work – a risk factor for human health?

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Shift work especially night shift work is a large challenge for the human circadian system that controls numerous body functions. Here we investigated the impact of night shift work on metabolism, the cardiovascular and immune system as well as brain function and morphology in the Heinz-Nixdorf-Recall and Multi-Generation cohort, as well as the 1000BRAINS study a subsample thereof. Participants who worked in night shift at time of investigation (PRESENT, n = 125) or before investigation (FORMER, n = 662) were compared to age and sex matched participants who NEVER worked in night shift.

Metabolic status was assessed by body mass index, waist-hip-ratio, hemoglobinA1c, blood glucose, total-, HDL- and LDL-cholesterol, triglycerides and uric acid. Systolic, diastolic blood pressure and HDL-LDL-ratio were determined as parameters for the cardiovascular system. Immune system markers included c-reactive protein, basophilic, eosinophilic, and neutrophilic granulocytes. Brain morphology and function were determined by resting-state functional connectivity (RSFC), cortical thickness and gray matter volume from magnetic resonance images. Differences between groups were tested using multivariate analyses of variance corrected for multiple comparisons.

Regarding the cardiovascular, metabolic or immune system markers we found no significant differences between PRESENT, FORMER and NEVER shift workers. Moreover, no associations between night shift work, RSFC and brain morphology were found after multiple comparison correction.

Our studies with a large population-based cohort (n = 5984) enabled examination of a variety of health-relevant parameters. The data do not support the hypothesis that night shift work elicits serious health problems.

The neuromuscular junction (NMJ) in vitro as an experimental model to study molecular mechanisms of neuromuscular transmission

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The main objective of our study is to generate neuromuscular junctions in vitro in a 3D cell co-culture model. We are most interested in the muscle endplate, where acetylcholine receptors are concentrated to bind the acetylcholine released from the activated nerve terminals. This model will help to identify postsynaptic molecular mechanisms of the neuromuscular transmission and explore potential cross talk in postsynaptic signaling pathways without the use of animals.
Moreover, the availability of such an in vitro experimental model can help to gain insights into the molecular mechanisms underlying NMJ imbalance in different pathological processes and potential therapeutic approaches, such as pharmacological interventions.

A murine myoblast and a neuronal cell lines grow in a 3D structure containing important factors of the extracellular matrix. At optimal cell density and in a specifically tailored environment, both cell types differentiate together and are able to make synaptic connections. These cultures can be fixed and investigated immunocytochemically by using NMJ specific molecular markers in combination with muscle and nerve specific differentiating markers. Additionally, whole/cell recordings allow us to assess the electrical cell-cell communication and thus confirm the existence of functional synapses.

We were able to establish a protocol to culture and maintain a co-culture within a 3D structure. Triple immunofluorescence labeling demonstrated that neurofilament-positive axonal nerve endings approached alpha-bungarotoxin-positive clustered acetylcholine receptors in desmin-positive or myosine-positive myotubes, indicating the establishment of NMJ-like structures in vitro. The measured distance of one synaptic cleft was approximately 87 nm, which is comparable to the native NMJ in vivo.

NMJ-like structures were present in our 3D co-culture system suggesting the viability of our experimental paradigm to generate specialized cell-cell contacts between neurons and muscle in vitro.

CEACAM1 metabolism in aortic endothelial cells

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Metabolic pathways have emerged as key regulators of many endothelial cell (EC) functions, including angiogenesis, inflammation, and barrier function processes. Emerging evidence demonstrates that perturbation of EC metabolism results in EC dysfunction and vascular pathologies. Cc1-/- mice exhibited early atherosclerotic lesions and vascular dysfunction specially impairment of EC functions. However, metabolic role of CC1 in vascular ECs remains unknown. Therefore, this study aims to investigate the metabolic role of CC1 in aortic ECs.

Aortic endothelial cells (AoEnds) were generated from mouse CC1 deficient mice (Cc1−/− AoEnds) and wild type (WT AoEnds). WT and Cc1−/− AoEnds were cultured in the presence of glucose-C13 and glutamine N15 and metabolites were detected by LC/MS mass spectrometry. Seahorse experiments were performed on Agilent XF system.

Our metabolic tracing studies revealed a significant decreased labelling for glucose 6-Phosphate in CC1-/- AoEnds in comparison to WT AnEnds. Further, products of the pentose phosphate pathway and nucleotide synthesis was decreased in CC1 deficient AoEnds, explains the less proliferative capacity of these cells. Moreover, a significant reduction in glycolysis and phosphatidyl choline phosphate labeling in CC1-/- AoEnds suggesting disturbed membrane composition leading to impaired barrier function in ECs.

We attempted for the first time to dissect the metabolic role of CC1 in ECs. Further, the present data hints another important function of CC1 in switching the ECs activation and back to quiescent state. In addition to the known functions, CC1 has potential importance in endothelial metabolism with novel signal connections highlighting the need to study metabolic therapeutic effects.

Continuous digital measurement of intratumoral fluid pressure revealing circadian oscillations

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Interstitial fluid pressure (IFP) is increased in almost all solid tumors, which leads to low penetration and heterogeneous intra-tumoral distribution of anti-cancer drugs. To date, only single-point measurements of IFP have been reported, mostly in anesthetized animals.

We established a new technique to continuously monitor IFP in conscious, freely moving mice using implantable telemetric transmitters (DSI TA11PA-C10 pressure transmitter). IFP was measured in immunodeficient pfp/rag2-/- tumor-bearing mice, subcutaneously xenografted with human HT-29 colon cancer or PaCa5061 pancreatic cancer cells. IFP was continuously recorded for 60 seconds every 5 minutes starting from the 7th day after implantation.

IFP curves of values registered for at least ten days were decomposed into a slowly changing trend component, a medium-scale component reflecting intra-day IFP changes, and high-frequency noise. Mean IFP trend values were 35.3 mmHg in HT-29 tumors and 12.3 mmHg in PaCa5061 tumors. The medium-scale component demonstrated distinct circadian oscillations with minimum levels approximately at midday and maximum levels approximately at midnight consistent with the passive and active behavioral phases of the nocturnal animals. Mean oscillation amplitude was equal to 23.5 mmHg for HT-29 tumors and 2.1 mmHg for PaCa5061 tumors.

Distinct circadian rhythm oscillations of IFP were uncovered in HT-29 and PaCa5061 xenograft tumors, demonstrating that IFP is not static but highly dynamic. As increased IFP critically affects the intratumoral distribution of anti-cancer drugs in solid tumors and thereby the therapeutic efficacy of chemotherapy, these observations may provide a mechano-physiological basis for chronotherapy of cancers.

Situs inversus - Macroscopic assessment and investigation of cerebral lateralization using manual and imaging techniques

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Situs inversus (SI) is a rare and only uncompletely understood phenomenon. Currently, our institute stores three SI body donors (year of death 2010/2013/2019) for teaching and research purposes. This study aims to add further data to the existing background, in particular on cerebral lateralization.

This study presents the case of an 85-year-old female SI body donor. The donor's medical history and its type of heterotaxy are presented. In order to detect possible lateralization of cerebral structures, manual and imaging techniques were employed and compared intra-individually and with ten situs solitus (SS) body donors. Vascular diameter, size of the anterior cranial fossa, bending, petalia, and results for MRI hemispheric volumetry were evaluated. In addition, DWI imaging using a 3T MRI was attempted to visualize cerebral pathway systems.

Dissection revealed a SI totalis with dextrocardia and -versia. All SI body donors showed a larger left anterior cranial fossa compared to the right fossa and to the control group. Frontal petalia were uniformly directed to the left, occipital ones to the right. No differences could be observed in bending. In two of the SI body donors, right cerebral hemispheres showed a higher volume in MRI images. However, the attempt of DWI MR-imaging of long-term fixed brains showed only weak and undirected signals.

In summary, this study provided a comprehensive macroscopic workup of a SI case. The results confirm previous data on body and cerebral asymmetry. The technique of DWI imaging has to be improved e.g. by a shorter postmortem intervals.

Bovine mammary epithelial cells in vitro - the future of milk production?

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Whilst in vitro production of meat is well advanced this is not the case for milk yet. Even plant-based alternatives' climate impact is bigger than expected. Could in vitro milk production be a possible solution? Aim of this study is to investigate the possibilities of in vitro milk production using bovine mammary epithelial cell cultures.

Samples of bovine mammary glands were enzymatically digested. Tissue was put through a cell strainer and single cells were seeded in cell culture dishes. Cells were incubated at 37°C, 5% CO_2 in basal medium. Later medium was changed to induction medium containing hormones to induce production of milk ingredients. For identification of cells immunohistochemistry was performed using EpCam, KRT18 and Vimentin as primary antibodies.

After two days of incubation primary cells formed a cobblestone-like confluent layer covering the cell culture dish. Cells in induction medium also developed cobblestone-like patterns. Whereas cells in basal medium kept their cobblestone-like morphology, cells in induction medium developed cellular heterogeneity, lumen-like structures and vesicle-like structures in varying sizes and shapes inside the cytoplasm. Cells in both media stained positive for EpCam and KRT 18 and negative for vimentin.

Different cell culture media cause varying cell morphology. Only cells incubated in induction medium developed lumen-like structures. Therefore, cells seem to begin to express cell polarity which later will be critical for mechanisms of milk secretion. The production of milk components (proteins and lipids) is under investigation using transmission electronmicroscopy, PCR and Western Blot.

HNE Provokes Pancreatic Islets Cyto-architecture Alteration in Japanese Macaques.

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To help advance the mechanism of Langerhans cell degeneration caused by free fatty acids, we aim to investigate the effect of free fatty acid derivatives, HNE in Langerhans cells of non-human primates.

In this study, three-Japanese macaques were utilized and randomly divided into 2 distinct groups of control (n=1) and HNE (n=2). After 5 mg/ week of consecutive HNE intravenous injection for 6 months, 3 pancreases were cautiously collected and studied by H&E staining, immunofluorescence histochemistry, transmission electron-microscopy and western blot.

The light microscopic examination revealed more abundant vacuole formations and nuclear condensations in the HNE-islet cells than in the control cells. The transmission electron microscopy (TEM) illustrated vacuolar alterations and a vast decrease of insulin secretory-granules occurring mainly in β cells of HNE islets. Moreover, HNE-islets exhibited marked increases in autophagosome/autolysosome numbers, lysosomal membrane ruptures and a mitochondrial degradation in comparison to control islets. The immunofluorescence reactivities demonstrated an increase of co-localization of activated μ-calpain and Hsp70 concomitant with Ctsb-Lamp2 followed by Ctsb release in HNE-islets. Western blots showed an increase of activated μ-calpain expression and Hsp70 cleavage (~30 kDa) after HNE injection compared to control monkey.

Our recent data highlights that HNE is a causative factor that can promote lysosomal membrane disintegrity and exacerbate Langerhans cells especially β cells degeneration in non-human primates.

Analysis of pancreatic cancer cell line and pancreatic cancer biopsies in 3D in-vivo tumor model

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Primary pancreatic ductal adenocarcinoma (PDAC) is one of the most lethal oncological conditions in Germany with 18.400 diagnosed patients in 2020. Despite enormous research efforts in the last decades, the prognosis has not improved, and the 5-year survival rate is below 10%. Due to demographic changes, an increase in PDAC related mortality is expected. Models enabling a deeper understanding of PDAC are needed to improve diagnostic and therapeutic options.

Pancreatic cancer cell line MIAPaca2 as well as primary PDAC biopsies were grafted onto the chorioallantoic membrane (CAM), a 3D-in-vivo-tumor model, for one week and treated with cisplatin or control. Tumor angiogenesis was measured using Laser speckle contrast analysis. Histological analyses of primary tissue and explanted CAM tumors utilizing H.E. staining and PAS reaction were performed. Engraftment rate and therapeutic effects were monitored immunohistochemically by Ki67 staining.

Engrafting of cell lines and primary material was successful in >70%. Inoculation of cell lines as well as primary material from surgery resulted in viable, growing tumors on the CAM. First histological analysis suggests main histopathological characteristics remained the same. Ki67 evaluation shows ongoing proliferation in MIAPaca2 based tumors and tumors from primary tissue. Chemotherapy shows significant effects in proliferation.

The CAM model might bridge the gap between cell culture and animal testing. It enables engraftment of primary patient cancer samples, allowing a more precise understanding of PDAC. It may serve as a personalized medicine tool, helping to quickly identify the most efficient therapy for a specific tumor.

Neuronal activity drives tumor microtube dynamics and generation in glioblastoma

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Glioblastoma are incurable primary brain tumors characterized by their infiltrative brain colonization. Previously, long membranous tubes called tumor microtubes (TMs) have been described in glioblastoma cells contributing to the invasion. Additionally, glutamatergic synaptic contacts on these TMs were identified in a subpopulation of glioblastoma cells. The effect of these synaptic contacts on TM dynamics and TM generation has not yet been characterized.

Cranial windows were implanted for chronic in vivo two-photon imaging of glioblastoma cells transduced with a membrane-bound fluorescent marker. During window implantation neurons were infected with an AAV mCherry-channelrhodopsin construct while the control group was infected with an AAV construct only expressing mCherry. Subsequently, a novel workflow using augmented microscopy was established allowing to analyze tumor microtube dynamics and generaton after neuronal stimulation. These data were subsequently augmented with a deep-learning based algorithm called enhance.ai (Nikon), registered and manually tracked.

This novel workflow revealed that TM dynamics consist of multiple mechanisms including protrusion, retraction and branching. Interestingly, the data showed that neuronal activity drives TM growth, branching and TM formation including the formation of small processes. Lastly, we also studied the dynamics of putative neurogliomal synapses on glioblastoma cells revealing that neurogliomal synaptic boutons are predominately transient.

Our work shows that glioblastoma cells adopt neuronal-like mechanisms to invade brain tissue. Furthermore, we identified a novel function of neurogliomal synapses inpromoting TM formation (Venkataramani et al., Cell, in press).

Adipocyte size in lipoedema patients

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Lipoedema is a female disease with a painful, disproportional accumulation of fat at the extremities. Several studies attribute the lipoedematous subcutaneous tissue to altered gene expressions, increased pro-inflammatory markers, or altered sensitivity to hormones. Nevertheless, not much is known about the basic morphological characteristics of this kind of fat. Especially, it is unclear, whether the adipocytes in lipoedema are hypertrophic or hyperplastic.

Biopsies from lipoedema patients undergoing lymphologic liposculpture were collected at the beginning of the procedure, fixed in 4% buffered formaldehyde and processed histologically. HE-stained slides (3 µm) were photographed and analysed in FIJI (ImageJ-package) for the cross-sectional area and maximum diameter (feret). The weight-to-height ratio was used to classify the patients from underweight to obese.

Biopsies of 57 lipoedema patients were analysed. Patients had adipocytes with an average cross-sectional area of 4635 µm² (normal weight), 4167 µm² (overweight), 4603 µm² (obesity grade I), and 4856 µm² (obesity grade II), respectively. Maximum diameters were on average 93 µm (normal weight), 88 µm (overweight), 93 µm (obesity grade I), and 96 µm (obesity grade II).

The adipocytes of the immediate subcutaneous tissue do not show the expected increase in cross-sectional area or maximum diameter with an increasing weight-to-height ratio. Assuming that adipocytes of normally weighted patients are not hypertrophic (as assumed in obesity), the data of overweighted or even obese patients show no hypertrophy. Therefore, the increase in limb volume in lipoedema should be contributed to an increase in the number of cells (hyperplasia).

Sex-dependent responsiveness of hippocampal neurons to sex neurosteroids: A role of Arc/Arg3.1

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Sex steroids, such as estradiol (E2) and dihydrotestosterone (DHT), regulate hippocampal plasticity and memory in a sex-dependent manner. Because the activity-regulated cytoskeleton protein Arc/Arg3.1 is essential for long-term memory formation and synaptic plasticity, we investigated the expression of Arc/Arg3.1 with respect to its responsiveness to E2 and DHT in male and female hippocampal neurons.

Primary hippocampal dispersion cultures from E18 rats were used to investigate the influence of neurosteroids on neurons. After stimulation with the neurosteroids and their inhibitors, quantitative real-time polymerase chain reaction, western blot analysis and quantitative immunoreactivity were used.

For the first time, we show that, in hippocampal neurons, Arc/Arg3.1 expression is sex-dependently regulated by sex steroids. No difference in the expression between sexes was observed under control conditions. Upregulation of Arc/Arg3.1 protein expression was observed in specifically female hippocampal neurons after application of E2 to the cultures. Conversely, upregulation of Arc/Arg3.1 was seen in specifically male neurons after application of DHT. A quantitative real-time PCR revealed that the sex-dependency was most pronounced on the mRNA level. Most importantly, the effects of E2 in cultures of female animals were abolished when neuron-derived E2 synthesis was inhibited.

Our results point to a potentially important role of Arc/Arg3.1 regarding sex-dependency in sex steroid-induced synaptic plasticity in the hippocampus.

Suprastructures of extracellular matrices: paradigms of functions controlled by aggregates, not molecules

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Extracellular matrices contain aggregates of macromolecules organised in a hierarchical manner into alloyed polymeric suprastructures that, in turn, form tissue or organ structures. Mainstream scientific approaches usually limit their attention to structures of molecules which then are extrapolated automatically to the architecture at higher levels. The lecture will focus on the significance of structure-function relationships occuring only at intermediate or tissue level.

Transmission electron microscopy, transgenic animals (K.O. of integrin alpha chains), tendons, cornea, reconstitution in vitro of matrix aggregates (e.g., collagen fibrils, proteoglycan aggregates, basement membranes)

- Basement membranes (BMs) consist of separable alloyed aggregates of multiple types of laminins or collagen IV variants connected by polymeric perlecan. Both networks contain nidogens-1 and/or -2 which are not involved in the structural continuity of the two composite networks.
- The epidermal BM is anchored in the dermal stroma by aggregates containing collagen VII (anchoring fibrils) that strongly interact with dermal collagen fibrils. These interactions do not occur at the level of single collagen I- and collagen VII molecules.
- Cells are tethered to their extracellular matrix by suprastructures containing integrins in their membrane spanning portions. Collagen-integrins (heterodimers of beta1- with alpha1-,alpha2, alpha10-, or alpha11-polypeptide chains) recognise several collagen sequences in a highly specific manner. These interactions are abolished when collagen molecules aggregate into fibrils.
- Mice lacking alpha1-, alpha2-, or alpha11-integrin chains have normal fibrillar architectures in their cornea or tendons. Hence, collagen integrins are dispensable for collagen fibrillogenesis in vivo.

Direct extrapolations from molecular interactions (interactomes) to tissue functions often require critical inspections.

Engineered matrices for musculoskeletal tissue regeneration

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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.

TFF1 in aqueous humor – a potential new biomarker for retinoblastoma

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Retinoblastoma (RB) is the most common childhood eye cancer. The expression of trefoil factor family peptide 1 (TFF1), a small secreted peptide, has been correlated with more advanced RB stages and it might be a promising new candidate as a RB biomarker. The study presented addressed the question if TFF1 is detectable in aqueous humor (AH) of RB patients` eyes, providing easy accessibility as a diagnostic and/or therapy accompanying predictive biomarker.

TFF1 expression status of 15 retinoblastoma AH samples was investigate by ELISA and Western blot analyses. The results were correlated with the TFF1 expression status in the tumor of origin and compared to TFF1 expression in established corresponding primary tumor cell cultures and supernatants.

Nine out of 15 AH patient samples exhibited TFF1 expression, which correlated well with TFF1 levels of the original tumor. TFF1 expression in most of the corresponding primary cell cultures reflects the levels of the original tumor, although not all TFF1 expressing tumor cells seem to secret into the AH.

Aqueous humor analyses and identification of tumor biomarkers have the potential to renew the management of retinoblastoma. TFF1 is ectopically expressed in a subset of more advanced RB tumors and its expression correlates with a higher risk for metastases. Taken together we provided evidence for TFF1 expression in the AH of RB patients, strongly suggesting TFF1 as a clinically interesting new RB biomarker.

Inflammatory Bowel Disease caused by desmoglein 2 cytoplasmic truncation

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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.

C

Eye immobilization but not visual deprivation affects palisade endings development

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Palisade endings are unique nervous end organs and are regularly present in extraocular muscles (EOMs) of frontal-eyed animals (human, monkey, cat, dog, ferret). In a previous study, we have demonstrated in cats that palisade endings develop postnatally in a heterochronic sequence. In the present study, we tested if visual deprivation and/or eye immobilization affect the palisade endings development.

The right eye of 3 newborn cats was covered until 30 days from postnatal day 7 (before eye opening) and both eyes of 3 newborn cats were covered until 45 days also from P7. To block eye movements, 7 cats received a Botulinum Neurotoxin-A (BoNT-A) injection at birth into the retrobulbar space of the left orbit and had a survival time of 45 or 95 days. For analysis, EOM whole-mount preparations were triple-immunolabeled with anti-neurofilament, anti-synaptophysin or anti-growth associated protein 43 (GAP43), and phalloidin, and examined with a confocal laser-scanning microscope.

Following unilateral and bilateral dark rearing, palisade endings were qualitatively and quantitatively equal to palisade endings from age-matched controls. After BoNT-A induced eye immobilization for 45 or 95 days, palisade endings were absent in the superior and lateral rectus and only present in the inferior and medial rectus. BoNT-A treated palisade endings were rudimentary and their number was reduced. Additionally, the expression of GAP43 was significantly reduced in BoNT-A treated palisade endings.

This study demonstrates that eye immobilization, but not visual deprivation, affects the development of palisade endings.

Characterization of circadian modulators in relay stations of prefrontal-to-hippocampal circuits via laser microdissection

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Circadian strains can impair cognitive function, possibly by disrupting prefrontal cortex (PFC) to hippocampus (HIP) information flow. To relay information from the PFC to the HIP two major pathways exist, involving projections from the supramammillary area (SUM) to CA2 and the dentate gyrus (DG) and from the Nucleus reuniens (NRe) to CA1 of HIP. In this study, we analyze the inputs of circadian neuromodulators to SUM and NRe cell populations participating in the PFC-HIP- circuit.

In order to identify receptors of the orexinergic, histaminergic and purinergic system in these pathways, we tagged cell populations in SUM and NRe with PFC-anterograde transsynaptic or DG-retrograde viral tracing. Tagged and non-tagged population cells were isolated using laser single-cell capture and the mRNA expression levels of selected receptors were determined with qPCR for each neuron population.

Neuron populations in NRe receiving projections from PFC showed significantly lower expression of Orexin receptor 1 mRNA and a significant increase in GABA-A receptor (Gabra2 subunit) mRNA compared to non-tagged neurons in the same area. However, SUM neurons that received innervation from DG had significantly less expression of Gabra2 and Orexin receptor 2 mRNA compared to non-tagged neurons.

Using these techniques, we were able to characterize different populations of SUM and NRe neurons and found differences in their expression of orexin receptors and Gabra2. These findings will be considered in future pharmacological studies of SUM and NRe relay function in cognition under circadian strain.

Fibrinogen Regulates Lesion Border-Forming Reactive Astrocyte Properties after Vascular Damage

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Reactive astrocytes at the border of damaged neuronal tissue organize into a barrier surrounding the fibrotic lesion core, separating this central region of inflammation and fibrosis from healthy tissue. Astrocytes are essential to form the border and for wound repair but interfere with neuronal regeneration. However, the mechanisms driving these astrocytes during CNS disease are unknown.

To identify the role of fibrinogen on astrocytic border formation, we used photothrombosis (PT), a mouse model for ischemic stroke that induces a defined cortical lesion with fibrinogen deposition and reactive astrogliosis.

Here we show that blood-derived fibrinogen is enriched at the interface of lesion border-forming elongated astrocytes after cortical brain injury. Anticoagulant treatment depleting fibrinogen reduces astrocyte reactivity, extracellular matrix deposition and inflammation with no change in the spread of inflammation, whereas inhibiting fibrinogen conversion into fibrin did not significantly alter astrocyte reactivity, but changed the deposition of astrocyte extracellular matrix. RNA sequencing of FACS-isolated astrocytes of fibrinogen-depleted mice after cortical injury revealed repressed gene expression signatures associated with astrocyte reactivity, extracellular matrix deposition and immune-response regulation, as well as increased gene expression signatures associated with astrocyte metabolism and astrocyte-neuron communication. Systemic pharmacologic depletion of fibrinogen resulted in the absence of elongated, border-forming astrocytes and increased the survival of neurons in the lesion core after cortical injury.

These results identify fibrinogen as a critical trigger for lesion border-forming astrocyte properties in CNS disease.

Reelin and glioblastoma - evidence that Reelin modulates gap junction activity in tumor cells

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The extracellular matrix protein Reelin, which plays a critical role during neuronal migration and whose malfunction has been associated with a variety of pathologies including Alzheimer's disease and epilepsy is poorly studied in the context of tumor research. The main characteristics of tumors are their autonomous growth, their capacity for unlimited cell division, and their invasive and metastatic behavior. Based on the hypothesis that Reelin not only affects glial cells but also influences astrocytic tumors such as glioblastoma multiforme (GBM). Therefore a glioblastoma cell line was investigated in this study, with focus on migration, invasion, metastasis and cell-cell communication.

In this work, a glioblastoma cell line was used and a variety of cell biological and molecular biological methods were applied. In addition to migration and invasion assays, Western blots were performed with antibodies against various proteins associated with migration and cell communication.

The present study shows that Reelin not only affects proliferation, migration, invasion, and adhesion in tumor cell lines, but in particular promotes gap junction-mediated cell-cell communication (GJIC). Immunohistochemical and quantitative protein expression analyses show that Reelin affects the expression of connexins and their transport to the plasma membrane. Furthermore, incubation with Reelin influences gap junction channel activity. Moreover, we found that recombinant Reelin stimulates signaling pathways that modulate migration, invasion as well as cell adhesion, independently of the canonical Reelin signalling cascade via Dab1.

The results of the present study imply that Reelin affects tumor cell motility and communication by adressing gap junctions, independently of the canonical signaling cascade.

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Connexin 43 levels of equine adipose derived mesenchymal stem cells after treatment with Interleukin-1β

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Osteoarthritis (OA), one of the most common joint diseases in humans and horses worldwide is characterized by progressive cartilage degradation, subchondral bone sclerosis and chronic inflammation [1]. Regarding the cellular level, the expression of Connexin 43 (Cx43) is significantly elevated in the osteoarthritic cartilage compared to healthy cartilage [2].
The aim of the present in vitro investigation was to elucidate whether proinflammatory cytokines such as Interleukin- 1β (IL-1β) might be responsible for the elevation of Cx43 in the damaged cartilage.

Mesenchymal stem cells (MSCs) which are the precursors of chondrocytes were isolated from adipose tissue of horses and cultured in standard medium. 1µl/ml IL-1β was added to the supernatant once a day over a period of three days. Cx43 protein levels were then investigated by means of immunofluorescence and quantitative Western Blot.

MSCs cultured in combination with IL-1β reveled higher Cx43 levels compared to negative controls.

Hence, it can be concluded that IL-1β, one of the main inflammatory cytokines of the osteoarthritic cartilage, leads to elevated Cx43 protein levels in MSCs in vitro. The elevation might cause formation of a high number of hemichannels that could be related to the alteration of cellular functions and tissue homeostasis of the damaged cartilage.

[1]van Weeren, PR.; Back, W. (2016): Musculoskeletal Disease in Aged Horses and Its Management. In: Vet Clin Equine 32:229–247.
[2]Mayan, MD. et al.(2013a): Human Articular Chondrocytes Express Multiple Gap Junction Proteins: Differential Expression of Connexins in Normal and Osteoarthritic Cartilage. In: Am J Pathol. 182(4):1337–1346.

Catecholaminergic system exists in the mouse tracheal epithelium

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Agonists of beta-adrenergic receptors enhance mucocilliary clearing and mucus secretion. However, the source of endogenous agonists acting upon the epithelium remains largely unknown. Here we investigated the expression of catecholaminergic enzymes and the distribution of catecholaminergic nerve fibers and beta-adrenergic receptors in the mouse trachea and isolated epithelial preparations.

Catecholaminergic and tyrosine hydroxylase (TH)-immunoreactive nerve fibers were visualized in cleared trachea whole mounts and tissue sections of C57BL/6J mice by glyoxylic acid-induced catecholamine fluorescence and immunohistochemistry, respectively. Expression of the adrenergic receptors beta1, beta2, and beta3 was investigated by RT-qPCR in the upper, middle and lower parts of the trachea and in abraded trachea epithelium. Expression of catecholamine synthesizing enzymes by the different tracheal cell populations was analyzed in silico using publicly available scRNAseq data.

A dense subepithelial network of TH-immunoreactive and catecholaminergic nerve fibers was identified in the intercartilagenous regions of the trachea, with some of them entering the epithelium. RT-qPCR showed abundant expression of adrenergic receptors beta2 and beta3, but only very low expression of beta1 in the whole trachea, whilst scRNAseq data suggest a rank order of abundance in the epithelium of beta2>beta1>>beta3. In silico analysis revealed prominent expression of AADC (aromatic amino acid decarboxylase) in neuroendocrine cells, but no other catecholamine synthesizing enzyme in any epithelial cell type.

While there is no evidence for local epithelial catecholamine synthesis, the murine tracheal epithelium receives an exceptionally dense catecholaminergic, presumably sympathetic, innervation, which may influence important epithelial functions like mucus secretion and ciliary beat frequency.

Glomerulopathy vs. tubulopathy – comparative pathological and functional analysis of nephrotoxicity caused by calcineurin inhibitors in the rodent model

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Chronic calcineurin inhibitor (CNI) nephrotoxicity is a major drawback in current immunosuppressive regimens. Pathology includes vascular and tubulointerstitial alterations. Although still commonly in use, cyclosporine A (CsA) is increasingly exchanged for tacrolimus (Tac). Data on their differential pathogenetic effects in the kidney are still scarce. We hypothesized that CsA and Tac may differ in their nephrotoxicity.

CsA and Tac were administered chronically in rats and mice. Animals were prepared for high-end morphological analysis, elective immunostaining, and high-throughput technology. Large scale electron microscopy (EM), confocal, stimulated emission-depletion (STED) and 3D-structured illumination (SIM) microscopy were used for pathology. Standard biochemistry, RNAseq, proteomic and phosphoproteomic technology were used to identify gene expressional difference.

In rats, CsA and Tac produced distinct alterations in glomeruli and tubulointerstitium. Both drugs caused □-SMA-positive fibrotic foci with sclerotic glomeruli and damaged tubules to similar extent. With CsA, proximal tubules showed widespread dysmorphic lysosomes with peripheral LAMP1 signal as well as autophagic vacuoles along with high apoptosis rate and diminished albumin uptake. Lysosomal exocytosis was sharply stimulated. KIM1 signal was moderate. With Tac, these changes were far less pronounced, but the incidence of glomerular damage was high. High throughput analysis showed differential changes between CsA and Tac with almost no overlap between the respective spectra. CsA caused upregulation of components of the unfolded protein response and apoptosis, whereas Tac caused excessive RAS stimulation and glomerular deterioration.

CNI nephrotoxicity presented with fundamentally different effects caused by CsA and Tac. While CsA mostly affected proximal tubular integrity, Tac was primarily acting on the glomerulus. Results may serve to better adjust immunosuppressive treatment options in transplant patients.

Foxg1 Organizes Cephalic Ectoderm to Repress Mandibular Fate, Regulate Apoptosis, Generate Choanae, Elaborate the Auxiliary Eye and Pattern the Upper Jaw

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Jaws, the defining feature of gnathostomes, are formed of articulated (hinged), appositional functional units – the upper and lower arcades – that largely arise during development from the cranial neural crest (CNC) of first branchial arch. Gnathostome jaw patterning involves focal instructive signals from the embryonic surface cephalic ectoderm (SCE) to a fungible population of CNC. The spatial refinement of these signals, particularly for those patterning the upper jaws, is not fully understood.

We conducted morphologic and molecular phenotypic analyses of the targeted loss of function in mice of Foxg1 (which is broadly expressed in the SCE overlying the upper jaw primordia but not in the CNC).

We document its requirement for neurocranial and viscerocranial development as well as for the appropriate development and elaboration of the LAMBDOIDAL-junction, choanae, palate, vibrissae, rhinarium, upper lip and auxiliary eye. Furthermore, we demonstrate that Foxg1 regulates intra-epithelial cellular organization, gene expression, and the topography of apoptosis within the SCE. Lastly, we show that Foxg1 controls upper jaw molecular identity and morphologic development by actively inhibiting the inappropriate acquisition of lower jaw molecular identity within the upper jaw primordia.

Our analysis substantiates significant, non-cell autonomous roles outside of neurogenic control for Foxg1 during craniogenesis and implicates focal control of apoptotic cell death in the SCE as a plausible patterning mechanism during jaw development. It further presents a caveat to investigations ascribing biological functions when utilizing the endogenous Foxg1 as a ‘Cre-deleter’ in genetic studies of both neural and non-neural cranial structures.

Bcl11a is essential for postnatal cerebellar development

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The transcription factor Bcl11a is essential for the development of distinct types of mid- as well as forebrain neurons in mice. Recently, mutations of the BCL11A gene in humans were shown to be associated with neurodevelopmental delay, intellectual disability and cerebellar hypoplasia. During human as well as murine cerebellar development, Bcl11a is specifically expressed in Purkinje cells. However, cellular and molecular functions of Bcl11a in cerebellar development remain to be identified.

To determine regulatory functions of Bcl11a in cerebellar development we generated mice with a hindbrain-specific mutation of the Bcl11a gene (Bcl11aflox/flox; En1-Cre) using the Cre-loxP system. For phenotype analysis we focused on cerebellar morphogenesis, proliferation and survival of neurons in Bcl11a mutant and control animals.

Until birth cerebellar development was found grossly normal in Bcl11aflox/flox; En1-Cre mutants, compared to controls. Between P4 and P7 we observed a significant and progressive size reduction of the vermis by 27-44%. Moreover, the overall length of the Purkinje-cell layer was reduced by 26-41%. To further characterize the mechanisms underlying cerebellar hypoplasia, we determined proliferation and cell survival during cerebellar development. At P0, apoptosis was massively increased within Bcl11a mutant Purkinje-cells. In contrast, cell proliferation was unchanged in Bcl11a mutant Purkinje-cells, however, significantly decreased in cerebellar granule cells at P4 and P7.

Our study identified important novel functions of Bcl11a in the control of postnatal cerebellar development in mice. Developmental defects observed in both, mice and humans show striking similarities suggesting Bcl11a knock-out mice to provide an excellent model for analyzing human disease.

Versican GAG-α domain deficiency causes developmental malformations of the retinal pigmented epithelium

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Versican, a chrondroitin sulphate proteoglycan is a major component of the extracellular matrix. It consists of four isoforms (V0,V1,V2,V3) that show molecular weight differences in their respective glycosaminoglycan (GAG) attachment domain. To learn more about the specific function of versican and its isoforms in the retina, we analyzed mutant mice with deficiency in isoform V0 and V2.

VCAN(tm1Zim) mice were investigated with a splice-variant specific gene inactivation of V0/V2 resulting in GAG-α domain deficiency. We analyzed the eyes of Versican-deficient mice in comparison to wildtype-littermates during development and adulthood. HE-stained paraffin- and semithin-sections were investigated by light microscopy. Optic nerve (ON) axon number and retinal thickness was quantified. The distribution of GFAP and the versican binding partner fibronectin was investigated by immunohistochemistry.

The loss of V0/V2 isoforms resulted in abnormalities and displacement of the retinal pigmented epithelium (RPE), leading to the formation of retinal rosettes, affecting the RPE, outer nuclear layer, and photoreceptor inner and outer segments. Retinal malformations ranging from mild to severe were first seen on postnatal day 1 in mutant mice. Rosette formation was frequently associated with detachment of the sensory retina in adult mice. GFAP immunoreactivity in Müller cells of mutant mice was identical to that seen in wildtype-littermates. Immunoreactivity for fibronectin was dramatically reduced in the retina. Retinal thickness and ON axons showed no difference in mutant mice compared to controls.

Deficiency of the versican isoforms V0/V2 causes retinal changes that indicate its important role in the development and/or differentiation of the RPE.

Impact of ionizing radiation and possible countermeasures on microglia cells in murine organotypic hippocampal slice cultures.

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Galactic cosmic radiation originates outside the solar system and consists partly of heavy ions with high charge and energy. Damage to the central nervous system caused by these ionizing particles is generally considered among the main health risks in long-term space missions. Our working hypothesis is that heavy ion radiation activates the immune cell of the brain, known as microglia. Their over-activation upon prolonged intense radiation exposure can cause secondary effects, like chronic pro-inflammatory signaling leading to neuronal damage. However, we are studying the radiation effect on microglia after a single heavy ion event, occurring during space missions, to investigate their role as a target for possible bio-pharmaceutical countermeasures.

Organotypic hippocampal slice cultures (OHSC) extracted from C57BL/6J pups are cultured for 30 min up to 4 weeks to investigate short- and long-term effects post irradiation. The OHSC are irradiated with a space relevant radiation dose with X-ray or Fe-ions and treated with two potential cannabinoid receptor agonists. The analysis includes immunohistochemical (IHC) and molecular methods to determine abundance, morphology and activation of microglia cells of the hippocampus. Furthermore, cytokines in the supernatant are analyzed to provide information about the inflammatory response.

OHSC cultures were successfully established for irradiation at the heavy ion accelerator facility at GSI (SIS18). IHC staining of the local microglia show morphological alterations after iron irradiation towards an activated phenotype but not after X-rays. The application of a cannabinoid receptor agonist seems to reduce this radiation-induced microglia over-activation.

Ionizing radiation leads to a long-term increased proportion of the activated microglia phenotype in all investigated hippocampal regions. The addition the of the cannabinoid receptor agonist prevents this effect.

Targeting Protein tyrosine phosphatase type E (PTPRE) and miR631 – new chances for chemoresistant retinoblastoma treatment?

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Retinoblastoma (RB) is the most frequent intraocular tumor in early childhood. Chemotherapy resistance diminishes RB treatment options, emphasizing the necessity for new therapeutic approaches. Protein tyrosine phosphatase type E (PTPRE) supports oncogenesis in several cancer entities. This study aims to investigate PTPRE expression, regulatory mechanisms and signaling pathways in etoposide resistant retinoblastoma.

PTPRE and microRNA (miR)631 expression levels were analyzed by Real-Time PCR and/or Western Blot and immunohistochemistry in RB cells and patients. Effects of PTPRE knockdown (KD) were investigated by cell viability, proliferation, apoptosis and in ovo CAM assays. Upstream regulation of PTPRE was investigated by transient miR631 transfection and PTPRE promotor methylation analyses via bisulfit conversion and sequencing. Additionally, phosphorylation status of different protein kinases was investigated by Western Blot analysis.

PTPRE expression is increased in etoposide resistant RB cells. PTPRE KD affects cell viability, proliferation and caspase dependent apoptosis in vitro as well as tumor size in vivo. MiR631 is downregulated in etoposide resistant RB cells and patients. Reduced PTPRE expression and induced apoptosis following miR631 overexpression indicate PTPRE regulation via this miR. Promotor methylation studies showed that PTPRE expression is not regulated by promotor methylation. Additionally, PTPRE KD leads to altered phosphorylation of protein kinases, thereby potentially influencing cell signaling pathways.

PTPRE expression, most likely regulated by miR631, seems to trigger the tumorigenic potential of etoposide resistant RB cells by altering phosphorylation of protein kinases. These results indicate a potential role of PTPRE and miR631 as novel targets for retinoblastoma treatment.

Microbiological evaluation of selected historical anatomical specimens.

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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).

AN ANATOMIST- DETECTIVE, the chemical techniques in the service of museologist

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During inventory work in the basement of the 19th-century building of Department of Anatomy, interest was aroused in the neglected collection of anatomical specimens. The extent of conservation care for them remained unknown.
An interdisciplinary team was formed to undertake conservation measures to save the collection and to carry out research bringing new observations on the effects of preservatives on the condition of the tissues. An important point of the project was the attempt to determine the chemical composition of the historical fluids of the samples

A specialised chemical analysis was carried out on the liquids taken from the specimens classified in both the study and the control group.
The samples were examined for the identity of the substances contained in the preservative fluids.
The analyses were carried out in three stages: in the first part, the liquids were characterised using qualitative chemical reactions with low execution cost. In this step, the pH of the preservation fluids was also determined.
To compare the accuracy of the results, qualitative and quantitative analysis was performed using gas chromatography coupled to mass spectrometry, Fourier transform infrared spectroscopy and optical emission spectroscopy.

The lack of conclusive effects of chemical analyses of the identity of the samples was demonstrated

The results are surprising to the authors of the project. Perhaps the heterogeneous results and the difficulties in interpreting them were due to the diversity of the material analysed. An undesirable effect of the lack of conservation supervision in the study group cannot be excluded either.

Effects of punicalagin on osteosarcoma in the 3D in-vivo tumor model

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Osteosarcomas are rare, malignant and heterogenous bone tumors. Despite current treatment options such as chemotherapy with doxorubicin, cisplatin and high-dose methotrexate, survival of patients has not improved significantly (Lei et al., 2021). In recent studies, punicalagin has reduced viability of osteosarcoma cell lines, tumor-associated inflammation, angiogenesis, and invasion in cell culture trials (Huang et al., 2020). The aim of this study was to examine the effects of punicalagin on osteosarcomas in a 3D in-vivo tumor model.

Human Osteosarcoma biopsies and the osteosarcoma cell line SaOs-2 cells were grown in the 3D in-vivo chorio-allantoic membrane (CAM) model. After a cultivation period of up to 72 hours, the tumors were treated daily with punicalagin or a solvent as a control. Laser speckle contrast imaging (LSCI) was used for blood flow measurements and the weight of the tumors was measured.

Both the SaOs-2 cells (n=10 out of n=15) and the primary material from osteosarcoma biopsies (n= 75 out of n=76) showed good tumor growth on the CAM. Treatment with punicalagin decreased tumor weight and caused a reduction in blood flow around the tumors.

Osteosarcoma cells and primary material were successfully cultivated in the 3D in-vivo CAM model. Treatment with punicalagin decreased tumor weight and reduced blood flow. Additional studies are recommended to further evaluate these aspects.

Function of filopodia like protrusions in chicken embryos

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Interaction between cells is mediated by direct cell-cell communication via cellular protrusions. These cellular protrusions either extend from the donor cell to release the ligand in close vicinity of the recipient cells. Vice versa, receptors, which are located at the protrusions, can be transported to the ligand secreting donor cells to fetch the signal. We identified and characterized cellular protrusions, so called filopodia, which carry the Wnt receptor Fzd7 and extend from the epithelial somites to the overlying ectoderm expressing Wnt6, during chicken embryonic development. These data suggest that Fzd7 positive filopodia fetch the Wnt6 signal in order to control the process of epithelial-mesenchymal transition, which is essential for somitic differentiation and accordingly for the formation of dermis and myotome.

In order to test this hypothesis we aimed to inhibit the formation of protrusions specifically and to analyze the subsequent somitic differentiation pattern. Since the formation of cellular protrusions requires the reorganization of the actin-cytoskeleton, which is controlled by RhoGTPases. We manipulated the RhoGTPase signaling pathway using electroporation in the chicken embryo.

We show that overexpression of the constitutive active forms of RhoGTPases Rac1 and Cdc42 leads to a significant reduction in the number of protrusions and results in disintegration of the somitic, epithelial structure.

These data confirm the hypothesis that the Fzd7 positive protrusions are required for proper somite differentiation.

Localization of mast cells in the rodent cochlea

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Mast cells (MCs) are myeloid leukocytes involved in the innate immune response and the immune reaction to allergens. They are localized in mucosal and connective tissue and have been detected in the endolymphatic sac of the inner ear. MCs could potentially be involved in inner ear homeostasis and maturation, but it is currently unknown whether MCs are also localized in the cochlea. This study aimed to assess rodent cochlear tissues for the presence of MC using immunohistochemistry.

Cryosections and flat cochlear explants were prepared from the C57BL/6 mice and Wistar rats (P3-P30). Samples were immunohistochemically stained using avidin and specific primary antibodies against c-Kit/CD117, chymase, tryptase, and FcεRIα and fluorescently labeled secondary antibodies. Stained sections were examined under the epifluorescent and confocal microscopes.

MCs were detected in the different regions of the rodent cochlea (P3-P30), particularly in the modiolus and the spiral limbus. Occasional avidin positive staining was also observed close to Reissner's membrane of the scala vestibuli. No MCs were detected in the organ of Corti. The cochlear MCs expressed c-Kit/CD117, tryptase, chymase, and FcεRIα. The reducing of MC numbers was observed with progressing postnatal maturation and after cisplatin exposure (40 µM during 24 h).

The results demonstrate the presence of MCs in the rodent cochlea´s modiolus, spiral ligament, and stria vascularis. We speculate that cochlear MCs are involved in the maturation and homeostasis of the auditory system. This discovery adds information to the field of cochlear physiology and may lead to the identification of novel roles of MCs in the cochlear development and pathophysiology. Future clinical studies should verify these results obtained from the animal models.

The concept of meso-esophageal excision and topographic-anatomical navigation in the surgical treatment of esophageal cancer.

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To develop topographic and anatomical navigation for performing video-assisted thoracoscopic extirpation of the esophagus, taking into account embryo-oriented surgery with the patient in pron-position.

An anatomical experiment was conducted, including 30 human cadavers to identify reference anatomical landmarks in the posterior mediastinum (in accordance with the protocol of the Local Ethical Committee № 01-21 от 22.01.2021) extirpation of the esophagus: 8 patients using topographic and anatomical navigation and 15 patients using the routine method.

The reference points visible before opening the mediastinal pleura are: v. azygos, arc v. azygos, pulmonary ligament, vagus nerve, esophagus. After opening the mediastinal pleura, access to the anatomical structures located in the fiber and not available for direct visualization is opened: tracheal bifurcation, thoracic aorta, superior vena cava, right recurrent laryngeal nerve, sulcus azygoaortalis, thoracic lymphatic duct, inferior pulmonary vein, lymph nodes of the posterior mediastinum.
Sequential tissue preparation - from superficial to deep - allows to perform an adequate amount of lymph node dissection (14±2 in the "without navigation" group vs 17±3 in the "anatomical navigation" group). Combination of the plane of surgical resection with the mesoesophageal layer allows to increase the adequacy of dissection of paraesophageal tissue with signs of perineural (12 (80%) vs 6 (75%)) and lymphovascular (11 (73.3%) vs 7 (87.5%)) invasion, which increases the oncological radicality.

Topographic and anatomical navigation makes it possible to combine the planes of surgical resection with the embryonic mesoesophageal layer during extirpation of the esophagus in compliance with the principles of oncological radicalism.

E

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

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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.

Mechanisms causing acantholysis in pemphigus-lessons from human skin

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To summarize our studies in which we characterized the roles of signaling pathways in the pathogenic effects of PV-IgG on desmosome ultrastructure.

Ex vivo human skin model
Transmision electron microscopy

Inhibition of p38MAPK, ERK and PLC/ Ca2+ to be protective in human epidermis. In contrast, inhibition of Src and PKC, which were shown to be protective in cell cultures and murine models, was not effective for human skin explants.

The ultrastructural analysis revealed that for preventing skin blistering at least desmosome number (as modulated by ERK) or keratin filament insertion (as modulated by PLC/ Ca2+) need to be ameliorated. Other pathways such as p38MAPK regulate desmosome number, size and keratin insertion indicating that they control desmosome assembly and disassembly on different levels.

Microglia mediate synaptic plasticity induced by transcranial magnetic stimulation

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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.

CRISPR Cas9-targeted Myostatin deletion improves myogenic differentiation and increases muscle mass of muscle stem cells in mouse

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Skeletal muscle wasting with ageing, muscular dystrophy and cancer cachexia causes a serious illness. Blocking of Myostatin (GDF-8) activity augments muscle mass through hyperplasia and hypertrophy of the muscle fibers. The present study investigates the impact of myostatin deletion on myogenic differentiation of C2C12 muscle stem cells by using CRISPR Cas9 gene editing.

Five different loci were targeted for Myostatin depletion using RGEN Cas-Designer. Myostatin locus oligonucleotides were designed and cloned into the px459 vector. After transformation, plasmids targeting Myostatin loci deletion were harvested. C2C12 cells were electroporated with deletion plasmids and single-cell clones were expanded. Myostatin knockdown (Mstn-/-) was evaluated on cell viability and myogenic differentiation compared with control (Mstn+/+) using morphometric analysis, immunohistochemistry and RT-qPCR. Data were statistically analysed.

By sequencing of clones, nucleotides deletion could be detected. Mstn-/- revealed enhanced cell viability and cell proliferation. Three Mstn-/- clones showed an upregulated MyoD expression compared with Mstn+/+. An increased Myogenin expression in both proliferating and differentiating cells of Mstn-/- compared with Mstn+/+ clones were detected. Mstn-/- clones showed an enhanced upregulation of fast myosin heavy chain expression compared with Mstn+/+. Mstn-/- showed a higher expression of ActRIIb and mTOR in the differentiated cells compared with Mstn+/+. Using immunohistochemistry, Mstn-/- showed increased MyoD and Myogenin positive cells in both proliferation and differentiation conditions.

Our data provide evidence that Myostatin deletion using CRISPR Cas9 improves myogenic differentiation thus, targeting myostatin could be a beneficial therapeutic strategy to restore muscle mass loss.

Fibronectin deficiency in newborn mice leads to the loosening of the interstitium and cysts formation in the kidney

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The ubiquitously expressed glycoprotein fibronectin (FN) is a central component of the fibrillar extracellular matrix (ECM) that is found in multiple sites throughout the body including the peritubular interstitium of the kidney. To learn more about the specific role(s) of FN in the kidney we generated and investigated Fn-deficient mice.

We generated CAGG-Cre-ERTM/Fnfl/fl mice which carry floxed Fn alleles and ubiquitously express Cre-recombinase after tamoxifen treatment. Newborn pups were treated with tamoxifen eye drops (2.5 mg/mL) to induce Fn deficiency. Conditional deletion of Fn was confirmed by quantitative real-time PCR, Western blot analysis and immunohistochemistry. The expression patterns of Fn were analyzed by in situ hybridization. Kidneys were investigated by light microscopy and immunohistochemistry.

The expression analyses and immunohistochemistry showed a significantly reduction of FN at postnatal day (P) 4. Loss of FN corelated with the formation of renal cysts at the corticomedullary border, which expand with increasing age. In situ hybridization demonstrated that on P4 Fn expression extends mainly from the pelvis to the corticomedullary border, whereas in 5-6 weeks old mice it is located only in the cortex. Immunohistochemistry and light microscopy showed a loosening of the renal interstitium and additionally an appearance of ECM proteins in the cysts.

We conclude that FN deficiency leads to the development of renal cysts, which occurs a few days after tamoxifen treatment and results in extensive loss of renal parenchyma a few weeks after birth. The results indicate an important role of FN for maintenance of kidney structure and function.

Elastic deformable human brain models copied from donor specimens: didactic value in neuroanatomy education as evaluated by students and teachers

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Thorough comprehension of the human brain is fundamental for neuroanatomy teaching. Fixated brain specimens are not ubiquitously available and rather fragile and inelastic. Plastic models provide excellent tools to demonstrate refined and complex structures, but with limited haptic experience. Practical haptic eperience was emphasized particularly in remote learning during Covid19-pandemic. We evaluated the usability of self-produced elastic brain models.

The models were developed at the Institute of Anatomy in Zurich and serve as complementary tools in neuroanatomy courses at the Universities of Zurich and Bern. For this study, medical students attending 2016-2018 the 2nd year neuroanatomy course voluntarily evaluated the use of the brain models for self-study by a questionnaire. A detailed questionnaire was answered by five experienced teachers.

The models are produced using elastomeric material, which allows exploration, deformation, and repeated manipulation. Their special advantage is the originality of anatomical structures, since they are reproducible copies of body donor brains with all variations. The majority of students rated the didactic value of the brain models very positively, particularly for deeper understanding, and to identify tiny or hidden structures, which they found difficult to detect on fixated anatomical specimens or models. The teachers compared the detectability of refined structures with those on original specimens and rated the models were positively.

We propose this elastic model as excellent tool to support students in neuroanatomy learning, particularly for self-study or limited access to original specimens. We aim at publishing the production procedure to enable reproduction and use by other teaching institutions.

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

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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.

F

Potential involment of the intestinal serotonin – macrophages axis in Parkinson's disease

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To date little is known about the potential alterations of the enteric neuroimmune system in Parkinson's disease (PD). In preliminary transcriptomic analyses, we observed an altered expression of the nonconventional macrophage marker LYVE1 and of HTR2B, a serotonin receptor that has been involved in the regulation of macrophages activity. In order to further investigate potential alterations of this serotonin-macrophage axis in PD, we aimed to characterize LYVE1 and HTR2B localization patterns in the human colonic mucosa and further assessed potential alterations of intestinal mucosal macrophage populations in PD patients.

HTR2B and LYVE1 localization pattern was assessed in full-thickness colon specimens of control subjects using immunohistochemistry. CD68-positive intestinal mucosal macrophages cellular density was assessed in deep rectal biopsies of PD patients in comparison to healthy controls using immunohistochemistry.

We observed that HTR2B, and LYVE1 stain different cell types in the human colonic mucosa, which mostly do not overlap with the macrophage population markers IBA1 or CD68, respectively. HTR2B was mainly localized within enteric neurons, as well as in smooth muscle cells, whereas LYVE1 mainly stained lymphatic vessels. CD68-positive macrophage density remained unchanged in rectal biopsies of PD patients in comparison to healthy subjects.

These preliminary results provide first information on the expression of LYVE1 and HTR2B in the human colonic mucosa under physiological conditions. Whereas intestinal CD68-macrophage density appears not to be altered in PD, further work is required to fully characterize potential alterations of the serotonin - intestinal macrophages axis, including macrophage subpopulations, in the context of PD.

Ultrastructural changes of organoids from head and neck squamous cell carcinomas following irradiation

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Achieving personalization in radiation oncology requires adequate models. Important tools are organoids derived either from fresh tumor tissue specimens of cancer patients or derived subclones from cell lines with different radiosensitivity. Until now, there is only sparse information on the ultrastructure of these organoids. Therefore, we examined untreated and irradiated organoids by transmission electron microscopy to investigate ultrastructural effects of irradiation.

Two patient-derived organoid models from head and neck squamous cell carcinoma (HNSCC) were irradiated with 0, 2 or 2 x 2 Gray. Seven days after delivering the final dose, the PDOs were processed for transmission electron microscopy. The same protocol was applied on the radiosensitive (C46) and intermediate radioresistant (C78) subclones derived from the HNSCC cell line FaDu.

The PDOs recapitulate aspects of the ultrastructural morphology of poorly differentiated squamous cell carcinoma, such as high nuclear to cytoplasmic index, segmented nuclei, well developed cell organelles and nucleoli, high abundance of polyribosomes and low content of heterochromatin. The most striking ultrastructural changes in irradiated organoids included increased number of fragmented cells, heterolysosomes, lipid droplets and membrane blebs. Upon radiation the quantity of microvilli diminished in cells showing signs of apoptosis or necrosis. In FaDu C46, those features were already detectable after a total dose of 2 Gy. The double dose was necessary to induce a comparable effect in FaDu C78.

Transmission electron microscopy reveals distinct ultrastructural changes and different responses to radiotherapy, opening opportunities to study personal radioresistance mechanisms in more detail.

CRISPR/Cas9 screening identified HDAC3 and KLF5 as novel regulators of desmosomal adhesion

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Desmosomes are essential junctions to facilitate strong intercellular adhesion. This adhesive function is impaired in severe diseases such as the autoimmune blistering skin disease pemphigus. We here used unbiased approaches to identify unknown regulatory pathways influencing desmosomal adhesion.

A whole-genome CRISPR/Cas9 screen was applied in a human keratinocyte cell line to identify novel regulators of desmosomal adhesion. Candidates were further analyzed by dissociation assays, promoter pulldowns, luciferase assays, flow cytometry, western blot and qRT-PCR.

The screen identified 56 candidates for positive and 27 candidates for negative regulation. Two potential positive regulators were histone deacetylase 3 (HDAC3) and the transcription factor Kruppel-like-factor 5 (KLF5). Knockout of HDAC3 or HDAC3 inhibition in independent cell lines confirmed a downregulation of mRNA levels of the desmosomal adhesion molecule desmoglein 3 (DSG3), reduced DSG3 levels at the cell membrane and impaired intercellular adhesion. A DSG3 promoter pulldown followed by mass spectrometry outlined KLF5 as putative direct regulator of the DSG3 gene. Knockout of KLF5 resulted in reduced DSG3 protein levels and a reduction of intercellular adhesion. HDAC3 knockdown led to KLF5 upregulation suggesting a functional link between HDAC3 and KLF5. Interestingly, treatment with autoantibody fractions from pemphigus patients strongly increased HDAC3 mRNA and protein levels. Under this disease setting, HDAC3 inhibition led to an induction of the DSG3 promotor and effectively restored intercellular adhesion impaired by pemphigus-IgG.

HDAC3 and KLF5 were identified as novel regulators of DSG3 gene expression and intercellular adhesion and are potential targets for pemphigus treatment.

Degenerative respiratory skeletal muscle as a cofactor of neuromuscular respiratory dysfunction post Covid-19 infection

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Dyspnea is common during and post Covid-19 infection (CI) coming along with cardiovascular dysfunction, whole-body sarcopenia and muscular degeneration (MD). How far MD can be attributed to direct effects of CI or immobility in intensive care and mechanical ventilation remains unknown. A specific evaluation of MD and regeneration in respiratory skeletal muscle (RM), as diaphragm and intercostal muscle, in CI is mandatory.

Pseudonymized pathological routine section-derived samples of RM of 60 diseased in-patients of University Hospital Augsburg, categorized in Covid and Non-Covid patients, including reports of temporary mechanical ventilation, comorbidities and biographical data as gender, age and BMI are collected. Immunohistochemical markers against cellular parameters of skeletal muscle type, intramuscular adipose tissue and satellite cells (skeletal muscle stem cells) and specific parameters of inflammation, degeneration, autophagy and oxidative stress and the evaluation of the ratio of skeletal muscle tissue and intramuscular adipose fat are applied. Stained RM are digitalized, expression profiles evaluated and statistical correlation with biographical data conducted.

Preliminary results will be shown at the poster session since the project and its funding by “Bayerisches Staatsministerium für Wissenschaft und Kunst“ was initiated in 03/2022.

Interpretation of cellular composition of RM in CI patients increases knowledge about principles of MD and neuromuscular dysfunction in the aspect of remodeling capacity and physiological structure of skeletal muscle fibers of the respiratory tract. The intention is to consolidate imaginable options for reducing MD in RM and improving rehabilitative respiratory therapy during and post CI.

Comments -->
'The Topic Area could be changed if desired. Immune Biology as well as Cell Biology would be appropriate.';

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

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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.

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

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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.

Changes in sciatic nerve histomorphometric parameters of the porcine sciatic nerve depending on preservation conditions after traumatic limb amputation

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This study is the first to present a histomorphometric analysis of a porcine peripheral nerve after limb perfusion following a traumatic amputation. The limb was amputated and preserved under different conditions for 6 h thereafter: static storage at room temperature, static storage at 4 °C, and limb perfusion with two different nutrient solutions.

Semithin cross sections (n = 3 / group) of (1) the proximal muscle branch of the sciatic nerve, (2) peroneal nerve, (3) tibial nerve, (4) caudal sural cutaneous nerve were analyzed for myelinated nerve fiber density, fiber diameter, axon diameter, thickness of the myelin sheath, and g-ratio. Values were compared to a non-amputated healthy control.

Preliminary results indicated that use of drugs-supplemented nutrient solution reduces the accumulation of fluids inside the epineurium of the nerve close to cannulation site. This was accompanied by a significant reduction in nerve fiber density in the proximal muscle compared to the healthy control. Further, we detected an increased number of fibers with swollen myelin sheath compared to limbs undergoing static storage at 4 °C, while samples harvested after static storage at 4 °C showed a significant enrichment of fibers with an impaired integrity of the myelin sheath. The paper will present new results comparing different perfusion conditions and solutions that a currently analysed.

The perfusion pressure and composition of the nutrient solution is likely to have an impact on fiber integrity and Schwann cell viability during limb perfusion after traumatic amputation.

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

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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.

Quantitative analysis of Bcl11b expression in GABAergic hippocampal interneurons

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Hippocampal interneurons differ in morphology, molecular properties, innervation patterns, as well as developmental origins. Among other classifications, GABAergic hippocampal interneurons are classified into seven subclasses according to their molecular profile: parvalbumin-, calbindin-, calretinin-, somatostatin-, VIP-, CCK- and NPY-positive interneurons. While the zinc-finger-transcription factor Bcl11b has been shown to be expressed by hippocampal projection neurons and to be critical for development and synaptic stability of the hippocampal mossy fiber system, little is known about the expression and putative functions of Bcl11b in hippocampal interneurons. In this study, the expression of Bcl11b in GABAergic hippocampal interneurons was determined.

GABAergic hippocampal interneurons were identified by GAD67-GFP transgene expression in mice. For quantitative analysis of subclass-specific Bcl11b expression in hippocampal interneurons coexpression analyses using antibodies against GFP, Bcl11b and subclass-specific interneuron markers were carried out.

Bcl11b was detected in 18.3% of GABAergic hippocampal interneurons. Bcl11b expression in interneurons was highest in the dentate gyrus (24.0%), and lowest in CA3 (8.6%). Bcl11b coexpression was detected only in NPY- (20.1%) and in calbindin-positive (14.1%) GABAergic hippocampal interneurons. The majority of Bcl11b expressing interneurons (53.7%) could not be assigned to any interneuron subclass. Interestingly, Bcl11b positive interneurons were preferentially located next the distal portions of dentate granule cell-, and hippocampal pyramidal cell dendrites

In this study, we provide the first, systematic and quantitative characterization of Bcl11b expression in hippocampal interneurons. Based on established subclass markers used in this study, most Bcl11b positive, GABAergic hippocampal interneurons remain unclassified and may represent a previously uncharacterized, discrete interneuron population.

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Repetitive transcranial magnetic stimulation (rTMS) induces plasticity of excitatory synapses in human cortical slices

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Repetitive transcranial magnetic stimulation (rTMS) is a non-invasive brain stimulation technique that is used in clinical practice for the modulation of cortical excitability. While rTMS-based therapies for depression and obsessive-compulsive-disorders are well-established, the cellular and molecular mechanisms of rTMS-induced plasticity remain poorly understood. In this study we sought to investigate how rTMS affects plasticity in human cortical tissue.

We used neocortical access tissue (obtained as part of routine neurosurgical procedures and usually discarded), and generated acute neocortical slices within 10 minutes of tissue extraction. Using single-cell patch clamp recordings, fluorescent and electron microscopy, and molecular biology techniques we assessed the effects of distinct rTMS parameters on synaptic plasticity of layer 2/3 pyramidal neurons in human neocortical slices.

We identified specific protocol parameters that affect the outcome of rTMS on synaptic transmission and plasticity. Particularly, we demonstrated that intermittent theta burst stimulation (iTBS) induces synaptic plasticity of excitatory synapses on layer 2/3 pyramidal neurons. The induction of plasticity in human cortical slices was accompanied by characteristic structural and molecular synaptic changes.

Our results provide the first direct experimental evidence that rTMS induces synaptic plasticity in human cortical tissue. Defining the effects of specific stimulation parameters and identifying the cellular and molecular mechanisms involved in rTMS-induced plasticity in human cortical tissue will support the design of more effective rTMS protocols in clinical settings.

Lipocalin 2 regulates blood-brain barrier integrity under inflammatory conditions

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Endothelial cells are an integral part of the blood-brain barrier. The pathogenesis of multiple sclerosis includes a significant contribution of vascular inflammatory processes in which circulating immune cells enter the CNS. In previous studies, by using mouse models for MS, we identified lipocalin 2 (LCN2) as a molecule that partly protects oligodendrocytes in the cuprizone model. Since LCN2 is a secreted protein and endothelial cells are known to express LCN2 receptors, we hypothesized that LCN2 might additionally stabilize the integrity of the BBB by restoring endothelial permeability under inflammatory conditions.

First, we analyzed astrocytic LCN2 expression at the Glia limitans perivascularis using transmission electron microscopy. Further, endothelial cell cultures were used to investigate LCN2 effects on endothelial permeability under inflammatory conditions. Pro-inflammatory stimuli resulted in a disruption of endothelial barrier as measured in transwell permeability assays and electrical impedance sensing measurements (EIS). Gene and protein expression levels of tight junction molecules and integrins were evaluated in response to inflammatory stimuli and/or additional LCN2 treatment.

Using in vivo MS models, we found astrocytes to express LCN2 in close spatial proximity to endothelial cells, indicating that astrocytes might secrete LCN2 at the astrocyte-endothelial interface. In vitro, endothelial permeability was improved by LCN2 treatment and EIS showed enhanced impedance after co-stimulation with LCN2. Tight junction molecules were stabilized in the cell membrane by LCN2 as observed in immunofluorescence-based time-lapse microscopy.

Taken together, our data indicate a possible role of LCN2 in maintaining BBB integrity during inflammatory lesion formation and progression.

The importance of PLVAP for maintaining peritubular capillary integrity in the kidney

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Plasmalemma vesicle-associated protein 1 (PLVAP) is required for the formation of diaphragms and fenestrae of fenestrated capillaries such as peritubular capillaries (PTCs) of the kidney. We analyzed the long-term effect of PLVAP deficiency on capillary integrity of PTCs in adult mice within a developmental-independent context.

We used 4-week-old CAGG-Cre-ER/Plvapfl/fl mice of C57BL/6 background to induce a PLVAP deficiency via tamoxifen administration (5mg/ml 3x/d for 5 days) and used their Plvapfl/fl littermates as controls. The PTCs were analyzed in 12-week-old mice. We confirmed the presence of PLVAP deficiency in kidneys via Western Blot and real-time RT-PCR. Immunohistological staining against PLVAP of kidney cross-sections was performed. The fenestration was analyzed via transmission electron microscopy.

Western Blot and real-time RT-PCR data confirmed a significant PLVAP reduction of 56-80 %. The degree of PLVAP loss differed between kidney compartments, whereby PTCs of the medulla suffered from a stronger loss than those of the cortex. PTCs showed in selected areas massive endothelial damage.

Our data suggest that some capillaries are more vulnerable to PLVAP loss than others. PLVAP seems to be important not only for maintaining endothelial diaphragms and fenestrae but also for the overall structure and health of the endothelium. In the future, we will use this mouse model to obtain more knowledge on the function of PLVAP and investigate the role of capillary impairment in diseases.

Easily detectable morphological features of the superficial zone of bovine articular cartilage: identifying the base line for the development of a diagnostic tool for early osteoarthritic tissue degeneration

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Aim of this study was to identify easily detectable morphological features of the superficial zone (SZ) of articular cartilage as a basis for the development of a diagnostic tool for early osteoarthritic degeneration.

We used laser-scanning microscopy for the detection of autofluorescence and second harmonic generation (SHG)-signals in a top-down-microscopy, as well as in classical transversal sections of macroscopically intact bovine femoropatellar cartilage. Transmission electron microscopy (TEM) was used for the detection of extracellular vesicles and light microscopy (toluidine blue) for standard histology.

Autofluorescence and SHG microscopy demonstrated layers within the SZ starting superficially with a cell-free collagen-poor layer followed by a distinct autofluorescent, branched net of (most-likely elastin) fibres, which was co-localized with a layer of SHG-positive matrix-components. Autofluorescent signals close to the most superficial cells were strong in belt-like signal accumulations at the cell sides, as well as in "snow cap-like" accumulations on top of the cells, which was absent in SHG-images. The source of these signals is unknown, but preliminary TEM data show increased amounts of extracellular vesicles in a belt-like manner and on top of the cells. The increased autofluorescent ratio on top versus below the cells was topographically specific: in proximal joint regions more than 90% of the cells showed “snow cap-like” autofluorescence whereas distally this feature disappeared, accompanied by changes in cell distribution and tissue layering.

We identified new easily detectable features of the SZ which now need further characterization of their origin and relevance in early degenerative tissue changes.

Vascular Bagging in the White Matter is Related to Aging but Not to Alzheimer-related Changes in the Human Brain

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We could previously show that numbers of vascular bags around microvessels in the white matter of the cerebral hemispheres, a novel microvascular pathology discovered in our laboratory, are significantly increased in aging and in subcortical cerebral small vessel disease (sCSVD) with white matter lesions (WML). Patients with Alzheimer's disease often have mixed vascular pathologies including wide-spread WML, leading to mixed dementia. Therefore, the goal of the study was to investigate the relationship between Alzheimer-related pathology and the development of vascular bagging in the white matter.

Autopsy tissue from a non-selected aged autopsy cohort as well as from an age-matched cohort with different stages of Alzheimer-related pathology was used in the present study. A mid-hemispheric formalin-fixed tissue block was embedded in polyethylene glycol and 100 m-thick hemisphere sections were obtained. Vascular bags were studied by using double-label immunohistochemistry for the simultaneous visualization of collagen IV (COLL4)-positive membranous vascular bags and the endothelial cell layer using UEA-l lectin that labels the endothelial glycocalyx.

Results showed that vascular bagging is a common finding in the white matter of a non-selected aged autopsy cohort and in a second cohort with Alzheimer-related changes. Quantitative analyses of vascular bagging in the frontoparietal white matter indicated a statistically significant correlation between age and the degree of vascular bagging. In contrast, frontoparietal vascular bagging was not associated with different stages of Alzheimer-related changes.

The risk for developing vascular bagging in the periventricular and deep frontoparietal white matter, which is associated with sCSVD, increases with age. However, vascular bagging is not increased in the frontoparietal white matter of randomly selected cases with a pathologically confirmed diagnosis of Alzheimer's disease.

CAR12N bioactive glass scaffolds, developed for cartilage tissue engineering, are biocompatible and allow tissue formation in vivo

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Bioactive glass scaffolds could support cartilage repair by continuous ion release. The novel degradable bioactive glass (patent DE 10 2018 114 946 B3, 2019) CAR12N was designed by the research team to specifically support cartilage formation without hydroxy apatite deposition. Since in vitro results with highly porous CAR12N scaffolds were very promising (Gögele et al., 2021, 2022), in vivo behavior and biocompatibility, were investigated in this study.

CAR12N, CAR12N enhanced by polymer (PLGA) infiltration (patent submitted) orsupplemented with CaO/MgO ions and the commercially available reference BG1393 were dynamically pre-colonized for 7 days with porcine articular chondrocytes (pACs) or undifferentiated human mesenchymal stem cells (hMSCs), before they were subcutaneously implanted (subnuchal) into nude mice for six week. Mice weight development was monitored. Explanted scaffolds were examined according to their size, shape, weight and vitality and histologically. Histopathology of explanted organs (axillary lymph nodes, liver, kidney, lung, spleen) was assessed.

Cell viability of pre-colonized scaffold before implantation was more than 85% and remained high after explantation Mice showed regular weight development and wound closure after scaffold implantation. Explanted organs appeared unaffected by the implants. In contrast to CAR12N variants BG1393, irrespectively whether precultured with cells or not, showed lesser size loss, and shape alterations and higher weights underlining its low degradability. ECM rich neotissue was formed within all scaffold variants with lower ECM density in BG1393 and presence of some foreign body giant cells in CAR12N+PLGA.

All tested BG scaffold were biocompatible allowing neotissue formation in vivo.

Suitability of Fix4Life Cadavers for Medical and Surgical Training and Comparison with Fresh Frozen and Formalin-Fixed Preservation Methods

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For centuries now, cadaver training has been a pillar of surgical education. Two methods of preservation for surgical training are ubiquitous nowadays: formalin-fixation and freezing (fresh-frozen cadavers). Fix4Life (F4L) is a novel fixation method, which promises a long-lasting and lifelike preservation of morphology. The purpose of this study is to evaluate the suitability of F4L cadavers for medical and surgical training and simulation.

A questioner, asking participants to evaluate and rate statements about the morphology and suitability of F4L, formalin-fixed, and fresh-frozen cadavers for surgical training, was given to everybody who has worked with F4L-fixed bodies in our institute during the last 2 years (19 Clinicians and Anatomists).

Of the 19 participants, 16 completed the questioner. F4L was ranked as the top method in terms of realistic gross anatomy, life-like tissue feel, life-like tissue look, and suitability for medical and surgical training. 100% of participants indicated that, given the choice between F4L, Formalin-fixed, and fresh-frozen cadavers, they would choose F4L preserved bodies for their future courses.

F4L provides a comparative, if not in certain aspects better, morphology to formalin-fixed and fresh frozen cadavers. Our survey showed that Fix4Life cadavers are not only a suitable model for surgical and medical training, but might even provide a better model than fresh-frozen and formalin-fixed cadavers. As this method of fixation provides benefits not conveyed by other methods of preservation, especially for training within the neurosurgical domain, further studies should be conducted to establish validation, safety, and standardization.

Vascular colored latex injection of the hand as a facilitator for the anatomical study

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Understanding the vascular anatomy of the hand is challenging for every medical student and medical resident. A comprehensive knowledge of anatomy is the centerpiece for good results at hand surgery, maximizing success and minimizing complications. The best study method is the careful dissection of human cadaveric specimens. The injection with colored latex of the vascular system allows arteries and veins to appear more prominently, resulting in a substantial facilitation of the dissection process.

Four cadaveric specimens underwent colored latex injection of the vascular system of the hand at the radial and ulnar artery and veins for subsequent dissection. A survey was carried out among students and medical residents comparing the easiness of anatomical dissection and study between hands with and without latex injection.

Tissue penetration of the latex was excellent in all specimens with a clear improvement of the malleability and vascular identification. Among the students and medical residents, 100% agreed that this technique improved the quality of the hand dissection and facilitated their anatomical study.

We consider that injection of colored latex, reaching small caliber vessels, and giving tissues a greater malleability and flexibility, allows facilitated dissections and exposure and makes them suitable for improved anatomy and surgery teaching.

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

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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.

Time lapse imaging of single granule cells in the mouse dentate gyrus after entorhinal denervation in vitro – identification of different response types to denervation

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The reorganization of synaptic connections is an important mechanism contributing to the recovery of neuronal networks following brain injury. In recent years, we established an in vitro denervation model using organotypic slice cultures to visualize structural changes of dentate granule cells (GC) following entorhinal denervation. Here we used this model to analyze spine density changes of denervated and non-denervated dendritic segments of single GCs.

AAV-injections were employed to transduce dentate granule cells with tdTomato and entorhinal projection neurons with GFP. This allowed us to visualize both innervating entorhinal fibers and their target neurons. Furthermore, we could readily distinguish segments innervated by entorhinal fibers (in the outer molecular layer) from those receiving other afferents (in the inner molecular layer). Entorhinal lesion was performed at 18-19 DIV and time-lapse confocal imaging was used to visualize single dendritic segments of the same neuron. Non-denervated cultures imaged at the same time points served as controls.

As previously reported, average dendritic spine loss was 30-40% of all spines (2-4 days post lesion) in the denervated zone, whereas average spine density remained constant in the non-denervated layer. Individual neurons showed a broad variability in their reaction to denervation in both layers. Different types of reactions were identified and distinguished.

Our data suggest that more extensive sampling strategies, i.e. sampling of several segments of one neuron, may result in more robust results than the sampling of single dendritic segments, since dendritic segments of a neuron may differ considerably in their response to denervation (supported by DFG).

Microstructure and mechanics of the periodontal ligament

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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.

Urethral chemosensory cholinergic cells express components of the leukotriene- and prostaglandin-synthesis pathway

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Urethral cholinergic chemosensory cells (UCCC) serve as sentinels initiating protective mechanisms. The aim of this study was to determine whether UCCC express the synthesis pathways for leukotrienes and prostaglandins, including 5-lipoxygenase-activating protein (FLAP) and cyclooxygenase-1 (Cox-1).

Next generation sequencing (NGS) data of UCCC were screened for expression of proteins relevant to prostaglandin and leukotriene synthesis. The (co-)expression of FLAP and ChAT, α-Gust, PLCβ2 and TRPM5 as well as Cox-1, ChAT and FLAP in UCCC was investigated immunohistochemically in tissue sections. The basal prostaglandin concentration was compared by LC-MS/MS (liquid chromatography with mass spectrometry), the cysteinyl-leukotriene concentration after stimulation with the bitter substance denatonium (5 mM) was investigated by ELISA (Cys-LT ELISA).

NGS revealed mRNA expression of proteins relevant for the prostaglandin and leukotriene synthesis. 50% (m, n=54 cells)/63%(f, n=30) of UCCC (marker ChAT+) were FLAP+. 54% of α-Gust+ cells, 74% (m, n=136)/69% (f, n=486) of PLCβ2+ cells and 69%(m, n=101)/49%(f, n=358) of TRPM5+ cells were FLAP+. ChAT and Cox-1 were colocalised in 63% (m, n=63)/75% (f, n=39), Cox-1 and FLAP in 63% (m, n=39)/69% (w, n=54). After stimulation with denatonium, there was no significant increase in Cys-LT concentration in the supernatant. LC-MS/MS showed no significant difference in prostaglandin concentrations between POU2F3-/- and C57BL/6 mice.

A subpopulation of UCCC expresses elementary components of the prostaglandin and leukotriene synthesis, both by expression analysis and immunohistochemistry. With an initial high baseline of measured leukotrienes, the precise function of the prostaglandins and leukotrienes released from UCCC remains unclear and requires further investigation.

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

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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

Morphological and Physiological Characterization of Subicular Principal Cells

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The subiculum is an output region of the hippocampal formation relaying information between the hippocampus proper and entorhinal cortex. While a divergence along its proximo-distal axis has been recognized by early studies, there is increasing evidence for further spatial subdivisions (Ishihara et al., 2020) and a high molecular heterogeneity of its principal cells (Cembrowski et al., 2018). Indeed, a neuroanatomical characterization of the subiculum indicates that the distal part of this area (subiculum-1) diverges in its cytoarchitecture, neuronal density and immunocytochemical staining pattern from the proximal parts (subiculum-2): it has a dense homogenous cell population, with consistent expression of fibronectin-1 (FN1) in contrast to the sparse, layered cytostructure largely lacking FN1 immunoreactivity. In contrast, parvalbumin positive axonal arborization of putative basket cells was found to be high in subiculum-2 but low in the subiculum-1, suggesting a differential organization of the inhibitory system in these two subregions.

Therefore, in this study we investigated the electrophysiological and morphological characteristics as well as the inhibitory input of its principal neurons of these two regions in a comparative manner by using whole-cell recordings in combination with intracellular filling in acute mouse slices. To analyze the inhibitory input from PV-positive basket cell, photostimulation was performed in slices from transgenic mice expressing channel rhodopsin-2 under the parvalbumin promoter.

Preliminary results indicate that neurons of the subiculum-1 and subiculum-2 diverge in specific morphological characteristics: cell body size, dendritic length and spatial spreading of the apical dendrite, but their intrinsic physiological properties are largely comparable.

Sub1 and sub2 neurons diverge in their morphological and electrophysiological properties.

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Role of the GIP receptor in retinoblastoma

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Retinoblastoma (RB) is the most common malignant intraocular tumor in early childhood. The gastric inhibitory polypeptide receptor (GIPR) is a member of the 7-transmembrane G protein coupled receptor family, expressed in beta-cells of the pancreas but also in the nervous system. GIPR was linked to human pathologies, particularly carcinomas. Most recent data by our group showed that GIPR is upregulated in RB cells following overexpression of trefoil factor family peptide 1 (TFF1), a potential new biomarker for more advanced RB subtypes.

In the study presented, the role of GIPR in RB cell tumorigenicity was investigated. Effects of lentiviral GIPR overexpression on RB cell viability, proliferation and apoptosis were analyzed by WST-1 assays, BrdU immunocytochemistry, DAPI cell counts and growth curve analyses. The in vivo tumor formation capacity of RB cells overexpressing GIPR was examined via chicken chorioallantoic membrane (CAM) assays. Additionally, GIPR downstream mediators were studied via real time PCR.

Functional analyses showed a significant reduction in cell viability, cell growth and proliferation, and a concomitant increase in apoptosis after GIPR overexpression. Furthermore, in ovo CAM assays revealed a decrease in RB tumor weight and size.

Overall, our results show that overexpression of GIPR decreases the tumorigenic potential of RB cells by diminishing cell viability, proliferation and survival. This indicates that GIPR and downstream mediators may be a new potential target for the treatment of retinoblastoma. Future studies will address further details in GIPR signaling pathways especially in the context of TFF1 and probably identify more targets for therapeutic interventions.

Responsiveness to and synthesis of sex hormones in male and female microglia

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Synaptic pruning after birth, aimed at elimination of weak synapses for the establishment of an adult synaptic network, has been shown to be a function of primarily microglia activity, aside from astrocytes and neuronal autophagy. Microglia, in turn, are differentiated in a sex-dependent manner. Given this background, we analyzed whether sex-specific differentiation of microglia includes sex specific sex steroid receptor expression, and/or sex specific differences in the equipment of enzymes, responsible for either estradiol (E2) or dehydrotestosterone (DHT) synthesis.

Using MACS sorted microglia (CD11b positive) from adult C57Bl6/J mice of both sexes, we analyzed transcription levels of the sex steroid receptors, estrogen receptor (ER) ALPHA and BETA, G-Protein coupled ER 1, androgen receptor and zinc transporter ZIP9 as well as transcription levels of aromatase, being essential for the synthesis of E2, and of 5 ALPHA-reductase 1-3, being essential for the synthesis of DHT

For none of these targets we found a sex specific difference in transcription levels, but independent of sex we found an unexpectedly high expression of 5-reductase 3 and ER ALPHA in sorted microglia as compared to remaining cells in the flow through. Consistent with previous studies we found no expression of aromatase, estrogen receptor-BETA and 5 ALPHA-reductase 2 in microglial cells from adult mice.

Surprisingly and unlike reports on sex differences in microglia, we found no sex differences in surveilling microglia regarding transcription levels of sex steroid receptors and sex steroid synthesizing proteins.

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

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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.

Solitary chemosensory cells in the respiratory tract of man

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Specialized sensory epithelial cells sensing noxious chemicals and initiating protective reflex are found along the mammalian respiratory tract. Studies in mice suggested the presence of at least two populations: 1) neuroendocrine cells (marker: PGP9.5), 2) solitary cholinergic chemosensory cells (SCCC) (brush or tuft cells; markers: GNAT3, PLCβ2, TRPM5, POU2F3). In humans, a positional and sensory signaling pathway focused inventory of SCCC is yet missing.

Single- and multiple-labelling immunofluorescence with relevant marker antibodies was performed on human vallate papillae (positive control) and respiratory tract (nose, trachea, lung) obtained from anatomy body donors and pathology. Pig trachea and lung were studied for comparison; TRPM5-eGFP reporter and C57BL6/J mice served as reference. Publicly available scRNAseq data were analyzed in silico.

PLCβ2-antisera labelled cells in human taste buds, but not in the respiratory mucosa; TRPM5-, POU2F3- and GNAT3-positive cells were not found. Accordingly, in silico-analysis revealed minimal expression of these markers in human epithelial cells, opposite to mice. Guided by scRNAseq data, LRMP-antibodies (lymphoid-restricted membrane protein) were used and labelled sensory cells in taste buds and rare slender epithelial cells along the entire airways with predominance in bronchioli (0.342 cells/mm; trachea: 0.046). Multiple-immunolabeling established them as separate entity, distinct from ciliated, secretory, neuroendocrine cells and ionocytes. In pig the distribution is similar to human. Mice LRMP was also localized specifically to SCCC and restricted to extrapulmonary airways.

These data identify chemosensory cells in human and pig airways with predominant intrapulmonary (bronchioli) localization, substantially different from mice.

A Novel High-throughput Screen to Identify Modulators of Cell-cell Adhesion

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Defective cell-cell adhesion contributes to the patho-mechanism of various diseases including Arrhythmogenic Cardiomyopathy (ACM). Here, mutations mainly in components of the desmosomal adhesion complex cause arrhythmia with ventricular fibrosis and impaired cardiac function up to sudden cardiac death. A mouse model recently developed by our group demonstrate that loss of cell-cell adhesion is an important initial step leading to the ACM phenotype. In this study we established a high-throughput screening method to identify pro-adhesive compounds from a drug library aiming for new therapeutics to restore intercellular adhesion under pathological conditions such as ACM.

We developed an adhesion-based high-throughput approach by adapting a standard cell-cell dissociation assay to 96-well plate format including establishment of an automated acquisition and analysis pipeline. This method was applied to screen a FDA-approved drug library with 1946 compounds in three concentrations. To model loss of intercellular adhesion, cells deficient for the desmosomal adhesion molecule desmoglein-2 were used.

After establishing settings to sensitively detect changes in cell-cell adhesion, the FDA-approved drug library was screened. Several new compounds strengthening intercellular adhesion were identified in addition to known pro-adhesive drugs, confirming the robustness of our assay. The increased adhesiveness of selected top hits was confirmed in cells expressing ACM patient mutations.

We developed an adhesion-based high-throughput approach capable of identifying adhesion modulators which have the potential to be used in therapy of diseases with defective desmosomal adhesion such as ACM.

GABAB Receptor-mediated effects in VIP-Expressing Interneurons of the Dentate Gyrus

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GABA acts in addition to ionotropic GABAA via metabotropic GABAB receptors. GABAB receptors mediate diverse effects in cortical networks and are highly expressed in INs, however their action in VIP-INs has not been studied.

Acute slices from transgenic mice VIP-ires-Cre :: Ai9 line (P18-25).
Whole-cell recording combined with intracellular biocytin-filling.
Bath application of baclofen (10 uM) to activate GABAB receptors; followed by the antagonist CGP 55945 (5 uM).

Bath application of the agonist baclofen (10 µM) induced a hyperpolarization and reduced excitability of VIP-INs. This effect was underlied by an outward whole-cell current of 25.12 ± 6.2 pA in DG VIP-INs which was fully reversed by the application of the antagonist CGP (5 µM).

These results suggest that postsynaptic GABABRs are highly expressed and modulate the activity of VIP-INs of the DG.

Role of BMPs for the development of the eye field

Stephan Heermann (Department for Molecular Embryology, Institute for Anatomy and Cell Biology, Freiburg)

Vision is our most prominent sense and a correct development of the light sensing organ, the eye, is at its basis. Previously, we identified a “bilateral neuroretinal flow” shaping the optic cup, a process sensitive to bone morphogenetic protein (BMP). A precocious arrest of this flow results in a specific form of coloboma, a morphogenetic coloboma. In general, coloboma can be associated with Holoprosencephaly (HPE) a failure in forebrain splitting. Here, we address whether forebrain splitting is sensitive to BMP signaling.

We use transgenic zebrafish (Danio rerio), in vivo induction of a BMP ligand, in vivo imaging and whole mount in situ hybridizations.

Early induction of a BMP ligand results in Anophthalmia with retinal precursors stuck in the brain. Analysis of the Anterior Neural Plate (ANP) indicates a defect in forebrain splitting but also a loss of the transcription factor rx3, a key factor of the eye field. Shh, a major player for HPE development is only affected mildly.

Our data strongly indicate that also the process forebrain- and eye field splitting is sensitive to BMP signaling indeed putting our data into the context of holoprosencephaly. It is yet unclear why the remaining retinal progenitors were incapable to form one central optic cup, and a cyclopic eye. In the future we are going to further address the mechanism underlying the phenotypes of anophthalmia and cyclopia both hallmarks of severe forms of holoprosencephaly.

The well known arterial variation Corona mortis is still an unpredictable source of bleeding at the pelvic inlet – an anatomical study

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The Corona mortis is a well known enlarged anastomosis between the territory of the obturator artery and the external iliac artery. Its injury can lead to death in the worst-case scenario. However, exact anthropometric data are still lacking. The aim of this study was to quantify the diameter and level of origin of the Corona mortis. In addition, the diameters of the inferior epigastric and regular obturator arteries were determined.

The external and iliac arteries and their branches were dissected bilaterally in 75 embalmed specimens (37 females, 36 males). Measurements were performed using two different methods.

The Corona mortis was present in 36 of the 150 hemipelves (24%), in one third of cases bilaterally. It originated between 4.4 and 28.3 mm from the commencement of the inferior epigastric artery. The mean diameters of the regular obturator artery (2.4 and 2.0 mm, respectively) and the Corona mortis (2.5 and 2.1 mm, respectively) were similar. The diameter of the inferior epigastric artery was significantly smaller distal to the origin of the Corona mortis.

The high incidence, non-predictable level of origin of the Corona mortis and its size similar to the regular obturator artery support its clinical relevance. Clinicians should always be aware of an additional arterial vessel close to the pelvic brim.

Immuno-electron microscopy of the extracellular matrix

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Extracellular matrix (ECM) is a complex network of material, in particular, proteins and polysaccharides that are secreted locally by cells and remain closely associated with these to provide structural and adhesive support. However, the ECM is more than a passive mechanical structure. Research in the last decades have demonstrated how the ECM dynamically regulates cellular phenotypes, including diverse cellular processes in development, homeostasis, and disease progression. In addition each tissue has an ECM with a unique composition and topology that is essential for dynamic, reciprocal dialogue between the various cellular components (e.g. epithelial, fibroblast, adipocyte, endothelial elements). ECM therefore offers an indispensable platform for cell-cell communication. Considering the fragile structure of GCX, electron microscopy (EM) seems to be the method of choice for the analysis of ECM morphology. Moreover, immuno-EM enables the study of alterations in the ECM composition during development as well as in pathological conditions.

For immuno-EM samples were HPF-fixed, freeze-substituted and processed for pre-embedding immunogold labeling to analyze the distribution pattern of ECM components.

Immuno-EM protocol optimized in our lab enables detailed ultrastructural analysis as well as high quality immunocytochemistry of well-preserved ECM.

In summary, immuno-EM enables monitoring of morphological changes of unique extracellular components that provides both architectural support and molecular signals to cells and modulate their behaviors. With a better understanding of the morphological homeostasis of ECM and how ECM morphological homeostasis is disrupted in diseases, the ECM is now emerging as a potential pharmacological target for innovative therapeutic interventions.

Collagen Type I Deficient Mice Show a Loss of Myelinated Optic Nerve Axons and Retinal Ganglion Cell Somata

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Susceptibility of optic nerve (ON) axons to glaucomatous damage is likely influenced by mechanical properties of the peripapillary sclera (PPS). Here we investigated mutant mice with a heterozygous deficiency in collagen type I (Col1), the major structural fibrillar molecule of the sclera.

Ubi-Cre3 col1a1 mice lacking one allele of Col1a1 were investigated. Col1a1 encodes for the pro-alpha1(I) chain that is essential for formation of triple stranded Col1. Knockdown of Col1a1 mRNA expression and protein synthesis was analyzed via real-time RT-PCR and Western blot. Ocular phenotype was analyzed by histological staining of sagittal sections. Axial length of enucleated eyes was measured with a digital caliper. Myelinated ON axons were counted in PPD-stained ON cross-sections, whereas RGC somata were quantified on retinal wholemounts with an immunofluorescence staining against RBPMS.

At three months of age, mutant mice showed a significant 50 % reduction of scleral Col1a1 mRNA that resulted in a 75 % reduction of translated scleral COL1A1 at five months of age. Other than a significantly reduced central corneal thickness, mutant mice had an unobtrusive ocular morphology compared to their control littermates. ON axon number of 2-month-old mutant mice was not different compared to their control littermates. However, 5-month-old animals showed a significant reduction in ON axons. The findings correlated with a significant decrease of RGC somata.

We conclude that reduction of Col1 causes a progressive loss of ON axons and RGC somata like that seen in glaucoma. These changes are likely induced by alterations of the mechanical properties of the PPS.

The subpopliteal fat body

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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.

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

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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.

Pemphigus Foliaceus Autoantibodies Induce Redistribution Primarily of Extradesmosomal Desmoglein 1 in the Cell Membrane

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The autoimmune dermatosis pemphigus foliaceus (PF) is predominantly caused by IgG autoantibodies against the desmosomal cadherin desmoglein (Dsg) 1. The exact mechanisms that lead to the characteristic epidermal blistering are not yet fully understood. In the present study, we investigated the fate of membrane-bound Dsg1 after incubation with PF patients' IgG.

We used a variety of biophysical methods such as atomic force microscopy (AFM), stimulated emission depletion (STED) super-resolution microscopy, fluorescence recovery after photobleaching (FRAP) and dispase based dissociation assays.

Dispase-based dissociation assays confirmed that PF-IgG reduced intercellular adhesion in a manner dependent on phospholipase C (PLC)/Ca2+ and extracellular signal-regulated kinase (ERK) 1/2 signaling. AFM revealed that Dsg1 binding on single molecule level paralleled effects on keratinocyte adhesion under the different conditions. STED super-resolution microscopy showed that under control conditions, Dsg1 was found to be in part co-localized with desmoplakin and thus inside of desmosomes as well as extra-desmosomal along the cell border. Incubation with PF-IgG reduced the extra-desmosomal Dsg1 fraction. In line with this, FRAP experiments demonstrated a strongly reduced mobility of Dsg1 in the cell membrane after PF-IgG treatment indicating remaining Dsg1 molecules were primarily located inside desmosomes. Mechanistically, experiments confirmed the involvement of PLC/Ca2+ since inhibition of PLC or 1,4,5-trisphosphate (IP3) receptor to reduce cytosolic Ca2+ reverted the effects of PF-IgG on Dsg1 intra-membrane mobility and localization.

Our findings suggest that during the first 24 h PF-IgG induce redistribution predominantly of membrane-bound extradesmosomal Dsg1 in a PLC/Ca2+ dependent manner whereas Dsg1-containing desmosomes remain.

MORPHOFUNCTIONAL ANATOMY OF OROPHARYNGEAL ISTHMUS DURING SINGING

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Our study aims to evaluate the adaptive changes taking place at the oropharyngeal isthmus during sustained phonation. We focused on exploring the dynamics of the oropharyngeal pavilion in voice professionals using Cone-Beam Computed Tomography (CBCT).

Our study included two women with soprano vocals and two tenor men in 3 different situations: mimed phonation in a neutral position, sustained phonation on vowel /i/, which brings the larynx in a high position and sustained phonation on the vowel /ä/ which brings the larynx in a low position.

By correlating these results with the morphometric and volumetric study of the buccopharyngeal space, we observed particular traits of the professional soprano voice, developing as a series of motor patterns that allowsthe singers to emit high-pitched sound and to maintain them for a long period of time. Our results show that the lateral wall of the isthmic space serves as a harmonica after adequate and prolonged vocal training. Vocal training seems to play a determining role in the functional synergy between the pillars of the palatine veil, which can be observed as a rhythmic motor pattern.

All the linear and volumetric differences we highlighted show important anatomical and functional differences among opera singers, which depend both on the gender of the subject and on their respective training period. The results of our study have direct applicability in practice, addressed to the field of anatomy, physiology, radiology, canto and phoniatry.

Changes in hyaluronan distribution during mouse eye development

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Hyaluronan is a glycosaminoglycan polysaccharide and is reported to have a substantial role in tissue remodeling during development, in tissue homeostasis and in disease, with implication in cell migration and proliferation. Although the multicellular development of the mouse eye is coordinated by a defined regulation of cell differentiation and proliferation, nothing is known about the presence and distribution of hyaluronan. Therefore, we investigated the changes in distribution of hyaluronan during mouse eye development.

Hyaluronan distribution was investigated in the eyes of C57BL6/J wildtype mice during development via immunohistochemistry using a biotinylated-hyaluronic-acid-binding-protein. We investigated mice on postnatal day (P)1, P5, P15, P21 and in adulthood. The different eye tissues were analyzed by fluorescence microscopy.

We could detect a hyaluronan fluorescence signal on P1 and P5 in the cornea, the ciliary body epithelium, the sclera, the choroid, and the inner plexiform and ganglion cell layer of the retina. In addition, on P5 hyaluronan was found in the iris. In contrast, at P15, the hyaluronan signal intensity was persistent in the ciliary body epithelium, sclera, and iris, but dramatically reduced in the layers of the retina and choroid and completely absent in the cornea. Furthermore, in the adult eye hyaluronan showed a similar distribution, but in addition it was detectable in the inner limiting membrane of the retina. Hyaluronan was not detectable in the interphotoreceptor matrix in all analyzed developmental stages.

The distribution of hyaluronan in the mouse eye changes during development indicating a role of hyaluronan in different morphogenic mechanisms.

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

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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.

Neurodevelopmental Cohen syndrome and VPS13B

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Four mammalian orthologues (VPS13A-D) belong to the Vps13 protein family, named after their common ancestor in yeast. Mutations in VPS13B cause neurodevelopmental Cohen syndrome, which is mainly characterized by postnatal microcephaly, intellectual disability, a typical facial gestalt, retinopathy, and neutropenia. Previously, we established VPS13B as Golgi-associated protein maintaining Golgi ribbon structure and showed that association of VPS13B with the Golgi complex demands RAB6 activity.

Here, we discuss our ongoing research on VPS13B in the context of the recent identification that Vps13 proteins tether membrane contact sites to facilitate lipid exchange.

We hypothesize that VPS13B serves as a tether protein between laterally associated Golgi membranes and between the Golgi and the endomembrane system in general. In addition to Golgi disruption, loss of VPS13B induces severe disorganization of cytoskeletal elements and diminishes microtubule-associated transport.

Thus, we expect that VPS13B also connects membranes to the cytoskeleton to maintain Golgi structure and Golgi-associated intracellular transport. Our results will significantly contribute to the general understanding of Golgi-associated processes in neurodevelopment and neurodegeneration. Together with proteomics of VPS13B and histological analyses of the Vps13b mouse model, our results will advance the pathomechanistic understanding of Cohen syndrome.

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Cholesterol-dependent cytolysins boost neuroinflammatory response in meningitis through enhanced endocytosis

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In the course of acute pneumococcal/bacterial meningitis, initial bacterial growth in the cerebrospinal fluid is followed by bacterial lysis, the release of toxic factors, and subsequent neuroinflammation. A key pathogenic process in this disease is the strong neuroinflammatory response. The aim of the current work was to study the role of the major neuropathogenic factor pneumolysin in the initiation of the neuroinflammation.

We used cell cultures, live imaging with fluorescence markers, electron microscopy and animal models to study it.

Primary murine astrocytes and microglia were exposed to Streptococcus pneumoniae (pneumococcal) lysates, leading to strongly elevated proinflammatory cytokine (TNF-alpha, IL-6) and chemokine (CXCL-2/MIP2) production. Pneumococcal lysates enhanced dynamin-dependent endocytosis, and dynamin inhibition blocked the neuroinflammatory response, confirming the importance of ligand internalization in both astrocytes and microglia. We identified the cholesterol-dependent cytolysin pneumolysin as the key pro-endocytic factor of the lysates. Knocking out pneumolysin eliminated their pro-endocytic effects and diminished the neuroinflammatory response. Only pore-competent recombinant pneumolysin enhanced cellular endocytosis in a dynamin-, PI3K- and potassium-dependent but calcium-independent manner.Endocytosis enhancement was limited to toxin-exposed parts of the membrane, suggesting a compartmentalized response. Finally, we treated mice with acute pneumococcal meningitis with the neuroleptic drug chlorpromazine, a therapeutic with a complementary endocytosis inhibitory effect that crosses the blood–brain barrier. The animals demonstrated a diminished neuroinflammatory response, confirming the pathophysiological significance of the mechanism.

Our work reveals a new pathogenic mechanisms leading to enhanced neuroinflammation by a cholesterol-dependent cytolysins, enhancing the endocytosis and the internalization of proinflammatory ligands into glial cells.

J

Impact of constant light on inflammasome expression in the mouse hippocampus

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Exposure to artificial light at night (ALAN) acts by inducing circadian disruption, and thus is linked to an increased incidence of diseases in modern society. Alterations of circadian rhythms and dysregulation of immune responses, including inflammasome activation, are common attributes of neurodegenerative diseases including Alzheimer's, Parkinson's and Huntington´s disease. Inflammasomes are multiprotein-complexes required for the processing of proinflammatory IL1beta and IL18. Activation of inflammasomes is a tightly regulated process that involves two steps: priming and activation. However, direct effects of circadian disruption on inflammasome activation in the CNS remains poorly understood.

Mice were exposed to standard light or ALAN conditions for 2 weeks. Methods including locomotor activity rhythms analysis, body weight control, white blood cell composition, Realtime PCR, Western Blot and Immunofluorescence were applied to study circadian disruption and inflammation.

Significant detrimental effects on behavioural parameters, such as reduced locomotor activity and decreased circadian power were observed. Other parameters, such as body weight and white blood cell composition were rather unaffected. Active caspase 1, but neither phospho-NF-kB nor interleukins were significantly elevated in hippocampal tissue from mice exposed to ALAN.

Our findings suggest that exposure to ALAN alone may lead to inflammasome activation, but not priming, which requires NF-kB activation and upregulation of precursor cytokines. However, an already assembled "ready to start" inflammasome in response to circadian disruption may intensifies neurodegenerative processes and associated inflammatory processes.

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

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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.

K

Transmembrane protein 119 is neither a specific nor a reliable marker for microglia

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Microglia are the resident innate immune cells of the central nervous system (CNS) parenchyma. To determine the impact of microglia on neurodegenerative and neuroinflammatory diseases, it is essential to distinguish microglia from recruited peripheral macrophages/monocytes. Several studies suggested transmembrane protein 119 (TMEM119) as a robust microglia marker that reliably discriminates resident microglia from blood-derived macrophages in the CNS. Recent studies indicated a decreased TMEM119 expression during early microglia activation. Therefore, we hypothesized that TMEM119 expression would decline as microglia activation increases under various neuropathological conditions.

In this study, we investigated the suitability of TMEM119 as a microglia marker in four in vivo models of neurodegeneration or neuroinflammation (i.e., cuprizone intoxication, experimental autoimmune encephalomyelitis, permanent filament middle cerebral artery occlusion, intracerebral 6-hydroxydopamine injections) as well as post mortem multiple sclerosis (MS) brain tissues by means of immunohistochemical labelling of ionized calcium-binding adapter molecule 1 (IBA1) and TMEM119.

In all applied animal models, we found increased densities of IBA1+ cells, paralleled by a significant decrease in TMEM119 expression. Decreased expression of TMEM119 was also found in areas of chronic active MS lesions. In addition, other cell types in peripheral tissues (i.e., follicular dendritic cells and brown adipose tissue) were also found to express TMEM119.

In summary, this study demonstrates that TMEM119 is neither exclusively expressed by microglia nor does it label all microglia, especially under cellular stress conditions. This should be considered when working with recently developed transgenic mouse lines using the TMEM119 promotor to label microglia.

Morphological variability of peroneus tertius tendon as a possible factor in fractures of the fifth metatarsal – an anatomical study

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Fractures of the fifth metatarsal are a common injury, in which the peroneus tertius muscle (PT) is suspected to play a role by putting torsional stress on the base of the bone, promoting fractures. However, data about the morphology of its tendons and especially its insertions are sparsely available, making it difficult to assess its role in causing said fractures. This study aims to measure relevant morphological features to help evaluate this theorized role.

50 fixed lower leg specimens were included (41 female, 9 male), age ranged from 62 to 101 years (mean: 84.08 years). They underwent dissection and measurement of length and width of both tendons and their insertions on the fifth metatarsal.

The distance from the base of the fifth metatarsal to the proximal tendon varied between 0 mm and 18 mm. The distance between the insertions of the PT tendons varied between 0 mm and 40 mm. This results in the most distal point of the anterior tendon relative to the length of the fifth metatarsal varying between 22% and 93%.

Our study shows that the PT's proximal insertion is consistently near enough to the base to support the theory that strain on this tendon can cause torsion in the fifth metatarsal. However, the variability of the distal insertion poses the question if the relationship between the two tendons has a, so far, neglected effect on the fractures: a wide-reaching distal tendon could distribute the strain on the bone over a larger area, minimizing torsional stress.

Changes to the subtalar joint in neutral position, plantarflexion, dorsiflexion and non-invasive distraction

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Arthroscopy of the lower ankle joint is a young technique compared to other arthroscopic surgical procedures, which was only described in studies in 1985 and limited in the 1990s in terms of indication and contraindication. The general level of research towards the subtalar joint lags significantly behind compared to that of the upper ankle joint. The purpose of the study is to depict the structural change in the joint gap of the lower ankle joint using radiological images of a 3D-C-arm, to compare them and to analyze the changes different standardised positions.

Twenty matched pairs (n=40) of anatomical ankle specimen were used. All specimens will be mounted in a standardized fashion, 3D radiography was performed in four defined positions (maximal plantarflexion, maximal dorsiflexion, neutral position and non-invasive distraction). All radiographs were be analysed and statistically compared.

Non-invasive distraction led to maximum expansion in the joint gap of the subtalar joint with an average increase of 1.03-1.05mm compared to the other joint positions. Likewise, there was an increase of 0.72-0.85mm on average in pars talonavicularis with non-invasive distraction. In the pars talocalcanearis of the lower ankle joint, plantar flexion led to maximum expansion with an increase in distance of on average 0.98-1.09mm. ($p<.001$)

Non-invasive distraction as well as plantar flexion led to a maximum increase in distances in the joint gaps. Intraclinically, this may enable better arthroscopic accessibility and insight into the lower ankle joint.

Perforasomes of the occcipital artery

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The aim of this study was to examine the perforators of the occipital artery (OA) and the perforasomes, which are perfused by them. The gained knowledge should lead to better understanding of the quantity, variability and localization of the artery's perforsomes and through this help design better flaps.

This study was performed on 39 (20 right, 19 left) occipital arteries of 20 fresh anatomical head and neck specimens. First, the occipital artery and its perforators were identified. Next, each perforator was injected with methylene blue to mark the perfused areas on the skin. The perforators and corresponding perforasomes were documented in standardized fashion. Location and size of the perforasomes were determined as well as the point of the perforator's origin.

The OA arised from the external carotid artery at a mean distance to the bifurcation of 1.59cm. Overall, 270 perforators were identified. 190 of them perfused a corresponding perforasome. The other 80 vessels didn't lead to a colored area on the skin after being injected. The mean diameter of the vessels was 0.08cm. The mean area of the perforasomes was 12.76cm2.

This study showed the arterial supply of big portions of the occipital and nuchal area by the OA. Additionally to perforasomes, which were already described in the literature, the study showed constant areas on the skin of the dorsal aspect of the sternocleidomastoid muscle, where perforasomes of the OA appeared.

Composition of gut microbiota affects myelination of the peripheral nervous system

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It has become more and more evident that the gut microbiota is responsible for essential functions in human health and disease. To our knowledge, no study was yet able to report if and how the gut microbiota impacts the development or physiological functions of the peripheral nervous system.

Stereological and morphometrical analysis was performed on median nerves of neonatal and adult of germ free (GF) mice, gnotobiotic mice colonized with oligo-mouse microbiota (OMM12) comprising 12 defined bacteria strains and mice colonized with complex gut microbiota (CGM). Further gen expression analysis of the dorsal root ganglia (DRG) was performed.

While no differences were observed between the neonatal nerves of the different groups, the median nerve of adult GF mice showed reduced nerve cross-sectional area, reduced number of myelinated fibers but an increased nerve fiber density. The axon diameter was reduced, however, thickness of myelin sheath did not diverge, resulting in a reduced nerve fiber diameter and reduced g-ratio. Gene expression levels for NRG1 Type III were upregulated in DRG of GF animals.

Taken together, the histomorphometrical results demonstrate that the absence of gut microbiota during peripheral nerve development results in hypermyelination of peripheral axons. The neuregulin system is likely involved in the observed changes.

The impact of immune cell-specific Nrf2 activity on disease progression in a combined multiple sclerosis mouse model

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Multiple sclerosis (MS) has neurodegenerative and autoimmune pathophysiological aspects. While the role of astrocytic Nuclear factor-erythroid 2 related factor 2 (Nrf2) activity as modulator of oxidative stress in MS has already been subject of our previous studies, the immunomodulatory aspect of Nrf2 during MS progression has not yet been examined.

An immune cell specific Nrf2 knockout was established using Cre/loxP transgenic mice. Knockout mice (Vav-Cre::Nrf2-loxP/loxP) and wild type littermates were analyzed in the combinatory cuprizone + EAE model. This model covers both, the autoimmune and the neurodegenerative, aspects of MS. One cohort was used for immunohistochemical staining to investigate the extend of oligodendrocyte death, perivascular infiltration of immune cells, microgliosis and astrogliosis, all typical hallmarks of MS lesions. A second cohort was used for gene expression studies to evaluate the brain-intrinsic inflammatory status.

The two genotypes showed notable differences in clinical scoring and immunohistochemical outcome. Vav Cre::Nrf2-loxP/loxP mice did not only reach higher clinical scores but also earlier than wild type littermates after treatment. In comparison to the wild type mice, Vav-Cre::Nrf2loxP/loxP revealed more severe microgliosis, more perivascular infiltrates and increased numbers of CD3+ T cells within the corpus callosum. There was no significant difference with respect to astrocytes between the groups.

We were able to show that immune cell-intrinsic Nrf2 deficiency intensifies the brain-intrinsic inflammation and thereby worsens the progression and outcome in the Cup+EAE model. These findings offer promising prospects for Nrf2-related immunotherapeutic interventions of MS in the future.

Thermosensitive TRP channels in the meibomian gland

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Hyperevaporation of the tear film is the leading cause of Dry Eye Disease (DED). Most commonly it is caused by a dysfunction of the Meibomian Glands (MGs), which provide the lipid layer of the tear film. The cation permeable transient receptor potential (TRP) channels are pharmacological targets that are functionally active in the lacrimal apparatus. They play an important role in the Ca regulation of cells and take part in several signaling pathways like nociception, thermoconception as well as apoptosis, cell growth and migration.

To verify the expression of TRP channels in human and murine MGs as well as in an immortalized human meibomian gland epithelial cell line (hMGEC), we performed RT-PCRs, western blots and immunofluorescence. In the second stage of the study we tested the gene expression regulation of TRPV1, -V4 and –M8 on hMGECs by stimulating them with various mediums and analyzed the differences by means of qPCR, which we also used to determine potential markers of differentiation.

Our results show that thermosensitive TRP channels -V1, -V3, -V4 and –M8 are expressed in human and murine MGs on mRNA and protein level. qPCR reveals TRPM8 as a potential marker of differentiation.

Thermosensitive TRP channels are expressed in the MGs and could be a promising new target for the treatment of DED.

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

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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.

Next-generation-analysis of equine mesenchymal stem cell secreted small extracellular vesicles

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With the help of next generation sequencing (NGS), large quantities of unknown gene sequences from a wide variety of sources can be analyzed. With a corresponding subsequent bioinformatics, special RNAs can be found from this. In our case, special attention was paid to the so-called micro RNAs. Among other things, this RNA form is packaged in so-called small extracellular vesicles (EV's). These EV's should have an essential aspect in the immunomodulatory communication between mesenchymal stem cells and cells of damaged tissue, for example, which should support healing and thus offer a starting point for the development of new therapy methods.

Using this method, small EV's were obtained from adipose tissue derived equine mesenchymal stem cells (eMSC) for NGS. For this purpose, eMSC's from three different horses were cultivated in serum-free culture medium for three days and the supernatants after filtration (0.4 µm) were sent to a commercial cooperation partner for NGS.

After the analysis, a total of 6254 transcripts could be found. 60 known miRNAs could already be identified, 21 of them in all three donors. The five most common miRNAs were eca-miR-125a-5p, eca-miR-99b, eca-miR-423-5p, eca-miR-100 and eca-miR-423-3p with 5.42, 4.51, 3.20, 2.84 and 2.18 transcripts per million.

With the help of this investigation, initial knowledge could already be gained in order to be able to further investigate the role of the miRNAs found. In a next step, differently treated MSCs will be examined in their expression pattern to find key miRNAs, which should offer a starting point for further investigations.

Cysteinyl leukotrienes and acetylcholine are biliary tuft cell cotransmitters

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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.

Marine Bone Graft Biomineral from coccoliths

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For fracture management of risk patients, the use of bone substitutes is required to promote the healing process.
Coccoliths are calcium carbonate plates produced by the red alga Emiliania Huxleyi.
Based on existing similarities in their structure and bone extracellular matrix, we aim to investigate the potential use of coccoliths as a bone substitute material in vitro studies

To evaluate the in vitro osteogenetic behavior of coccoliths we used mice osteoblasts cell line MC3T3-E1. A cytotoxicity assay was performed using cell titer Glo® cell viability assay after treatment of the cells with different concentrations of coccoliths. The ALP assay was performed after 14 and 21 days of threatening the cells with and without coccoliths. Alizarin-Red staining was used to determine the calcification of osteoblasts after 21 days.

the optimal concentration of coccoliths was determined at a high level of 0.1 mg/ml using a cytotoxic assay. This concentration was used for further experiments. Long-term experiments showed an increase in Alkaline Phosphatase (ALP) activity by incubating the MC3T3-E1 with the coccoliths. A calcification of cells was observed after incubation of the cells with coccoliths after 21 days using Alizarin red staining

The osteoinductive properties of coccoliths allow them to be considered as an attractive "Vegan" substitute material for bone -without animal products-, however, these new findings should be followed up by further fundamental studies.

Characterization of the Localization Pattern of Alpha-Synuclein in the Enteric Nervous System of Patients with Parkinson's disease

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Misfolded alpha-Synuclein (aSyn) is a main neuropathological hallmark of Parkinson's Disease (PD). aSyn is expressed not only in brain tissues, but also in the enteric nervous system (ENS). Here, we aimed to reevaluate the localization pattern of aSyn in the ENS of PD patients and control subjects.

Formalin-fixed and paraffin-embedded (FFPE) full-thickness colon resections were used to characterize aSyn localization pattern in the ENS of control subjects. Additionally, aSyn localization pattern was assessed by immunohistochemistry in FFPE deep rectal biopsies of PD patients using conformation-specific aSyn-antibodies and compared to healthy controls. Seeding potential of aSyn aggregates from FFPE biopsies was further analyzed by protein-misfolding-cyclic-amplification assays (PMCA).

Characterization of the physiological localization pattern of aSyn in the ENS confirms that aSyn is widely detected in enteric neurons within myenteric and submucosal ganglia. While intraganglionic enteric glial cells (EGC) remained aSyn-negative, aSyn immunoreactivity was observed in mucosal EGC. This localization pattern remained unchanged in PD patients in comparison to controls. However, PMCA analyses confirmed the presence of misfolded aSyn in FFPE biopsies of PD patients, but not in controls.

These results confirm that aSyn is not only detected in enteric neurons, but also in mucosal EGC, indicating that this glial subpopulation may contribute to the regulation of aSyn life-cycle. Unlike immunohistochemistry, PMCA may be used for the detection of pathological aSyn in rectal biopsies of PD-patients with a high sensitivity. Development of such methodologies may help to better understand PD pathogenesis along the gut-brain axis in manifest and prodromal stages of PD.

TNF-α-mediated CEACAM1 expression in endothelial cells

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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.

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

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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.

Differentiation of olfactory placode organoide

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Olfaction is a fairly underrated sense compared to other sensory organs like the eye or the ear. Still, it became more prevalent since the outbreak of COVID19 due to the fact anosmia is one of the most common symptoms.
Apart from that, the sense of smell is greatly important for our everyday life and the knowledge of the development, and the function of the olfactory system remains hardly existent in humans and needs to be improved.

Induced pluripotent stem cells are used to create organoids – in vitro cell constructions resembling the morphology, structure, function, and physiology of the target organ – mimicking the development of the olfactory epithelium undergoing different states. To show these steps during development and to prove the olfactory fate, immunofluorescence stainings and RNA analysis of tissue specific markers are used.

With immunofluorescence stainings and RNA data of specific marker, we could show crucial steps in the development of the olfactory placode and further developmental stages. We can show areas with surface epithelium, preplacodal region, anterior placode, olfactory placode, forebrain, and mesenchyme at different stages.

With this approach we aim to create olfactory organoids resembling the structure and function of the olfactory epithelium, including olfactory receptor neuron like cells. With these olfactory organoids we can not only improve the understanding of the olfactory development, which is rarely investigated in the human system, it is also possible to use them as a drug testing devise, allowing substances to enter the central nervous system directly via olfactory receptor neurons.

SARS-CoV2 viral entry proteins ACE2 and TMPRSS2 occurrence in human tissue

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COVID19 is still present two years after the beginning of the pandemic situation. Respiratory symptoms such as fever, cough or cold, as well as other symptoms, like anosmia or hair loss are frequently observed. The infection of the host cell is mediated by two viral entry proteins: Angiotensin-converting enzyme 2 (ACE2) and transmembrane-serine Protease 2 (TMPRSS2). In this project we wanted to identify ACE2 and TMPRSS2 in different human tissues and propose a hypothesis for the SARS-CoV2 mediated anosmia and hair loss.

Immunostaining of ACE2 and TMPRSS2 expression in human post mortem tissue of the nasal cavity (olfactory epithelium, respiratory epithelium of the nasal septum, nasal conchae and the paranasal sinus) and the olfactory bulb.
Immunostaining and RNA analysis of both viral entry proteins in human hair, keratinocytes and keratinocyte-derived induced pluripotent stem cells (kiPSCs).

ACE2 and TMPRSS2 protein expression was solely detected in the sustentacular and gland cells of the olfactory epithelium and not in the primary sensory neurons.
Both viral entry proteins are highly expressed in the basal layer of the outer root sheath of the hair follicle.

Our results show that the olfactory neurons are not directly attacked by SARS-CoV2. It seems that anosmia is not associated with the damage of the sensory neurons, the function of the supporting and glandular cells is impaired, which can lead to anosmia.
In the hair follicle, SARS-CoV2 probably attacks the basal cells of the outer root sheath directly, which in turn could explain the symptoms of hair loss.

Metatarsal blood supply - where is the problem in foot surgery?

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Clinical forefoot pathologies are among the foremost musculoskeletal pathologies. Surgical procedures are numerous, and osteonecrosis is mentioned frequently among the different side effects. The belonging prevalence data are highly contradictory. The number of publications on vascularization of the metatarsal bones is acceleratedly increasing. However, quantifications and microscopic analyses in humans are lacking.

We measured the foramina and vasa nutricia of 33 forefeet from the Düsseldorf body donation program, microscoped the metatarsal bones, and quantified the Havers system at defined positions by image analysis.

Vasa nutricia typically occur in the lateral intermediate shaft with two clusters and two safe zones and are singular in approximately one-third of cases. We identified Vater-Pacini corpuscles associated with the vascular bundle in approximately half of the specimens. Caput and base show significantly smaller Haversian canals (r=0.743) in contrast to the corpus with little change in density (r=0.287).

In the shaft, the Haversian system serves beside the local nutritive supply for longer-range blood distribution. The metatarsal head, in particular, depends on local vessels because of less transosseous blood supply. Accordingly, distant disturbances may emerge in the base when damage occurs in the corpus, whereas local nutritive disruptions are more likely in the caput. Based on the hitherto undescribed observation, we hypothesize that the Vater-Pacini corpuscles participate in the regulation of the bony blood flow. Detachment of the soft tissue mantle may affect this regulation in addition to the associated disruption of metatarsal vascular supply.

Using three-dimensional in vitro cultures in medical device testing.

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Modern medical device testing is a lengthy and costly multi-stage process, including cytocompatibility testing, which is usually performed in 2D cell culture, despite compelling evidence that 3D cultures are more predictive of (pre-)clinical success rates. We thus aimed at developing a spheroid-based biomaterial co-culture system for biomimetic ex vivo biocompatibility testing of Orthopaedic implants.

MC3T3-E1 pre-osteoblasts were cultured (37°C, 5% CO_2), in alpha-MEM for up to 28 days, in non-adherent plates to form scaffold-free 3D spheroids. Osteogenic differentiation (10mM b-glycerophosphate, 50µg/ml ascorbic acid; Alizarin Red/Von Kossa), mineralization stage (marker gene expression, alkaline phosphatase activity, calcium deposition), and mineralization quality (FTIR, micro-CT, SEM) were assessed. Cytotoxicity (FACS, CytoTox-Glo) and degradation of absorbable implants (EDS, SEM/-EDX, µCT) was evaluated under static and, to comply with ISO/TS 37137-1:2021, under dynamic conditions in custom-built perfusion bioreactors. Statistics comprised 2-way ANOVA with post-hoc tests.

Expression profiles of selected early- and late-stage osteoblast differentiation markers indicate a well-developed 3D biomineralization process with strongly upregulated Col1a1 and Bglap ($p<0.05$, $p<0.001$), and Alp ($p<0.001$) mRNA levels, and type I collagen- and osteocalcin-positive IHC. Biomineralization was quantified (day21, $p<0.05$; d28, $p<0.001$) and a bone-like hydroxyapatite deposition confirmed (SEM-EDX, FTIR). Biomimetic degradation of absorbable implants was monitored over 28 days under quasi physiological conditions and responses to static and dynamic culture conditions were significantly different. Incubation time-dependently decreased cytocompatibility (>2-fold, $p<0.001$) under static conditions, which improved under dynamic flow.

Differentiated osteoprogenitor cells formed a biomineralized bone-like matrix in a quasi-physiological scaffold-free 3D culture and hold potential to improve cytocompatibility evaluation in bioreactor systems.

In vivo Ligamentogenesis in Embroidered Poly(lactic-co-ε-caprolactone) / Polylactic Acid Scaffolds Functionalized by Fluorination and Hexamethylene Diisocyanate Cross-Linked Collagen Foams

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MK and CG contributed equally

Autografts are the gold standard for anterior cruciate ligament (ACL) reconstruction. Nevertheless, tissue-engineered ACLs are a perspective to minimize donor site morbidity and limited graft availability. In this study, a dynamic nude mice xenograft model was used, to characterize the ligamentogenesis in embroidered poly(L-lactide-co-ε-caprolactone) (P(LA-CL)) / polylactic acid (PLA) constructs.

(P(LA-CL))/PLA scaffolds were either untreated (co) or functionalized by gas fluorination (F), collagen foam cross-linked with hexamethylene diisocyanate (HMDI) (coll), or F combined with foam (F+coll). Cell free constructs or those seeded for 1 week with lapine ACL ligamentocytes, were implanted in nude mice for 12 weeks. After explantation, cell vitality and content, histo(patho)logy of scaffolds (including: liver, kidney, spleen), sulphated glycosaminoglycan (sGAG) contents and biomechanical properties, were assessed.

Animal weight development and organs were unaffected, indicating scaffold biocompatibility. Scaffolds maintained their size and shape and reflected a high cell viability prior and following implantation. Coll or F+coll scaffolds seeded with cells yielded superior macroscopic properties compared to the controls. Mild inflammation signs (foreign-body giant cells, hyperemia) were limited to scaffolds without collagen. Microscopical score values and sGAG content did not differ significantly. Although remaining stable after explantation, elastic modulus, maximum force, tensile strength and strain at Fmax were significantly lower in explanted scaffolds compared to those before implantation, but did not differ between scaffold subtypes, except for a higher maximum force in F+coll compared with F samples (in vivo).

Scaffold functionalization with fluorinated collagen foam provides a promising approach for ACL tissue engineering.

MicroRNAs determine structure and function of a central synapse.

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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.

Evaluation of the circadian expression of orexin receptors in the mouse brain by RNAscope®

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Orexin A and B are wake-promoting neuropeptides that originate from hypothalamic neurons and project to diverse brain areas widespread throughout the central nervous system. They modulate various physiological functions including sleep-wake rhythm but also cognitive function via their orexin 1 (OXR1) and 2 (OXR2) receptors. The expression of orexins varies over the course of a day, peaking during the active awake phase. In this study, we now investigate circadian and region-specific expression differences of orexin receptors.

OXR1 and OXR2 mRNA was analyzed in subareas of the dorsal hippocampus and medial prefrontal cortex at four different timepoints over the course of 24 hours using RNAscope®, a novel multiplex in situ hybridization technique.

The percentage of orexin receptor mRNA expressing cells was constant over time within brain areas, but significant expression differences between brain regions and subareas were evident. The highest percentage of OXR1 mRNA-positive cells was observed in the hilus of the dentate gyrus and the stratum pyramidale of the CA3 region, while the highest percentage of OXR2 mRNA-positive cells was seen in the stratum pyramidale of the CA1 and CA3 regions of the dorsal hippocampus. In subareas of the PFC, expression of both receptor subtypes was lower.

Detecting orexin receptor mRNA expression using RNAscope® provides high selectivity and great spatial resolution. The distinct expression profiles of both receptor subtypes within hippocampal subareas provide an interesting basis for future interventional studies of orexin receptor function in spatial and emotional memory.

Inhibitory synapse diversity in health and disease

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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.

Entorhinal cortex lesion induces homeostatic synaptic plasticity of CA3 pyramidal neurons

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A common aspect of many neurological diseases is the denervation of brain regions because of demyelination or cell death. Nonetheless, the underlying mechanisms involved in lesion-induced reorganization of neural networks warrant further investigation. In this study, we assessed the effects of a partial denervation on excitatory synaptic transmission of hippocampal neurons.

Lesion of the entorhinal cortex in organotypic entorhino-hippocampal tissue cultures — prepared from mice of both sexes — was used to denervate distal apical dendrites of hippocampal granule cells and CA3 pyramidal neurons. Changes in excitatory neurotransmission were assessed with single and paired whole-cell patch-clamp recordings, and morphological alterations were analyzed with electron microscopy. Moreover, region-specific transcriptome as well as compartmentalized FISH analyses were performed.

Partial denervation resulted in homeostatic synaptic adaptions of dentate granule cells and CA3 pyramidal cells. These changes in excitatory neurotransmission occurred predominantly in the strongest synapses as shown by a hierarchical analysis of spontaneous excitatory postsynaptic currents. The homeostatic adjustment was accompanied by characteristic region-specific transcriptomic changes. Consistent with these findings, paired recordings of dentate granule cells and CA3 pyramidal neurons and ultrastructural analyses of mossy fiber synapses revealed denervation-induced compensatory structural and functional changes at the single synapse level. These adjustments seem to depend on the presence of the actin-binding protein synaptopodin, a key regulator of synaptic plasticity, since they were not evident in synaptopodin-deficient tissue preparations.

Lesion-induced synaptic plasticity of dentate granule cells and CA3 pyramidal neurons depends on synaptopodin-mediated homeostatic hetero- and transsynaptic adaptations in hippocampal networks.

Alcohol consumption alters Fibroblast Growth Factor-2 signaling and expression of alcohol metabolizing enzymes in the murine liver

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Alcohol use disorder (AUD) is one of the most frequent mental disease worldwide, which is accompanied with behavior changes and alterations in peripheral organs. Therefore, the liver, the main alcohol metabolizing organ, is of great interest. Although, the underlying biological pathways are mainly unknown, it has been shown that neurotrophic factors, such as Fibroblast Growth Factor-2 (FGF-2), are involved in chronic alcohol consume behaviors.

Here, we investigated the livers of adult wild-type and FGF-2 knockout mice treated acutely (intraperitoneal ethanol injection) or chronically (intermitted access to ethanol in a two-bottle-choice paradigm for four or six weeks) with ethanol. For the former time point, mice were euthanized 900 or 1800 seconds after injection. Chronic treated animals had repetitive access to water and ethanol for 24 h followed by a 24 h withdraw period with water only. Then, livers were harvested and prepared for protein analysis.

We showed that acute and chronic ethanol consumption reveals effects on the FGF-2 signaling due to changed activation of its binding partner FGFR1 and down-stream Erk1/2 and Akt signaling cascades. Moreover, we identified different protein expression patterns of alcohol and fatty acid metabolizing enzymes, partially in a sex-dependent manner. Most differences were observed after acute and four weeks of ethanol treatment.

Our findings suggest that FGF-2 itself and the duration of alcohol consumption influences FGF-2 signaling as well as expression of alcohol metabolizing enzymes in the liver. This further supports the hypothesis that FGF-2 plays a role in the pathogenesis of AUD.

Role of ADAM10 in pemphigus vulgaris

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The severe autoimmune blistering disease Pemphigus vulgaris (PV) is mainly caused by autoantibodies against desmoglein (Dsg) 3 and Dsg1. Mechanisms leading to blister formation are not fully understood, but intracellular signaling seems to play an important role. Sheddases ADAM10 and ADAM17 are involved in the turnover of the desmosomal cadherin Dsg2 and ADAM10 has been shown to contribute to acantholysis in a murine pemphigus model. In the present study, we further examined the role of ADAM10 and ADAM17 both in keratinocyte adhesion and in the pathogenesis of PV.

Immunostaining, Western Blotting, Dissociation Assay, human ex-vivo pemphigus model

We found that inhibition of ADAM10 enhanced adhesion of primary human keratinocytes but not of immortalized keratinocytes. Furthermore, inhibition of ADAM10 shifted keratinocyte adhesion towards a hyperadhesive state. However, ADAM inhibition did neither modulate protein levels of Dsg1 and Dsg3 nor activation of EGFR. In primary human keratinocytes, inhibition of ADAM10, but not ADAM17, reduced loss of cell adhesion and fragmentation of Dsg1 and Dsg3 immunostaining in response to a PV1-IgG from a mucocutaneous PV patient. Similarly, inhibition of ADAM10 in dissociation assay decreased fragmentation of primary keratinocytes induced by a antibody against Dsg3 and by PV-IgG from two other patients both suffering from mucosal PV. However, such protective effect was not observed in both cultured cells and ex vivo disease models, when another mucocutaneous PV4-IgG containing more Dsg1 autoantibodies was used.

Taken together, ADAM10 modulates both hyperadhesion and PV-IgG-induced loss of cell adhesion dependent on the autoantibody profile.

Reelin signaling and posttranslational protein modifications

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The extracellular matrix protein reelin is a central regulator of neuronal migration and organization during brain development. Beside its action during brain development, it is also known that reelin fulfills important actions in the mature brain. A main aspect of reelin's action in the adult brain is modulation of neuronal signaling, thereby influencing neurophysiological processes, such as learning and memory. Previous work could show that reelin modulates excitatory glutamatergic as well as inhibitory GABAergic signaling by changing the phosphorylation status of neuronal receptors in various ways. These findings raise the question whether reelin signaling might also target other posttranslational modifications, such as protein acetylation.

By using dissociated neuronal cell cultures, tumor cell lines, as well as rodent brain slice cultures we are currently studying whether reelin signaling targets different posttranslational protein modifications that have not been studied before in this context. Furthermore, by using calcium imaging techniques, we want to address the question how reelin's action impacts cholinergic signaling, which has not been extensively examined so far.

We expect that these experimental approaches will complement our knowledge of reelin's action in both the developing and the mature brain.

Furthermore, our results will contribute to a better understanding of the interplay between posttranslational protein modifications, their intracellular pathways and neuronal signaling processes.

First results from the Integrated virtual and traditional anatomy (IN VITRA) project: A pilot for supporting teaching anatomy basics in a virtual reality (VR) setting.

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Objective
Virtual reality (VR) is an innovative technology. Together with the Comenius University Bratislava we launched the IN VITRA project (https://anatomieundzellbiologie.meduniwien.ac.at/abteilungen-wissenschaft-forschung/abteilung-fuer-anatomie/in-vitra/), a cross-border Interreg - "European territorial cooperation“. In its scope, we did a pilot, aiming at gathering first experience in VR hands-on teaching of medical students in their first semester.

Methods
A Virtual Reality (VR)-Facility was established and equipped with a total of 20 virtual reality headsets (Oculus Quest) operating with high end anatomy education software (Human Anatomy VR 2022), made by our collaboration partner in Bratislava. All 740 1st year-students had to use the facility. They were provided with educational objectives in the form of documents, listing basal structures in advance. Using VR equipment with its three-dimensional (3D) computer models, they had to study the relationship of relevant structures in a self-coordinated, but supervised practicum (total of 8 academic hours). Video sequences introducing the use of the software and hardware were recorded and provided in advance and on-site in the platform “Moodle”.

Results
All students successfully passed the practicum. They all met the educational targets and claimed to have profited from the 3D visualization tools. In general, their oral and written feedback via Moodle was predominantly positive.

Conclusion
Passing 740 students in parallel through a self-coordinated, hands-on VR practicum is possible. The setting excellently fits for teaching basic anatomic knowledge and first year students, not yet in the dissection classes, profit in preparing for their annual written exams. Additional applications remain to be evaluated.

A culture model for the assessment of phenylalanine neurotoxicity in phenylketonuria

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Phenylketonuria (PKU) is caused by a specific mutation of the phenylalanine hydroxylase (PAH) gene. The deficiency of PAH results in high phenylalanine levels (Phe), low tyrosine levels (Tyr), and reduced catecholamine neurotransmitters leading to mental retardation in PKU patients. The specific contribution of high Phe and low Tyr levels in mental retardation is largely unknown. In this study, we used organic hippocampal slice cultures in an optimized medium as an adequate culture model to decipher the precise role of high Phe and low Tyr levels on synaptic and glial integrity in PKU.

Using capillary western blot analysis and immunohistochemistry, followed by quantitative image analysis, we tested the expression of various pre- and postsynaptic proteins (PSD95, synaptopodin, SNAP25, synaptophysin), glial cell markers (GFAP, Iba1, P2Y12, CD68, C3b), and the morphology of glial cells.

We found a downregulation of PSD95 and SNAP25 in the presence of high/low Phe/Tyr levels after 3 weeks, which, then however, recovered after 6 weeks in culture. Furthermore, no change in the expression pattern of glial proteins was observed.

Our results show that high Phe levels/low Tyr levels alone are unlikely to substantially contribute to mental retardation in PKU. The direct neurotoxic potency of high Phe/low Tyr concentrations is almost negligible since the effects are transient. The transient character in the presence of unchanged levels of high Phe/low Tyr points to a role of reduced catecholamine derivate neurotransmitters, rather than of high Phe/low Tyr levels in PKU.

L

Classifications of the monopodial bronchopulmonary vasculature – A cluster based verification approach

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The pulmonary artery tree of mammals is composed of functionally different sub segments, whose design changes along the path through the organ. A morphological analysis of the vessel tree is thus advanced by grouping its branch segments into biologically similar groups. In contrast to the human lung with its dichotomous branching pattern, the lungs of the most widely used animal models of pulmonary disease have a monopodial branching pattern which exhibits a high degree of asymmetry. Thus, established methods of classification deliver widely different results when employed on the same monopodial lung. We formulate and demonstrate a workflow to quantify the precision of various grouping methods employed to the monopodial lung.

A mouse lung was imaged using synchrotron micro computed tomography. The resulting volume image was digitally segmented and the vessel tree extracted. Measurements were acquired for each of its segments. Different grouping algorithms taken from the literature, such as (Strahler) order and (fractal) generations were then employed on the vessel tree. Additionally, a grouping approach based on morphological features, as opposed to location in the vessel tree, was employed for comparison purposes.

Significant differences in method performance were observed. The Strahler order could be identified as the best performing method of the tested set. Standard methods for characterizing human lung structure, were unable to provide results of comparable precision.

Most grouping methods designed for the human lung do not perform well in the monopodial lung. A morphology based clustering approach could help to create classifications that are more precise.

Role of HCA2 receptor in brain and skin inflammation

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Chronic inflammatory skin diseases such as atopic dermatitis (AD) and psoriasis are associated with a high co-morbidity with neuropsychiatric disorders like anxiety and depression, suggesting a link between pathophysiological processes in the skin and the brain.
G-protein coupled receptors, such as the Hydroxy-Carboxylic Acid Receptor 2 (HCA2R), which is expressed on brain, skin and immune cells, could mitigate such interactions. In this study, we investigated cytokine expression profiles and neuroinflammation in HCA2R-/- mice, with and without chronic skin inflammation.

HCA2R-/- mice and their wildtype littermates were challenged with the hapten DNFB (1-fluoro-2,4-dinitrobenzene) to induce AD-like allergic skin inflammation. Gene expression of cytokines and microglia markers were quantified with qPCR in the prefrontal cortex, ventral and dorsal hippocampus. Microglia cell density was further determined using immunohistochemistry.

Chronic skin inflammation elevated the expression of IFN-gamma and CCl8, a chemokine induced by IFN-gamma, in the prefrontal cortex and hippocampus of wildtype mice. This proinflammatory response was further enhanced especially in the hippocampus of HCA2R-/- mice. This was associated with increased density of the microglia marker Iba1 in layers of the dorsal hippocampus of HCA2R-/- mice.

The HCA2 receptor may modulate proinflammatory responses to peripheral skin inflammation in brain areas important for emotional regulation and cognitive performance. Further studies are needed to investigate the role of this receptor in anxiety, learning and memory and its mechanisms of actions along the skin-brain axis. Ultimately, the pharmacological activation of the HCA2 receptor may provide treatment options also for psychiatric co-morbidities of skin inflammations.

The influence of calcitriol and calcipotriol on podocyte differentiation in situ and in vitro.

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Podocyte de-differentiation with specific changes of the complex 3D-morphology is mainly responsible for the pathogenesis of chronic kidney diseases (CKD). Since there is no causal therapy for CKD, inadequate and late treatment lead to end-stage kidney disease, subsequently making renal replacement therapy inevitable. To address this, novel screening strategies for the discovery of new treatment options are of vital significance.

We combined podocyte de-differentiation with a semi-automated imaging procedure using the GlomAssay. Cultured glomeruli isolated from transgenic mice expressing CFP podocyte specifically, were treated with calcitriol and its analogue calcipotriol. GlomAssay uses the nephrin promoter-controlled CFP fluorescence as a readout for podocyte de-differentiation. Calcitriol- and calcipotriol-treated glomeruli were investigated by RNA-Seq and LC-MS/MS. Cultured murine podocytes were treated with calcitriol and calcipotriol to elucidate morphological and molecular changes by immunofluorescence staining, RT-(q)PCR and Western blot.

Calcitriol- and calcipotriol-treated glomeruli showed a significantly higher CFP fluorescence intensity which indicates a higher nephrin expression compared to controls. This was verified by RT-(q)PCR and Western blot for nephrin and CFP expression. Additionally, we found an up-regulation of the vitamin D receptor (VDR) in calcitriol- and calcipotriol-treated glomeruli. By transcriptomic and proteomic analysis, we identified treatment-specific molecular patterns for several pathways like Wnt-signaling, actin cytoskeleton reorganization, focal adhesion formation and the slit membrane components. This is confirmed by in vitro experiments, where we additionally could detect changes of the podocyte morphology.

Our results show that calcitriol and calcipotriol influence podocyte differentiation in situ and in vitro by the regulation of specific signaling pathways.

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

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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.

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

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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.

Metalloprotease meprin BETA plays a minor role in fibrosis development in mouse colon

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The metalloprotease meprin BETA is a sheddase with a variety of substrates, leading to its versatility in various physiological and pathological scenarios. BETA-1 integrin, associating with the extracellular matrix components to maintain tissue homeostasis, is also one of the substrates of meprin BETA. It has been reported the deficiency of BETA-1 integrin can cause fibrogenesis in arteries and intestines. Preliminary data has shown in cell experiments and heterozygous mouse models, overexpression of meprin BETA leaded to a pronounced decrease of BETA-1 integrin, which can trigger the onset of fibrosis development. Thus, it is of high interest to further investigate the role of meprin BETA in fibrosis development in mouse colon.

Homozygous mouse lines overexpressing meprin BETA in smooth muscle cells were generated. Mice were euthanized in different ages to create a timeline of development of fibrosis. Colon tissues were conducted with Hematoxylin and Eosin staining, Azan staining, and Sirius red staining to mark out the fibrotic areas. And RT-qPCR was applied targeting several fibrosis-related genes with the RNA isolated from mouse colon tissues.

With RT-qPCR and multiple histological staining, trivial fibrotic symptoms were observed. However, fibrosis-related gene expression and the area of Sirius-red-stained smooth muscle layer reached their peaks at the age of three-month-old or six-month-old.

All these results taken together incline a network of fibrosis regulation and meprin BETA´s minor role in mouse colonic fibrosis development.

Mechanical properties of Achilles tendon and metabolites of exercise

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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.

Comparative characterization of human meibomian glands and sebaceous glands using different biomarkers

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Investigate the similarities and differences between meibomian glands (MG) and hair-associated sebaceous glands (SG) and other "free" SG using several biomarkers, which may provide new clues for possible future treatment strategies for dry eye disease (DED).

For immunohistochemistry, MG from eyelids, “free” SG from nasal wings, lips, external auditory canals, and hair-associated SG from scalp tissues of 4 human body donors (3 males, 1 female, aged 68 to 79 years) were used.

Cytokeratin (CK) 1 was expressed in the hair-associated SG acinus, while CK10 was expressed in both free SG and hair-associated SG acinus, but both markers were absent in the MG acinus and were expressed on the central duct of the MG and SG. CK14 was expressed in basal and mature cells in SG, but only in basal cells of MG. Stem cell marker Keratin 15 was expressed only in MG connecting ductules, but in both central ducts and connecting ductules in SG. Expression of cell-cell contacts like desmoglein1, desmocollin3, desmoplakin, plakoglobin, and E-cadherin was expressed in basal, differentiating and mature cells, and excretory duct cells of the MG and SG.

The expression of CK and stem cell markers differed markedly between MG and SG, but the expression of cell-cell contacts was similar between the two groups of SG. The findings highlight that MG is a unique subtype of SG. Intensive characterization of the MG will help us gain deeper insights into the structure and function of the MG - the main cause of the evaporative form of DED.

Morphological analysis of different types of hippocampal neurons in reelin conditional knockout mice

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Reelin is an extracellular matrix glycoprotein that has an important function in mammalian brain development. In the absence of reelin, neuronal migration, dendritic growth and synaptic development are disturbed, leading to abnormalities in the lamination of the cerebral cortex. In our study we aimed to investigate the effect of tamoxifen induced conditional reelin deficiency on the morphology of different neuronal cell types in the hippocampus.

Newborn pups where fed with tamoxifen from P1 for 5 consecutive days to induce Cre activation and knockout of the floxed reelin gene. The genotype of the animals was determined with PCR. At the age of 4 weeks, the animals were deeply anesthetized with isoflurane and decapitated. Brain hemispheres were separated, shortly dropped into Gey's Balanced Salt Solution, and proceeded to Golgi staining by means of a commercially available Golgi Staining Kit. Golgi-stained neurons in 90µm vibratome sections were morphologically analyzed under a microscope using Neurolucida Software.

Our preliminary results indicate a decreased size of pyramidal cell perikarya in the CA1-CA3 regions of conditionally reelin deficient brains when compared to wildtype Moreover, subtle changes of the dendritic tree morphology could be also observed in the hippocampus of knockout brain samples. In turn we found, so far, no difference between cell body parameters of hippocampal interneurons in the two groups.

Conditionally induced reelin deficiency at postnatal age appears to differentially affect the morphology of different neuronal cell types in the hippocampus, which may lead to functional disturbances of the excitatory-inhibitory balance.

Designing Anatomy facilities for integrated preclinical education in the Middle East

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We describe the establishment of state-of-the-art anatomy laboratories at the newly founded College of Medicine and Health Sciences at Khalifa University, Abu Dhabi, United Arab Emirates and how these facilities align with evidence-based best teaching practices.

A list of best practices for anatomy education in a modern medical curriculum is summarized. For each “instructional method” a list of required facilities/resources was established.

Our educational modalities include a broad range of teaching approaches. Prosected and plastinated specimens are housed in the Instructional Studio, where cadaveric dissections are performed. A large variety of plastic models and an Anatomage® virtual dissection table are situated in each of our three Dry Laboratories, where five CTOUCH® interactive smart screens allow for active learning and interaction between small student groups. Gross anatomy is taught in parallel with diverse imaging modalities: The Imaging Center is equipped with a Sectra® medical educational platform, giving the students the option to generate a three-dimensional appreciation of anatomy, based on a variety of normal and pathological radiological images. In addition, it houses a CAE Vimedix® Virtual Medical Imaging Ultrasound Training System and Philipps Lumify® Ultrasound devices to train students to conduct and interpret sonographic images. Moreover, the Complete Anatomy® program is made available to all our students.

The broad range of educational modalities and teaching approaches is highly appreciated by our faculty and students. Moreover, these technologies allowed for a relatively smooth transition from on-site anatomy teaching to online education during the COVID pandemic.

Postsynaptic GABAB receptors inhibit synaptic plasticity at the excitatory input of dentate gyrus basket cells

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GABAB receptors are important modulators of synaptic plasticity in principal cells, where they promote plasticity via disinhibition of the network. In inhibitory interneurons, GABAB receptors inhibit plastic changes but this role is less clear due to the diverse synaptic properties and function of interneurons. Therefore, their modulatory role on the neuronal network may differ depending on the region and cell-type. Here, we aim to determine the effect of GABAB receptors in modulating synaptic plasticity of basket cells of the dentate gyrus (DG) circuit.

To address this, we performed in vitro electrophysiological recordings to induce long-term potentiation (LTP) at the postsynaptic basket cells of the DG by delivering non-associative theta burst stimulation to their mossy fibre input. To investigate how GABA modulates plasticity in these cells, we pharmacologically manipulated GABAB receptors using Baclofen. We dissected the cellular mechanism involved in this modulatory effect by applying drugs to interfere at different stages of the GABAB receptor signalling cascade. Using electron microscopy, we confirmed the synaptic location of the GABAB receptors.

Under control conditions, LTP remained stable for a minimum of 30 minutes (230.8±45.25). Bath-application of Baclofen resulted in strong inhibition of LTP (119.9±12.57, $p=0.0493$), whilst co-application of SCH-23390 and Baclofen resulted in reversal of inhibitory effects on plasticity (192.7±20.07, $p=0.0146$). Intracellular perfusion of Gallein mildly affected recovery of plasticity (163.6±26.76), but not significantly.

These results show that at the excitatory input synapses of basket cells in the DG, GABA modulates plasticity via post-synaptic GABAB receptors, which inhibit LTP induction when activated.

M

Depletion of CD44 impairs metastasis formation of colorectal cancer by reducing hypoxia-mediated EMT induction in xenograft primary tumors in vivo

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To determine the functional role of CD44 for distant metastasis formation in human colorectal cancer (CRC).

We used a CD44 knockdown (kd) approach in a cell line/ xenograft model (HT-29) that spontaneously metastasizes to multiple sites in vivo. Transcriptomics, proteomics and kinomics were used in addition to corresponding validation steps to explain the observed effects. Findings were further verified by 3D in vitro culture and immunohistochemical stainings.

CD44 kd xenografts show a decrease in primary tumor growth and - independent of that - a marked reduction in distant metastasis formation, although CD44 is per se not expressed in lung metastases of control xenografts. CD44 is induced in the paranecrotic, hypoxic regions of control primary tumors. CD44 kd results in improved tumor angiogenesis in the paranecrosis, accompanied by reduced HIF-1α, EMT, and CEACAM5 expression. Vice versa, mitochondrial genes and proteins are induced upon CD44 kd as is oxidative phosphorylation. Hypoxia increases VEGF release from 3D HT-29 spheres, which is strikingly enhanced in CD44 kd spheres.

In a clinically relevant CRC xenograft model, CD44 kd evidently impairs metastasis formation by improving VEGF release and thus angiogenesis in hypoxic conditions, thereby decreasing hypoxia, EMT, stemness, and promoting mitochondrial metabolism.

The rare villin-expressing cell of the murine lower airway is a neuroendocrine rather than a brush cell

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Previous studies reported an enigmatic rare cell in the mouse tracheal epithelium displaying an apical tuft of villin-immunoreactive microvilli. This cell has a morphology similar to the brush cell. Unlike brush cells, however, the transcription factor Pou2f3 is not required for its development. This study aimed to clarify the identity of this ill-defined cell type.

Wild-type and genetically modified mice, including Pou2f3-/-, ChAT-eGFP, Vil1-Cre/ROSAmT/mG and Avil-Cre/ROSAmT/mG mice, were used to assess villin expression in airway epithelium, using electron microscopy, immunohistochemistry and in silico-analysis of publicly available sequencing data.

Electron microscopy revealed cells with neuroendocrine cell features (i.e. large dense core vesicles) as the only rare microvillous epithelial cell type in the trachea of mice lacking brush cells (Pou2f3-/-). Immunolabeling revealed extensive co-labeling of villin and neuroendocrine-specific-cell markers in wild-type tracheas (64% of PGP9.5+ cells), but only minimally in lungs (≈1% of CGRP+ cells). Vil1-Cre activity was detected in 22% of tracheal PGP9.5+ and 47% CGRP+ bronchopulmonary neuroendocrine cells. Brush cells, in contrast, were only rarely villin+ (2% of GNAT3+ cells), but advillin+ (85% of ChAT-eGFP+ cells). Accordingly, Avil-eGFP was detected in 75% of TRPM5+ brush cells, but not in neuroendocrine cells. In silico-analysis of publicly available sequencing data of murine tracheal epithelial cells revealed Vil1-mRNA expression in neuroendocrine cells while Avil-mRNA is expressed in brush cells.

The present study identifies the enigmatic villin-expressing cells in the lower airways as a cell population hidden amongst the neuroendocrine cells. Then, advillin, rather than villin, is a brush cell marker in the airway.

Molecular features of the glia limitans perichoroidalis

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The choroid plexus (CP) is an entry point for peripheral immune cells into the central nervous system (CNS). While immune cell migration has been described via the CP epithelium, direct migration from the CP stroma into the CNS parenchyma at the attachment point of the CP has not been addressed. Our group recently demonstrated the existence of a glial barrier at the CP attachment point at the third ventricle which we termed glia limitans perichoroidalis. We hypothesize that the glia limitans perichoroidalis also shows features of a glial barrier on the molecular level.

The glia limitans perichoroidalis of the third ventricle was analyzed in C57BL/6 mice by immunohistochemical labelling of glial and basal lamina marker proteins and by transmission electron microscopy. Furthermore, three regions (glia limitans perichoroidalis, periventricular fibriae hippocampi, glia limitans superficialis) were extracted from cryo-natively embedded murine brain sections by laser capture microdissection and analyzed for gene expression levels of tight junction proteins, astrocytic and basal lamina components.

The glia limitans perichoroidalis was located at the attachment point of the CP in the third ventricle and marked by an increased GFAP and laminin immunoreactivity. Semiautomatic optical density measurements in concentric semicircles revealed an increase of anti-GFAP immunoreactivity compared with periventricular reference regions that was confirmed by accumulation of intertwining astrocytic processes on the ultrastructural level. Results of gene expression quantification were still pending at the time of abstract submission.

The glia limitans perichoroidalis is a glial barrier structure with possible influence on immune cell migration under inflammatory conditions.

Multimodal Treatment for ALS – The only option?

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Amyotrophic lateral sclerosis (ALS) is the most common lethal motor neuron disease in adults, without causal treatment options yet. One reason for this is the large causal heterogeneity of the disease, where the cause remains unknown in up to 80% of ALS patients. ALS refers to a very heterogeneous group of neurodegenerative disorders that are triggered by an interplay of different molecular signaling pathways in motor neurons and neighboring cells such as microglia and astrocytes. However, numerous studies show evidence of oxidative stress in postmortem neuronal tissue, cerebrospinal fluid, plasma, and urine from ALS patients as well as in vitro and in vivo disease models.

We use the Wobbler mouse as a model organism for ALS and combine molecular and protein biochemical methods with modern imaging-techniques such as live cell imaging, SIM/LSM and electron microscopy to address our hypotheses.

Our studies demonstrate an abnormal mitochondrial morphology in motor neurons, which correlates with an increased ROS production. These cannot be adequately detoxified due to insufficient function of the major antioxidant system, the glutathione system. This oxidative stress leads to increased DNA damage in motor neurons, which could be counteracted using ROS scavengers.

Our results indicate that a focused intervention in one pathomechanism might be insufficient in ALS therapy, as multiple systems are affected, so that multimodal therapy will be necessary to prolong the average lifespan of motor neurons and thus slow down the progression of the disease.

Morphological characterization of pyramidal neurons in the medial prefrontal cortex of CYLD knockout mouse model.

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Cylindromatosis lysine 63 deubiquitinase (CYLD) is highly expressed in the brain, particularly at the postsynaptic density (PSD). CYLD plays a key role in the brain by modulating the mammalian target of rapamycin (mTOR) signaling and autophagy at the synapse. CYLD deletion in mice causes a reduction in hippocampal network excitability and long-term potentiation together with a decrease in dendrite total length and in the total number of basal spines CA1 pyramidal neurons.
Furthermore, CYLD Knockout (KO) mice display autism-like phenotypes including impaired social communication, increased repetitive behavior, and cognitive dysfunction.
To clarify the role of CYLD in dendrite and spine formation and to identify a possible morphological correlation with the observed behavioral abnormalities in the CYLD KO mouse model, the morphology of medial prefrontal cortex (mPFC) pyramidal neurons has been analyzed in young adult CYLD KO Thy1-GFP mice.

A double transgenic mouse line CYLD KO Thy1-GFP line M was used to characterize the excitatory cortical neurons in the mPFC.

In the mPFC of CYLD KO mice, the loss of CYLD reduces basal dendritic arborization of pyramidal neuron shape but has no effect on dendritic spine production.

These preliminary findings suggest that the deletion of CYLD may contribute to the reduced neuronal complexity, but other molecular abnormalities are needed to produce morphological alteration in spines.

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

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

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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.

NEURONS OF DEVELOPMENT IN THE ADULT BRAIN

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Objective: The lowermost cell layer of the adult cerebral cortex that contains interstitial white matter cells in humans and layer 6b in rodent. These cells are the remnants of the subplate cells that are present in large numbers and play key role in the formation of cortical circuits but a large fraction of them die during postnatal development. The adult population that remains in all mammals and display unique conserved gene expression and connectivity. These neurons are very abundant during development and express higher proportions of susceptibility genes linked to human cognitive disorders than any other cortical layer and their distribution is known to be altered in schizophrenia and autism (Hoerder-Suabedissen et al., 2013; Bakken et al., 2016; Molnár et al., 2020). In spite of these clinical links, our current knowledge on these neurons is limited.

Methods: We study their input and output using combined anatomical, genetic and physiological approaches. Selected cortical areas, relevant for sensory perception, arousal and sleep (V1, S1, M1, prefrontal cortex) are studied using chemogenetic and optogenetic methods.

Results: Members of my laboratory identified intracortical and thalamic projections from a subpopulation of layer 6b cells that might regulate both cortical and thalamic arousal of cortical areas that are involved in higher cortical functions (Hoerder-Suabedissen, et al., 2018).

Conclusions: Our results suggest that 6b is not just a developmental remnant cell population in the adult, but a layer that plays a key role in cortical state control, integrating and modulating information processing (Guidi et al., 2016; Horvath et al., 2022).

Enthesis Repair Through Decellularization-Demineralization

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Surgical reattachment of tendon/ligament to bone after injury is problematic, as the repair heals through scar tissue rather than the specially adapted enthesis. A novel approach to scaffold development for enthesis tissue engineering exploits the collagenous extracellular matrix and structural integrity of animal bone, fully decellularized and partially demineralized for bone-soft tissue continuation, and we are characterizing this design in the repair/bridging of significant rotator cuff tears.

Cortical and cancellous porcine bone samples were cut to an anatomical design and subjected to a variety of decellularization and partial demineralization procedures for optimization versus controls. Characterizations were performed through histology, matrix and cellular content, mechanical testing, mineral composition and computed tomography imaging.

Decellularization was improved by ultrasonication and negative pressure, and confirmed through reduced or absent cellular staining and DNA content, with a mild overall decrease in glycosaminoglycans matrix content. Reference point indentation measurements associated with toughness and elasticity showed no consistent changes upon decellularization, but a dramatic decrease after demineralization. X-rays demonstrated predictable control of a mineralized-demineralized interface position, whilst a change in mineral content and mechanical and structural properties across the interface was confirmed.

Initial optimization trials show proof-of-concept of a decellularized-demineralized soft-hard hybrid scaffold as an immune compatible tissue engineered xenograft for enthesis repair. Decellularization was achieved without discernible mechanical property changes or considerable matrix compromise, whilst partial demineralization produced a soft-hard transition in mechanical and structural properties. Further characterizations are ongoing to assess validity before in vivo animal trials and potential clinical translation.

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

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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.

Development of Therapeutic Platforms for Osteoporotic Impaired Vertebral Bone Regeneration

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Osteoporosis is the most prevalent metabolic bone disease in the world, causing fractures worldwide at a rate of 1 every 3 seconds[1]. 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 life[2]. 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 vertebrae[3]. 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.

1. Foundation, I.O. *IOF: A Fracture Every 3 Seconds Worldwide - That's Osteoporosis*. 2019; Available from: https://www.prnewswire.com/news-releases/iof-a-fracture-every-3-seconds-worldwide---thats-osteoporosis-300934719.html.
2. Sözen, T., L. Özışık, and N. Başaran, *An overview and management of osteoporosis.* Eur J Rheumatol, 2017. **4**(1): p. 46-56.
3. McCall, T., C. Cole, and A. Dailey, *Vertebroplasty and kyphoplasty: a comparative review of efficacy and adverse events.* Current Reviews in Musculoskeletal Medicine, 2008. **1**(1): p. 17- 23

Neuroanatomical investigations in the BTBR model for idiopathic autism spectrum disorder (ASD) at adulthood

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ASD comprises multifactorial neurodevelopmental disorders characterized by defective social interaction and communication and repetitive behavior, whose pathogenesis is not yet fully understood. On the one side, we aimed to define the basal receptor density profile in adult BTBR mice, a model of idiopathic ASD. On the other side, we planned to identify differential brain area activation during social interaction by combining behaviors and morphofunctional neuroimaging.

12-weeks old C57BL6/J and BTBR mice underwent 5 trials of 10 minutes of social interaction with an age- and sex-matched C57BL6/J partner. In the last trial, the interaction partner was changed. During the first, third and fifth social interaction trials, 99mTc-HMPAO was injected in order to acquire images in a NanoSPECT/CTTM scan. Social interaction time and total distance travelled were measured. The basal binding density to the main ionotropic excitatory and inhibitory receptors was also quantified through in-vitro receptor autoradiography.

Throughout all the behavioral trials, the BTBR mice spent significantly less time interacting with the partner compared to the C57BL6/J. Interestingly, the lack of social interaction was accompanied by a significant increase of the distance travelled. Increased GABA-A binding density was found in the dorsal hippocampus and prefrontal cortex.

The BTBR mice confirmed to have a robust social deficit phenotype. The SPECT/CT images, which will be correlated to the behavioral and autoradiographic outcomes, will be of crucial importance to grasp the neuroanatomical fundament underlying social deficit in ASD.

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

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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.

Impact of voluntary running on adult neurogenesis in BDNF-deficient mice

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The subgranular zone (SGZ) of the hippocampal dentate gyrus (DG) is one of a specific brain area where adult neurogenesis can be observed. It is known that voluntary running has positive effects on cognition and prevents cognitive decline. Brain-derived neurotrophic factor (BDNF), as a member of neurotrophin family, has neuroprotective and –trophic properties. Within the postnatal hippocampus BDNF is involved in neuronal plasticity and in processes related to learning and memory. We investigated the role of BDNF as a key regulator of adult neurogenesis in respect to voluntary running.

Therefore we used six month old male BDNF-deficient mice (i.e. heterozygous BDNF knockout (+/-) and heterozygous knockout mice with an additional BDNF knockout in neurofilament L-expressing neurons (d/fl)) and their C57BL/6N wildtype littermates (+/+) with unlimited access to a running wheel in their cages for three weeks.

Analyzing individual parameters we found that the genotypes differ in their mean weight. The three different mouse lines did not differ in food intake during the three week voluntary use of running wheels. As compared to heterozygous BDNF knockout and wildtype mice, the conditional knockout mice display reduced running distances and times and, unexpectedly an increase in their body weight. Counts of doublecortin positive cells revealed that voluntary wheel running increases adult neurogenesis within the DG in wildtype and heterozygous BDNF knockout, but not in the conditional BDNF knockout mice.

Our data indicate an important and crucial involvement of BDNF in beneficially effecting adult neurogenesis due to the use of voluntary wheel.

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

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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.

C1q-TNF-related-peptides 1, 6 and 8 promote corneal wound healing by targeting relaxin receptor RXFP1

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Corneal wound healing is partly mediated via relaxin/insulin-like family peptide receptor 1 (RXFP1) pathway. C1q/tumor necrose factor related peptides (CTRPs) 1, 6 and 8 have been proposed as novel interaction partners of RXFP1. We previously were able to detect CTRP1, CTRP6 and CTRP8 expression in cell lines and human tissue of the ocular surface and lacrimal apparatus. In this study, we investigated the effect of CTRP1, CTRP6 and CTRP8 on ocular surface wound healing and its dependency on the RXFP1 receptor pathway.

In vitro ocular surface wound modeling was performed using a scratch assay. We analyzed the effects of different concentrations of recombinant CTRP1, CTRP6 and CTRP8 on cell proliferation and migration in human corneal (HCE) and conjunctival (HCjE) epithelial cell lines. By adding a specific inhibitory anti RXFP1 antibody, we determined whether these effects depend on the RXFP1 receptor pathway.

The application of 100 ng/ml recombinant human CTRP1, CTRP6 or CTRP8 resulted in a significantly increased surface defect healing rate in HCE cell line by a factor of 1.41 ± 0.08 (mean ± SEM; $p<0.0001$), 1.20 ± 0.09 (mean ± SEM; $p=0.0110$) and 1.24 ± 0.05 SEM (mean ± SEM; $p=0.0015$) respectively, but not in HCjE cell line. The addition of a specific inhibitory anti RXFP1 antibody diminished the effect of each of the respective CTRPs on the surface defect healing rate in HCE cells.

Our results suggest a novel role for CTRP1, CTRP6 and CTRP8 at the ocular surface by (partly) targeting the RXFP1 receptor pathway.

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

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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.

Frequency of interclinoid bridging in Bulgarians

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Ossification of the ligaments between the clinoid processes of the sphenoid bone gives rise to bony bridges, which enclose additional foramina. Such structures could have an impact on various neurosurgical procedures in the sellar region.

The study was performed on head CT scans of Bulgarians. The images were generated using CT system Toshiba Aquilion 64. The sample included 291 individuals (136 males and 155 females). All CT scans were obtained for diagnostic purposes. Interclinoid bridging was classified into 4 main types, which include subtypes.

The most common type of interclinoid bridging was between the anterior and middle clinoid processes forming a complete or incomplete caroticoclinoid foramen. In both sexes, the bilateral cases of interclinoid bridging were more frequent than the unilateral ones, which were more commonly observed on the right side. There were no significant bilateral differences of the interclinoid bridging in males and females. The comparison of the combined data for the right and left sides showed significant sex differences in the distribution of the interclinoid bridging by types.

The frequency of the interclinoid bridging in Bulgarians is comparable with previously reported one for different population groups. Interclinoid bridging appears to be a common variation in the sellar area and should be kept in mind in surgical interventions in this region.
This study was supported by the Bulgarian National Science Fund, Grant DN01/15-20.12.2016.

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

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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.

Deficiency for FTSJ1 affects neuronal plasticity within the hippocampal formation of mice

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Neuronal plasticity describes our brain's capability of neuronal rearrangement and is thus essential for every learning and memorizing process. An intellectual disability (ID) phenotype
could emerge from abnormal cellular processing leading to pre- and/or post-synaptic dysfunction. Since mutations in the X-linked tRNA methyltransferase FTSJ1 can cause non-syndromic ID, it might be possible that FTSJ1 has an impact on neuronal plasticity.

Serial sections of the hippocampal formation were made and the thickness of different hippocampal layers were analysed.
Using Golgi-impregnated material, dendritic spines were analysed in area CA1 and the dentate gyrus.
Long-term potentiation (LTP) was used to examine synaptic plasticity.

Within area CA1 a reduction in the mean thickness of the basal layers (Stratum radiatum and Stratum lacunosum moleculare) was seen in the FTSJ1 deficient mice as compared to age-matched littermates. In addition, we could show that dendritic spine densities were altered in the FTSJ1 deficient mice, suggesting a possible alteration in plasticity on the synaptic level. Therefore, we investigated whether changes in synaptic plasticity can be observed on the electrophysiological level by analyzing LTP. Stable LTP can be induced in area CA1 of FTSJ1 deficient mice and remained stable. However, LTP was significantly weaker as compared to the age-matched littermates.

These results suggest that FTSJ1, at least in the hippocampal area CA1 of mice, has an impact on neuronal plasticity, both on the morphological and physiological level. Comparable changes in other cortical areas might accumulate in disturbed learning and memory functions in general.

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Modelling air-liquid interface cerebral organoids of SHANK3 deficient patients to study neural cell populations

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A syndromic form of Autism Spectrum Disorder, namely Phelan-McDermid Syndrome (PMDS) is caused by the functional loss of SHANK3, an important synaptic scaffolding protein which leads to the development of a variety of symptoms such as intellectual disability, speech delay and muscular hypotonia in the patients. The objective of the study was to model a translational system for understanding the contribution of different neural cell types in the development of PMDS.

Cerebral organoids derived from hiPSCs of SHANK3 deficient patients and healthy controls were used in this study. The organoids were grown as whole and later sectioned and cultured as air-liquid interface organotypic sections. Immunohistochemistry was performed to analyze the various cell populations.

The neuronal cell population and axonal fiber bundling showed to be similarly distributed in control and patient cell lines whereas the glial cell population was found to be altered. The radial glial and astrocyte cell population was found to be lower whereas the population of the oligodendrocyte progenitor cells were seen to be significantly higher in the PMDS patients when compared to the controls- indicating a dynamic shift in the pattern of the glial cell distribution at an early stage.

These findings support the idea of different biological populations being involved in contributing to a disorder that was previously understood to be mostly neuronal. This study presents an idea of exploring the various cellular and physiological changes that occur in a neuro-developmental disorder such as PMDS by using the advancements in the field of stem cell biology.

The impact of hypoxia on the obese lung

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Obesity is correlated with a higher risk for lung diseases; however, the underlying pathophysiological mechanisms are still unknown.

Here, C57BL/6N mice were fed either control diet (CD, 10% fat) or high fat diet (HFD, 60% fat). After 27 weeks, half of each diet-group was treated with hypoxia (-Hyp, 13% O2) for 3 weeks. After 30 weeks, lung mechanics, ultrastructure and proteome were assessed.

In comparison to CD, HFD resulted in lower arterial pCO2, thicker septal endothelium and interstitium, and an upregulation of proteins associated with lipid metabolism. Regardless of diet, hypoxia led to increased levels of hemoglobin and hematocrit, lower arterial pCO2, and higher lung volumes. Compared to CD, CD-Hyp leaded to decreased elastance H and increased static lung compliance. Additionally, proteins regulating vascular cell surface interactions and extracellular matrix (ECM) organization were downregulated and the alveolar endothelium was thickened. Along with the changes in lung mechanics under CD-Hyp, hypoxia under HFD also caused lower hysteresis, pointing to surfactant alterations. While the interstitial thickness was decreased under HFD-Hyp, the fat-related thickening of the endothelium was not exacerbated. Also, proteomics implicated downregulation of platelet degranulation pathways.

Thus, both chronic hypoxia and high fat intake alone resulted in thicker alveolar endothelium and lower arterial pCO2, indicating higher ventilation rates to achieve adequate oxygen saturation. Besides red blood cell adaptations and higher lung volumes, hypoxia altered the pulmonary elastic recoil. Analyses of the lung proteome suggested dietary variations in hypoxia adaptation, particularly cell-surface interactions/ECM organization under CD and platelet degranulation under HFD.

Ultrastructural characterization of the cellular contacts between alveolar epithelial type 2 cells and fibroblasts

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Communication between alveolar epithelial type 2 cells (AE2) and fibroblasts (FB) is involved in inflammatory response and fibrogenesis in the lung. Previous studies mentioned cellular contacts between AE2 cell and FB through apertures in the basal lamina, however, the precise ultrastructure of these contacts have not been investigated.

In this study, lung parenchyma from a healthy human donor lung was analyzed by focused ion beam scanning electron microscopy. The high-resolution data was utilized to reconstruct a 3-dimensional model of a septal region spanning two AE2 cells and their corresponding basal lamina and, two fibroblasts.

We observed eight and eleven contact sites per AE2 cell. The ultrastructure of the contacts is comprised of foot processes of AE2 cells with or without cytoplasmic extensions of FB. One complex contact area consisting foot processes of AE2 cell, cytoplasmic extension of two FBs and one prominent elastic fiber was found. AE2 cell foot processes generally appear as clusters, which scatter over the basal surface of these cells. Interestingly, some of them coil underneath the intact basal lamina. The foot processes show differences in morphology (e.g. length, orientation, and branching).

Despite the low number of investigated AE2 cell and FB, our results reveal the diversity and the special ultrastructure of the cellular AE2-FB contacts, the possible number of contact site per cell, the actual shape and size of the aperture in the basal lamina. These findings complement the previous descriptions and define the precise morphology of the cellular contacts between AE2 cell and FB.

Analysis of retinal glia cells in an animal model for primary open angle glaucoma

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Primary open angle glaucoma (POAG) is a progressive optic neuropathy leading to visual impairment. Increased TGFβ2 concentrations in the aqueous humor of POAG patients is a key finding, whereas Decorin (DCN), an antagonist of TGFβ, was shown to be significantly decreased. DCN regulates collagen fibrillogenesis and can directly interact with several growth factors. Implication of TGFβs to the activation of glia cells during the pathogenesis of glaucoma is assumed, but whether DCN is involved in the regulatory mechanism is not known. To study the role of DCN in the regulation of retinal glia cells, we analyzed a DCN knockout mouse line, a mouse model for POAG.

The activation of glial cells was analyzed in sagittal cryo-sections and retinal flatmounts by immunohistochemical staining against IBA1 (8- and 12-week-old mice) and GFAP (12-week-old) in DCN deficient and wildtype mice. Retinal flatmounts were used to study the morphology of astrocytes and microglia. Quantification of mean pixel intensity was done with Fiji/ImageJ.

Microglia showed an increased activity in 8- and 12-week-old DCN knockout mice, which was observed by a significantly increased IBA1 staining in the ganglion cell layer as well as in the outer plexiform layer. Immunohistochemical analysis of GFAP showed a significant enhanced staining intensity and pronounced morphological differences in retinal astrocytes of 12-week-old DCN knockout mice compared to wildtype littermates.

We conclude that DCN deficiency induces the activation of microglia cells and astrocytes, which can under pathological conditions induce retinal ganglion cell death and contribute to the development of glaucoma.

Making Connections: Anatomical design for musculoskeletal tissue engineering

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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 connection[1]. 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 phalanx[2]. 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

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

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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.

Tachykinin NK3 receptors in mouse gallbladder motility

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Motility of the gallbladder is affected by circulating hormones like cholecystokinin, motilin or gastrin. However, a well spread ganglionated nerve plexus exists within the wall of the gallbladder that is likely to play an important role in gall bladder function. Here we focused on the tachykinins, known as important excitatory neurotransmitters in the enteric nervous system involved in coordination of gastrointestinal motility.

Immunohistochemistry was used to visualize substance P- (SP), neurokinin B- (NKB) and calcitonin gene-related peptide- (CGRP) immunoreactive nerve fibers in cleared gallbladder whole mounts of C57BL/6J and nAChRa3-eGFP mice, in which all postganglionic autonomic neurons express GFP. Expression of the tachykinin receptors was investigated by qPCR. The effect of the NK3 receptor agonist senktide on gallbladder contraction was investigated in organ bath experiments.

SP-, NKB- and CGRP-immunoreactivities were identified in a dense network of nerve fibers all over the gallbladder wall. While about 40 % of the SP- and NKB- positive fibers were colocalized with nAChRa3-eGFP, there was almost no colocalization with CGRP. In the gallbladder, qPCR showed an about 7fold higher expression of the NK3 receptor compared to NK1 and NK2 receptors. In urinary bladder and duodenum, NK1 and NK2 were the predominant receptors. In organ bath experiments, senktide (100 nM) induced a tetrodotoxin-insensitive contraction in gallbladder, but not in urinary bladder and duodenum.

The NK3 receptor is the predominant tachykinin receptor in the mouse gallbladder. Its activation causes gallbladder contraction. A dense network of autonomic tachykininergic nerve fibers is an endogenous source of NK3 receptor ligands.

Mapping of proprioceptive receptors in the human pericardium: topographic and gender differences and their significance for the cardiac cycle

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Proprioception describes the unconscious and conscious perception of tissues and organs' spatial and mechanical states. Different stimuli are selectively converted into impulse patterns by various peripheral receptors and transmitted via afferent fibers. The proprioceptive receptors have already been described for many structures of the musculoskeletal system, but their occurrence in internal organs has hardly been considered in the literature so far. This study aimed to map the pericardium's neuronal proprioceptive morphological entities in terms of their occurrence and frequency in a topographical context.

Eight complete human pericardia (4 female and 4 male samples) were histologically processed and closely evaluated using various histological standardized staining methods (including Elastica van Gieson and Azan) and immunohistochemistry (including pan-neuronal andmyelin basic protein).

Ruffini bodies, pressure receptors of self-perception, were found in large numbers adjacent to all eight pericardia. Furthermore, topographical and gender-specific differences in the density of receptors could be determined.

In summary, it can be said that the pericardium is subject to proprioceptive neuronal control. These undescribed findings may be essential for controlling and regulating of cardiac activity during the cardiac cycle.

New, Innovative 3D-in-vivo-Model for High-level Microsurgical and Supramicrosurgical Training - A Replacement for Animal Models

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Microsurgery and supermicrosurgery are surgical subdomains necessary for a large variety of surgical disciplines. So far, there is no training model for lymphatic surgery or perforator flap surgery and the most commonly used microsurgical training models are living animals. However, the ethical principles of replacement, refinement, and reduction (3R´s) of living animals for training purposes were implemented, highlighting the necessity of an animal-sparing microsurgical training model.

Formed during
embryogenesis, the chick chorioallantoic membrane (CAM) resembles a highly vascularized, non-innervated membrane within fertilized chicken eggs. Aim of this study was to utilize the CAM model as an innovative and versatile training model for (super-)microsurgery that can reduce the number of animals used for these purposes.

The variety of different sized vessels for the implementation of an anastomosis proved the CAM model as a well-functioning (super-)microsurgical training model. The circulatory system is resilient enough to withstand the mechanical stress applied to the tissue and the patency of the implemented anastomosis can be tested for the verification of the procedures.

In summary, the integration of the CAM model into a
surgical training program can benefit its quality by representing a realistic anatomical and physiological model with a high variety of vascular structures. Moreover, the CAM model satisfies the principles of the 3 R´s as an animal-sparing model, indicating the potential of this model as an innovative microsurgical training model for the improvement of surgical skills.

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

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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 function to differences between patients.

Transcriptomic profiling after microglia-specific Smad4 deletion in early postnatal and adult mice

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Microglia are the resident immune cells of the CNS and TGFb signalling has been proven to be essential for the regulation of microglia. We have recently shown that microglia-specific deletion of Tgfbr2 leads to impaired microglial homeostasis and loss of maturation. However, the role of Smad4, a common downstream signalling molecule for TGFb superfamily members in microglia remains elusive.

We have generated tamoxifen-inducible mutant mice with microglia-specific Smad4 deficiency. Total tissues from frontal cortices of 28 days (P28) and adult Smad4 mutants, were used for total RNA extraction. RNA was used for subsequent analysis with nCounter® Mouse Glial Profiling Panel and differential gene expression was analysed.

Microglia specific Smad4-deletion lead to impairment of homeostatic microglia markers such as P2ry12, P2ry13, Hexb, sall1 and Gpr34 at both timepoints. Microglial activation markers such as Cst7, Cybb and Grm are increased in mutant mice. Moreover, astrocyte genes such as Axl, Apoe, Aqp4 and Gfap are increased in p28 KO and to a lesser extent in adult mutant mice

In conclusion, Smad4 mutants show a loss of Tgfb-regulated homeostatic microglia gene signature suggesting that the homeostatic microglia signature is dependent upon the Tgfb-Smad4 axis. Increased expression of microglia activation markers indicates an essential role of Smad4 in regulating microglia reactivity. Finally, our data show that reactive astrocytosis is driven by activated microglia in Smad4-defecient mice.

Sexually differentiated microglia and hippocampal postnatal synaptic pruning

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Synaptic pruning after birth, aimed at elimination of weak synapses for the establishment of an adult synaptic network, has been shown to be a function of primarily microglia phagocytotic activity. Microglia, in turn, are differentiated in a sex-dependent manner. In this study we tested whether sexual differentiation of microglia results in differences in postnatal synaptic pruning in CA1 pyramidal cells of the hippocampus of male and female mice.

The stereological counting of synapses in the electron microscope showed no difference in synapse densities between male and females during adolescence, puberty and adulthood. Only on postnatal day (P) 14 was the density of synapses significantly higher in the female than in the male hippocampus, which supported results of previous studies. A consistent difference in microglia surface to volume ratio was seen on P14, with a lower ratio in males than in females. Such a difference was neither observed at P7 nor at P21. Similarly, expression of CD68 as a marker of active microglia was stronger in females than in males at P14. P2Y12 immunofluorescence in the hippocampus of Thy1-eGFP mice allowed for the localization of microglia in relation to dendritic spines.

Importantly, we found a significantly higher percentage of spines which were encapsulated by microglia in females than in males at P14. Since CD47 expression, the “don’t eat me” signal of neurons, was similar in male and female hippocampus at P14, our results argue for a delay in microglia differentiation in the female hippocampus as compared to males at the end of the second postnatal week.

Overall, despite the important role of microglia in synaptic pruning the sexual differentiation of microglia does not lead to long lasting sex-related differences in synaptic numbers in the hippocampus.

Determining the intramuscular course of nerve branches using the modified Sihler's technique – an overview of practical applications

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During different surgical approaches muscles often need to be split. As their innervating nerves do not end by entering the muscle bellies, their branches may be harmed resulting in partial or total loss of function of the respective muscle. To minimize this risk, knowledge of the exact intramuscular branching patterns of muscular branches is important. The aim of this research is to determine the intramuscular course of nerves innervating muscles at high risk during surgical procedures.

The modified Sihler's technique was performed on each eight to ten vastus medialis, pectoralis major, and deltoid muscles. The muscles and their nerve branches were harvested from embalmed specimens after regular dissection courses.

The exact intramuscular course of the nerve branches within these muscles was thus observed. In principle, a characteristic pattern with some small variations is recognizable for each muscle. The entering nerves usually branch very strongly and even form intramuscular anastomoses in some of the muscles. In the case of the vastus medialis muscle, some nerve branches extended out through the muscle into the fibrous capsule of the knee joint.

In spite of its time-consuming nature, the modified Sihler's technique is ideally suitable for visualizing intramuscular nerve courses in three-dimensional detail. However, the direction of the anastomoses and the quality of the individual nerve branches cannot be determined.

PROJECTION TARGETS OF EFFERENT NEURONES IN THE INTERNAL BRANCH OF THE SUPERIOR LARYNGEAL NERVE : STRONG EVIDENCE OF DOUBLE INNERVATED POSTERIOR CRICOARYTAENOID MUSCLE

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For over two centuries, the traditional view on the motor innervation of the larynx has been in question. Namely, the intrinsic laryngeal muscles may not solely receive motor innervation from the inferior laryngeal nerve (ILN). Moreover, through tracer studies over the past several decades, the internal branch of the superior laryngeal nerve (SLNib) has been shown to not only carry sensory but also motor neuron fibres. As such, our aim was to investigate the potential target(s) of the SLNib efferent fibres in vivo and ex vivo in rats and rabbits.

Twenty male albino rats underwent glycogen depletion of the laryngeal muscles. In addition, electromyogramm recording was performed in five times each albino rats and rabbits. Furthermore, the fiber distribution within the internal branch of the superior laryngeal nerve was studied immunhistochemically in additional four male albino rats.

All results revealed a double innervation of the ventrolateral part of the posterior cricoarytenoid muscle by the internal branch of the superior laryngeal nerve and the inferior laryngeal nerve.

As this part of the muscle is the true abductor of the vocal folds, the ability to retain vocal fold abduction by the posterior cricoaryaenoid muscle in the setting of paralysis of the recurrent or inferior laryngeal nerve can possibly be explained by intact innervation of that muscle by efferent neuronal fibres of the internal branch of the superior laryngeal nerve.

The effect of stress on prolactin immunoreactivity in rat adenohypophysis: immunohistochemical and electron microscopical study

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Recent studies demonstrated increased serum prolactin levels upon stress. Glucocorticoids, liberated from adrenal cortex due to hormonal signals from pituitary corticotrophs are known to play a key role in systemic stress response. Previously we found evidence that corticosteroid binding globulin (CBG) is involved in rapid, membrane-mediated actions of adrenal steroids. Here we studied in greater detail immunostaining for prolactin and for CBG in pituitaries of rats, which were exposed to various stressors.

In our study, we compared male rats after osmotic challenge (N= 3, N=3 controls) with late pregnant (N=3), parturient (N=3), and early lactating rats (N=3), assuming that parturition represents a strong physiological stress. We employed immunoperoxidase staining of semithin sections, double immunofluorescence and double immunoelectron microscopy.

In stressed males we detected increased prolactin immunofluorescence associated with membranes while in controls this staining was predominantly cytoplasmatic. CBG immunofluorescence was found in almost all prolactin cells of stressed males while such double staining was only occasionally observed in controls. Similar observations were made in females: While parturient rats showed intense membrane associated double staining for both antigens, late pregnant and early lactating animals showed patterns like male controls. Immunoelectron microscopy revealed increased exocytosis of prolactin containing vesicles in lactating rats. CBG was localized on cell membranes and additionally within prolactin vesicles.

Our observations suggest prolactin liberation from pituitary lactotrophs along with CBG upon systemic stress response. Late pregnancy and early lactation as well as osmotic challenge showed a similar effect, whereby osmotic challenge appears to be a more intense stressor.

Mechanical stress and lysosomal increase in the development of Focal Segmental Glomerulosclerosis

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Focal Segmental Glomerulosclerosis (FSGS) is one of the most common causes of nephrotic syndrome (NS) characterized by proteinuria, edema, hyperlipidemia, hypoalbuminemia and hypertension. FSGS is characterized morphologically by sclerotic lesions in a subset of glomeruli. The mechanisms underlying glomerular damage and disease progression are not fully understood and are the aim of this work.

The FSGS mouse model was characterised at different time points during FSGS development. Renal function analysis, immunohistochemistry, western blot, histological analysis were performed. Additionally, immortalized mouse podocytes were used for RT-PCR, western blot and immunohistochemistry analysis following the application of mechanical stretch.

Morphological analysis of kidney sections of FSGS and control groups revealed augmented number of lysosomes in podocytes of FSGS mice already at day 9 (+104%) as well as at later time points at day 17 and 28 (+185% and +187%) after induction. Moreover, immunohistochemistry analysis revealed increased TFEB, a master regulator of lysosomal biogenesis, and Galectin 3, lysosomal damage marker, in podocytes of FSGS group compared to control. In addition, immortalized podocytes showed significant increase in lysosomal marker LAMP1 5 days after mechanical stress induction.

Our data suggest that mechanical stress leads to lysosomal increase and this lysosomal increase is one of the factors promoting podocyte injury. Additionally, observed increase in lysosomal biogenesis and – damage during FSGS development suggest that an additional event is required for the glomerular disease progression in FSGS.

Ultrasound detection of the axillary arch in a prospective dissection-controlled cadaver study

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A potential causality for the thoracic outlet syndrome (TOS) is the presence of an axillary arch. The aim of this study is to investigate the effectiveness of ultrasound in detecting such a variation.

200 axillae from 100 body donors were investigated. The arms were abducted and the axillae were examined with a standard ultrasound apparatus for the presence of an axillary arch. Then the axillae were carefully dissected. Descriptive statistics was used for presenting and comparing the results.

Ultrasound was capable of detecting an axillary arch in body donors (2 unilaterally and 3 bilaterally). Subsequent dissection revealed the presence of 12 axillary arches in a total of 8 body donors. Hence, the sensitivity of ultrasound was 67% for all types of axillary arches (muscular and connective tissue). On contrast, ultrasound had a sensitivity of 87% for detecting axillary arches solely comprised of muscle tissue. The positive predictive value was 50% and the negative predictive value was 97%.

Our data show that ultrasound is capable of correctly diagnosing axillary arches. However, they also demonstrate that arches, in particular those without muscle tissue might be missed. Therefore, ultrasound can be recommended as screening tool for identifying the causality in patients diagnosed with thoracic outlet syndrome, but it cannot rule out the existence of an axillary arch.

Structural connectivity differences reflect microstructural heterogeneity of the human insular cortex

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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.

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The effects of LIPUS on the proliferation and differentiation of MC3T3 cells; in vitro study

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A deficient healing can lead to the absence of bridging bone between the fracture fragments, a so-called Pseudoarthrosis.
Low-Intensity Pulsed Ultrasound (LIPUS) is a technology that could bring benefits to the fracture healing process through nano-motions, matrix synthesis, and cell proliferation. In this study, we aimed to demonstrate the efficacy of the effects of LIPUS on MC3T3-E1 cells in an in vitro study.

MC3T3-E1 cells were stimulated with LIPUS twice a day for twenty minutes each. Proliferation was detected with the CyQuant cell proliferation assay. The Osteoblast differentiation was assessed using Immunofluorescence staining against Osterix, Runx2, VEGF, and using Alizarin-red staining. Released VEGF R1/Flt, VEGF, and IL-6 in the culture medium was quantified using ELISA

: When we compared the proliferation and cell-differentiation between the control groups and the treated groups with LIPUS, We observed higher cell proliferation after LIPUS treatment in osteoblasts, but no effect was detected in cytokine Both immunofluorescence of Osterix and Runx2 and Alizarin-red staining showed the same pattern of osteogenic differentiation and calcification

our data indicate minor effects of short-term LIPUS treatment in monolayer cell culture. further long-term experiments and possibly three Dimension culture will be performed in the near future to determine the long-term effects of LIPUS.

Desmosomal adhesion and cytokeratin dynamics are regulated non-canonically by Dolichyl-Phosphate Mannosyltransferase 1 (DPM1) through regulation of SERPINB5

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Desmosomes are complex multiprotein units that are vital mediators of intercellular adhesion and their dysregulation causes severe pathogenic conditions. However, the regulation of desmosome complexes and the adhesive function are only partially understood.

A CRISPR/Cas9 whole-genome screen was applied to identify novel modulators of desmosomal adhesion. Independent knockout cell lines were generated for potential hits such as dolichyl-phosphate mannosyltransferase (DPM1KO). Cellular characteristics were determined by immunostainings, Western blot, FRAP, and dispase-based dissociation assays. Differentiation of epidermis was studied by generating 3D reconstructed human epidermis (3D-RHE). Proteomic analysis was performed by mass spectrometry.

DPM proteins are known regulators of glycosylation, which is required for intracellular trafficking and protein turnover. DPM1KO enhanced desmoglein 3 levels on the cell surface, but surprisingly reduced cell-cell adhesion in HaCaT cells and primary human keratinocytes. A deeper characterization of DPM1KO cells showed reduced levels and mobility of other desmosomal components such as desmoplakin (DSP) or desmoglein 2 at the cell membrane, indicating profound impairment of normal desmosome composition. Moreover, we noted reduced keratin bundles, demonstrating cytoskeletal alterations in DPM1KO cells. Pilot observations of DPM1KO indicated an impaired differentiation in 3D-RHE. Proteomic analysis of DSP interactors showed a serine protease inhibitor SERPINB5 bound to DSP in control cells but not in DPM1KO cells. SERPINB5 overexpression in DPM1KO background significantly restored cell-cell adhesion, desmosomal and cytoskeletal alterations.

These data suggest a non-canonical role of DPM1 in differentially modulating distinct subsets of desmosomal molecules.

Spinal cord obtained from human body donors is suitable for multicolor immunofluorescence stainings

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Immunohistochemistry is a powerful tool to study neuronal tissue from humans at the molecular level. Harvesting fresh neuronal tissue from human organ donors is difficult and sometimes prohibited. In anatomical body donors, neuronal tissue is dedicated to research purposes and because of the easier availability, it may be an alternative source for research. This study aimed to determine how long after death, valid immunohistochemistry is possible in spinal cord from body donors.

Spinal cord were harvested from a single organ donor 2 hours (h) postmortem and from body donors 24, 48, and 72 h postmortem. We used a series of general neuronal markers (antibodies against neurofilament, synaptophysin, and neuronal nuclear protein), specific neuronal markers (antibodies against choline acetyl transferase, vesicular acetylcholine transporter, and calcitonin gene related peptide), and glial markers (antibody against myelin basic protein) to visualize different subsets of neurons by multi-color immunofluorescence or immunoperoxidase.

We showed that it is possible to visualize molecularly different neuronal elements with high precision in body donor spinal cord 24h postmortem and the quality of the image data was comparable to those from the fresh organ donor spinal cord. High contrast multicolor images of 24h spinal cords allowed accurate automated quantification of different neuronal elements in the same sample. Over postmortem interval we found antibody specific signal reductions.

This study has defined a postmortem time window of at least 24h during which valid immunohistochemical information can be obtained from body donor spinal cords.

3D-Printing and DIY Ultrasound Phantoms as a Versatile and Cost-effective Alternative to Commercial Models for Healthcare Education

David RESUEHR (Cell, Developmental and Integrative Biology, School of Medicine, UAB, Birmingham)

In this presentation I would like to demonstrate some of the methods we have used in teaching anatomy and basic ultrasound to students of healthcare professions at the University of Alabama at Birmingham in the past years. The aim is to highlight the relative simplicity with which medical educators can be creative by making their own, versatile, affordable and efficient teaching tools.

We used several different filament 3D-printers (Lulzbot Taz6, Raise3D E2 and Flashforge Adventurer 3) to create anatomical models. Files of anatomical models were retrieved from the NIH 3D Printing collaborative and Thingiverse. Ultrasound (US) phantoms were made with ballistic gelatins (Vyse and Humimic Medical) and were customized depending on the training objective e.g., vessel trainers, foreign object recognition etc.

We have now for several years been able to use homemade US phantoms in teaching ultrasound workshops and courses to students of healthcare professions such as Medicine, Nurse Anesthesia and Physician Assistant. 3D-printed anatomical models have been piloted in several courses and well received by students. In some instances, we were able to create hybrid US trainers that included 3D-printed items or molds to pour gelatin into, to create realistic looking anatomical structures.

The availability and inexpensiveness of 3D-printers and materials along with the increasing ease of use has made this technology very useful for medical educators. The possibilities it opens up are near limitless. Merging 3D-Printing with US-phantoms has further increased our abilities to create powerful, affordable and intuitive teaching modalities.

Interactions of antibodies against Listeria monocytogenes with the low-density lipoprotein-receptor-associated-protein-1 due to molecular mimicry correlate to higher β-amyloid levels in human neuroblastoma cells

Bernhard Reuss (Institute for Neuroanatomy, Universitary Medical Center Göttingen, Göttingen)

In the brains of Alzheimer's disease (AD) patients, β-amyloid (Aβ) is known to accumulate in senile plaques, causing neurodegeneration and cognitive decline. This depends on effective Aβ-clearance, in which the LDL-receptor-related-protein-1, and its regulator, the low-density-lipoprotein-receptor-associated-protein-1 (Lrpap1) are critically involved. Thus in AD patients reduced Lrpap1 levels correlate to increased Aβ-accumulation. A link for this could be the interaction of an antiserum to the Gram-positive bacterium Listeria (L.) monocytogenes with Lrpap1, functional effects of which are characterized here at more detail.

For this, interactions of antibacterial antibodies with neural proteins were investigated using the HexSelect multiprotein array (engine, Berlin). Interactions were further confirmed by Western blotting with recombinant Lrpap1-proteins (Origene, Rockville), and with neuroblastoma cell lysates using Lrpap1-specific antibodies. Aβ-accumulation in antibody treated neuroblastoma cells was assessed by immunocytochemistry and Western blotting. For functional tests by calcium imaging, antibody-treated neuroblastoma cells, stained with Fluo3 were imaged during stimulation with acetylcholine.

The obtained results demonstrate for the first time that antibodies to L. monocytogenes are indeed able to interact with Lrpap1, and that this contributes to increased extracellular Aβ-levels in human SH-Sy5y-, and SiMa- neuroblastoma cells. In addition, an antibody dependent decrease in cholinergic signaling can be observed in these cell lines.

Therefore, these in vitro findings open the possibility that infections with L. monocytogenes and the resulting immune responses could lead to an inactivation of Lrpap1 and consequently to impaired Aβ-clearance. Although highly speculative, this mechanism could be of pathogenic relevance in at least a subset of idiopathic AD-cases.

MORPHOLOGICAL CHANGES IN THE STRIATED MUSCLE CAUSED BY COMPONENTS OF THE THIEL EMBALMING METHOD

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Thiel-fixed body donors are highly valued for medical training courses. The reason for the flexibility of Thiel-fixed tissue remains unclear and the histologically visible fragmentation of striated muscle has been postulated as the cause. The aim of this study was to analyze whether a specific ingredient, pH, decay, or autolysis could be responsible for the fragmentation. Elucidating the cause could lead to modulating the Thiel-solution and adapting specimen flexibility specifically to the needs of different courses.

Striated mouse muscle was fixed for different time periods in formalin, Thiel-solution, and the individual ingredients, and then compared by light microscopy. Furthermore, the pH-values of Thiel-solution and its ingredients was measured to investigate a correlation with the fragmentation. In addition, unfixed muscle tissue was histologically analyzed including Gram staining to investigate a relationship between autolysis, decomposition, and fragmentation.

Muscle fixed with Thiel-solution for 3 months showed a slight increase in fragmentation compared to muscle fixed for 1 day. Three of the individual ingredients, all of which belong to the group of salts, showed a slight fragmentation. Decay and autolysis had no effect on fragmentation. Fragmentation occurred regardless of the pH of all solutions.

Fragmentation of Thiel-fixed muscle is dependent on fixation time and most likely occurs due to salts present in the Thiel-solution. Adjustment of the salt composition in the Thiel-solution with verification of the influence on the fixation effect, fragmentation and flexibility of the cadavers could be performed in further studies.

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

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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.

The expression of surfactant proteins A and B during postnatal development of rat lung

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The surface active agent (surfactant) reduces the surface tension on the inner surface of the alveoli and prevents them from collapsing. Among the surfactant are the four surfactant-specific proteins (SP), which are responsible for the functional and structural integrity as well as for the stabilization of the intraalveolar surfactant-film. The aim of this work was to investigate whether the postnatal morphological maturation of the rat lung is associated with characteristic changes in surfactant-protein expression. The hydrophilic SP-A and the hydrophobic SP-B were investigated.

Using immunohistochemical and molecular biological methods (Western Blot, RT-qPCR), the two SPs of adult rat lungs and of those with different postnatal developmental stages (3, 7, 14 and 21 days after birth) were characterized.

The results show SP-dependent and development-dependent differences. The significantly highest relative surface fractions of SP-A labeled alveolar epithelial cells type II (AE II) are found together with the highest relative SP-A gene expression before the alveolarization (3 days after birth). With the downregulation of SP-A gene expression during and after alveolarization (7 and 14 days after birth), the surface area of the SP-A labeled AE II also decreases, so they are lowest in adult animals. The surface fraction of SP-B labeled AE II, the protein expression as well as the SP-B gene expression is comparable before, during and after the alveolarization. A significantly larger surface fraction of the SP-B marked AE II is only detectable in adult lungs.

The maturation of the rat lung is associated with differential changes of SP-A and SP-B.

Interclavicularis anticus digastricus muscle in a female body donor

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Variations of the muscles of the ventral thoracic wall are divers and can be observed in many cases. Due to there size and location they might be of clinical interest.

Here we describe an unusual muscular variation of the thoracic wall which was discovered during dissected of a West-European female body donor.

During dissection, an interclavicularis anticus digastricus muscle was found. It originated from the manubrium sterni and span bilaterally to the clavicles. A tendon on the ventral surface of the manubrium sterni connected the two muscle bellies. Furthermore, the innervation of both parts of the muscle was ensured by branches of the lateral pectoral nerve.

Variations of the ventral thoracic wall including the interclavicularis anticus digasticus muscle are of unknown prevalence. However, the knowledge about this muscular variation might be of clinical interested regarding orthopedics and thoracic surgery. Due to its localization, it might be a vulnerable structure during infraclavicular insertion of a subclavian vain catheter or clavicular fractures.

Towards a characterization of CD cell surface antigen expression in the human spinal cord

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We aim to comprehensively characterize the expression pattern of a broad array of cluster-of-differentiation (CD) surface antigens to further characterize functional network relationships and to refine the anatomical cartography of the human spinal cord.

Immunofluorescence (IF) analysis was performed on 8-10 m thick frozen human spinal cord specimens (cervical region). In brief, sections were air-dried (1h), fixed (2 min in 4% paraformaldehyde) followed by permeabilization/blocking (1h) in fish-gelatin-solution containing 1% bovine serum albumin (BSA) and 0.1% Tween®20 in TBS. Primary antibodies at 1:50 dilution in 1% BSA/TBS were incubated overnight at 4°C. After washing, sections were incubated with the corresponding secondary antibodies for 7h at 4°C. Whole specimen IF images were taken using an Olympus VS120 slide scanner at 20x magnification.

A select panel of >30 CD surface antigens has been analyzed to date. Besides some anticipated expression patterns, as e.g. for the integrin family member CD49b, others showed specific and not previously described distribution. CD15 (Lewis-X) staining was exclusively discernable at the ependymal layer of the central canal, whereas the heat-stable antigen, CD24, showed a pronounced expression around the central canal as well as in Rexed lamina II. CD90 (Thy-1) was predominantly expressed throughout the gray matter, yet sparing Rexed lamina X. In contrast, CD99 (MIC2) within the gray matter appeared to be confined to lamina X, whereas CD200 (OX-2) was expressed in the entire gray matter.

Prospectively, the ongoing expression screen for >200 CD antigens will help to elucidate the cellular cartography of the human spinal cord.

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

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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.

Developing a 3D brain model as an online learning tool for health profession students

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Studying the morphology of the brain is challenging for medical students. To improve understanding of the multiple neuroanatomical structures, a virtual 3D-brain model was created.

The model is based on a freely available ultra-high resolution ex-vivo 7T MRI dataset of the human brain from the study “7 Tesla MRI of the ex-vivo human brain at 100 micron resolution” published October 2019 in Nature. Over the course of a year, our team from the Paracelsus Medical University (PMU) has been working on an innovative and original interactive 3D-model of the human brain. Specific structures were segmented in brain images using the open-source medical image analysis software ITK-SNAP. Repairing and smoothing the 3D-mesh was performed via Autodesk Meshmixer (Autodesk Inc., CA). The finalized mesh was imported into Unity (Unity Technologies, CA) to create the 3D-model.

The completed interactive brain model is freely available on the PMU website (https://www.pmu.ac.at/anatomie) to help medical students visualize and memorize challenging neurological brain anatomy such as the corpus striatum, ventricular system, limbic system and cerebellum.

We envision that this brain model will improve understanding of human brain anatomy in conjunction with other already existing 3D-applications and virtual learning tools.

Analysis of microglial synaptic engulfment during postnatal CNS development

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During the development of the CNS, microglia are important for neural differentiation, the establishment of neuronal circuits, and synaptic pruning. Synaptic pruning is defined as the removal of a subset of synapses, while others keep maintained. The aim of this study was to analyze at which timepoints during postnatal CNS development microglia are actively engulfing synapses

Brains from NMRI and C56BL/6 wild-type mice at different postnatal time points were used to isolate total proteins and microglia. Developmental changes in synaptic proteins of cortices and hippocampi were analysed using western blotting at postnatal days (P) 0, 7, 14, 21, 28 and 56. Same time points were used for immunohistochemistry to label synaptic markers Synapsin and PSD95. Flow cytometry was established to directly quantify microglia with engulfed Synapsin.

During the first postnatal weeks, the total levels of synaptic proteins in the cortex and hippocampus increased and later decreased to reach a steady state. Using the newly established flow cytometry protocol, we were able to demonstrate that this method is specific and sensitive to detect and quantify microglia with engulfed synapses. Using this approach, we show that the maximum of phagocytic microglia corresponds with the observed decrease in synaptic markers during postnatal CNS development.

Together, our data indicate that microglia actively remove excessive synapses during postnatal brain maturation. Disturbances of microglia functions and phenotypes at critical developmental timepoints could have severe implications for postnatal CNS development

Characterization of the role of sensory neurons for the induction of neurogenic inflammation in the airways using a Trpa1-DTR mouse model

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Previously, we have shown that tracheal brush cell (BC) activation via denatonium leads to transient receptor potential membrane channel 5 (TRPM5)-dependent release of calcitonin-gene-related-peptide (CGRP) and substance P (SP) resulting in neutrophil recruitment. Here, we explore sensory nerve fibers' involvement in neurogenic inflammation-induction in the airways.

We generated mice that express the diphtheria-toxin (DT)-receptor (DTR) in Trpa1+ neurons by cross-breeding Trpa1tauGFP-IRES-Cre mice with Rosa26iDTR. Mice were injected with DT (20 ng/kg body weight, i.p.) on three consecutive days. Trpa1+ nerve fibers depletion was characterized by immunohistochemistry, [Ca2+]i-imaging, and real-time RT-PCR. Next, mice with depleted Trpa1 sensory innervation received Evans blue (EB, 20-mg/kg, i.v.). 30 min after inhalation of 1, 10, and 20 mM denatonium (4 µl) tracheas were explanted, and EB and neutrophil extravasation was estimated.

Immunohistochemistry of dorsal root ganglia (DRG) from DT-treated Trpa1tauGFP-DTR mice revealed a loss of Trpa1tauGFP+ neurons. TRPV1-expressing neurons were decreased by 80%. CGRP+/SP+ double-immunoreactive neurons were almost abolished. Supportively, the CGRP+/SP+ nerve fibers volume was reduced in tracheal whole-mount preparations of mice depleted from Trpa1+ neurons. A scarce number of primary neurons isolated from DRG and jugular-nodose-complex responded to the TRPA1-agonist cinnamaldehyde and to TRPV1-agonist capsaicin. RT-PCR demonstrated that the Trpa1- and Trpv1- expression were significantly down-regulated in both sensory ganglia. DT-treated Trpa1tauGFP-DTR lacked an EB extravasation and neutrophil recruitment after BC-stimulation.

Taken together, our Trpa1-DTR mouse model can be successfully used for ablation of sensory innervation. The BC-induced protective neurogenic inflammation depends on transmission to/from sensory nerve fibers.

THE SIGNIFICANCE OF ANATOMICAL VARIANTS IN THE CIRCLE OF WILLIS IN CEREBROVASCULAR DISEASES - AN AUTOPSIC STUDY

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The aim was to analyze the anatomical variants of the circle of Willis (CoW) and the histology of its arteries in relation with the causes of death in patients with cerebrovascular disease.

We realized a retrospective analysis of all cases with cerebrovascular disease admitted in „Prof. Dr. N. Oblu” Emergency Clinical Hospital from Iași, Romania. The research was based on the macroscopic and microscopic analyses of CoW specimens obtained at autopsy over a period of 30 months.

Out of a total of 96 human brains taken into study, only 28 cases (29.17%) presented anatomical variants of CoW. The anatomical variants of anterior part were absence, hipoplasia, fenestration and duplication, but only ACA hypoplasia was associated with the patient's death due to a ruptured ACoA aneurysm. The posterior part presented aplasia, hypoplasia, and fetal type as anatomical variants of its arteries. Anatomical variants of the posterior part of the CoW were associated with ischemic strokes if there was occlusion of the internal carotid artery or basilar artery, and with hemorrhagic strokes in the conditions of vascular changes caused by hypertension and atherosclerosis in the constituent arteries of the CoW. The histopathological and immunohistochemical exams revealed the replacement of smooth muscle fibers by fibrous connective tissue and the presence of the atherosclerotic plaques.

There was a great heterogeneity of ther causes of death associated with anatomical variants in the CoW and significant risk factors (such as arterial hypertension, diabetes mellitus type II, and liver cirrhosis).

Ambivalent functions of Dickkopf1 in postnatal enteric progenitor cells derived from mice and human

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Neurogenesis in the mature enteric nervous system is controversially discussed. Deciphering the molecular-regulatory network of enteric neurogenesis is still in its infancy. Since the Wnt-antagonist Dickkopf1 (DKK1) is well established in tissue homeostasis, we proposed the hypothesis that DKK1 could function as a negative regulator of enteric neurogenesis in the postnatal gut of mice and human.

We used proliferation- and cell-death assay, immunohistochemistry, in-situ-hybridization, qRT-PCR, as well as Western-Blot analysis.

We detected the expression of DKK-ligands and corresponding receptors within submucosal and myenteric ganglia of the murine and human intestine. Moreover, molecular biological evaluation revealed that postnatal ENS-progenitors are equipped with receptors and signaling cascade components essential for pharmacological probing. On a cellular level, DKK1-stimulation increased the proliferation of murine and human ENS-progenitor cells in-vitro. However, this was accompanied by an increased cell-death in the P75-positive neural cell population via a Caspase-3/7-dependent mechanism resulting in less differentiated enteric neurons and glial cells. Intriguingly, we were able to rescue this effect by co-applying a pan-Caspase-inhibitor.

The ambivalent functions of DKK1 on postnatal ENS-progenitors presented here are the first step to deepen our understanding of how the ENS is maintained during postnatal maturation. Although we had to reject our initial hypothesis, DKK1 proofed to have a strong, though unexpected regulatory influence on the murine and human ENS-progenitor pool in-vitro. Ongoing studies address the DKK1-function in the ENS of the living animal.

Gender Diversity in anatomical teaching

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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.

The potential role of SP-G and PLUNC in tumor pathogenesis and wound healing in the human larynx respectively the vocal fold

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Objective
Surfactant proteins (SP) well known from lung have meanwhile been detected in other parts of the human body, e.g., in the larynx. Beside their surface activity they have been described to play important roles in the innate immune system. The objective of this study was to detect and characterize the expression and distribution of SP-G and PLUNC in the human larynx.

Methods
Expression and distribution of SP-G and PLUNC in human tissue of vocal-folds and in an immortalized human laryngopharyngeal cell line (FaDu HTB-43TM) was analyzed using immunofluorescence. The possible role of the SP-G and PLUNC during wound healing was investigated by means of in vitro wound healing assay using ECIS. Furthermore, FaDu cells were treated with scratch or stimulation with cortisol, serotonin and bombesin, followed by quantification of mRNA-expression of SPs using qRT-PCR.

Results
The presence of SP-G and SP-H was demonstrated in squamous epithelium of the vocal fold and in FaDu cells. The wound healing assay showed a beneficial influence of SP-G and PLUNC during wound healing. Mechanical stress (scratch assay) and stimulation with cortisol and serotonin induced the expression of SP-G and PLUNC in the laryngeal cell line.

Conclusions
The results demonstrated that SP-G and PLUNC are present within the epithelium and mucus of the vocal fold and therefore part of the human larynx. Based on our results, we assume that the proteins may play an important role during wound healing and tumor pathogenesis in the human larynx respectively the vocal fold.

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

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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.

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

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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.

Lipofibroblasts in structurally normal, fibrotic and emphysematous human lungs

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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.

Dsg1 and Dsg3 composition of desmosomes across human epidermis and alterations in pemphigus vulgaris patient skin

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Desmosomes are important epidermal adhesion units and signalling hubs, which play an important role in pemphigus pathogenesis. Different expression patterns of the pemphigus autoantigens desmoglein (Dsg)1 and Dsg3 across different epidermal layers have been demonstrated. However, little is known about changes in desmosome composition in different epidermal layers or in patient skin. The aim of this study was thus to characterize desmosome composition in healthy and pemphigus skin using super-resolution microscopy.

Methods: Immunostaining, STED microscopy, co-localization analysis
Use of human material in ex vivo human skin model, and (pemphigus) patient skin samples

An increasing Dsg1/Dsg3 ratio from lower basal (BL) to uppermost granular layer (GL) was observed. Within BL desmosomes, Dsg1 and Dsg3 were more homogeneously distributed whereas superficial desmosomes mostly comprised one of the two molecules or domains containing either one but not both. Extradesmosomal, desmoplakin (Dp)-independent, co-localization of Dsg3 with plakoglobin (Pg) was found mostly in BL and extradesmosomal Dsg1 co-localization withPg n all layers. In contrast, in the spinous layer (SL) most Dsg1 and Dsg3 staining was confined to desmosomes, as revealed by the co-localization with Dp.
In pemphigus patient skin, Dsg1 and Dsg3 immunostaining was altered especially along blister edges. The number of desmosomes in patient skin was reduced significantly in basal and spinous layer keratinocytes with only few split desmosomes found. In addition, Dsg1-Pg co-localization at the apical BL and Dsg3-Pg co-localization in SL were significantly reduced in patients, suggesting that that extradesmosomal Dsg molecules were affected.

These results support the hypothesis that pemphigus is a desmosome assembly disease and may help to explain histopathologic differences between pemphigus phenotypes.

Ablation of scleral TGF-BETA signaling increases IOP induced optic nerve damage

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It is unclear if scleral stiffening in human glaucoma causes or contributes to optic nerve (ON) axonal damage. Alternatively, scleral stiffening might provide a beneficial adaption that attenuates the intraocular pressure (IOP)-induced axonal damage. Scleral stiffening in glaucoma is most likely driven by TGF-BETA signaling that induces the synthesis of fibrillar ECM molecules. We followed up on the hypothesis that TGF-BETA signaling is part of a mechanism to induce molecular changes in the extracellular matrix of peripapillary sclera that affect IOP-induced ON axonal degeneration.

We induced tamoxifen dependent deficiency of TGF-BETA receptor type II (TGF-BETA RII) that is essential for TGF-BETA signaling in fibroblasts (including those of the sclera) of mutant mice. After treatment with tamoxifen, ocular hypertension (OHT) was induced using a magnetic-microbead-model. After six weeks of OHT, ON axons were PPD-stained and quantified and retinal ganglion cells (RGC) were quantified.

Following treatment with tamoxifen, TGF-BETA RII and its mRNA was decreased significantly in the sclera. After six weeks of OHT reduced numbers of OH axons were seen in OHT eyes in comparison to un-injected contralateral eyes Moreover, OHT also led to a decrease of retinal ganglion cell somata as seen in RBPMS stained retinal whole mounts. Intriguingly, axon loss and RGC loss were significantly higher in mice with a fibroblast specific deficiency of TGF-BETA RII in comparison to control animals.

We conclude that scleral TGF-BETA signaling plays an important part in mechanisms that induce resistance to IOP-induced damage in ON axons.

Paradoxical effects of acute ethanol exposure on neuronal morphology

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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.

Molecular subgroups of mouse cortical VIP neurons – laminar distribution and optical stimulation

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Vasoactive intestinal polypeptide (VIP)-expressing neurons belong to the class of GABAergic interneurons in the mouse neocortex and are predominantly located in layer II/III. VIP neurons are integrated in different neuronal networks and were previously considered to be predominantly inhibitors of other GABAergic interneurons (called as disinhibitors). Recent studies demonstrate that they can also directly inhibit excitatory principal neurons. Molecular analyses have further differentiated VIP interneurons based on their co-expression of cholecystokinin (CCK), calretinin (CR), and choline acetyltransferase (ChAT). In our study we want to investigate the laminar distribution and the target cells of these subsets of VIP neurons in the mouse barrel cortex.

We used different intersectional mouse lines for VIP interneurons in fluorescent-insitu-hybridization (FISH). Sections of the barrel cortex was analyzed for the markers VIP, CCK, CR and ChAT. In acute brain slices of a new optogenetic mouse (Ai211) we performed whole-cell patch-clamp recordings.

VIP- and CR-expressing cells (VIP/CR) are predominantly located in the deeper portion of layer II/III whereas VIP- and CCK-expressing cells (VIP/CCK) preferred the region close to layer I. We found a 33% overlap between VIP/CCK and VIP/CR neurons, accounting for 23.5% and 46.5% of the total VIP cell population, respectively. Optogenetic stimulation of VIP/CR and VIP/CCK neurons in the Ai211 mouse lead to cell body limited generation of action potentials.

We expect a different laminar distribution and partial overlap of the molecular subgroups of VIP neurons. In electrophysiological experiments we will explore to what extent the subgroups belong to the disinhibitors or inhibitors of excitatory cells.

Gelsolin modulates wound healing in patients with periodontal disease

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Periodontitis is an acute or chronic inflammation caused by bacteria, which if left untreated leads to inflammation and lesions on the periodontium. The aim of therapy is to restore the form and function of the periodontal tissue. Gelsolin is an actin regulator of cell motility and regulates apoptosis and inflammatory processes. Gelsolin could contribute to reduce the inflammation and close lesions. The aim of this project is to investigate the importance and regulation of Gelsolin as a regeneration promoting and apoptosis regulating protein with regard to a possible therapy of gingival wound healing processes.

Saliva and tissues were collected for the study from healthy patients (180), patients with periodontal diseases (98) and patients with autoimmune diseases (66). Enzyme-linked immunosorbent assay (ELISA) and qRT-PCR were performed to quantify GSN in the saliva and tissue samples. In addition, wound healing studies with GSN were performed on gingival keratinocytes.

Our results indicate that Gelsolin promotes wound healing of the gingival epithelium. ELISA measurements show that gelsolin can be used as an indicator of periodontitis. The saliva of patients with chronic autoimmune diseases such as rheumatoid arthritis reveal reduced GSN levels.

The results underline that gelsolin could have a significant role in the prophylaxis and treatment of inflammatory processes of the periodontium.

Investigations on the influence of Nrf2 in TAMs using multicellular 3D spheroids

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Tumor associated macrophages (TAMs) undergo a polarization switch from an anti-tumorigenic to pro-tumorigenic phenotype during tumor progression. These cells can infiltrate the tumor and thereby make up to 50% of total tumor mass. Literature suggests that this phenotype switch may be influenced by Nuclear factor-erythroid 2 related factor 2 (Nrf2), but the precise impact of Nrf2 on TAMs is still unclear. Therefore, a realistic co-culture model using TAMs and tumor cells is required to investigate this effect.

A novel multicellular 3D spheroid model was established. To do so, the non-alcoholic steatohepatitis-derived hepatocellular carcinoma cell line (N-HCC25) and bone marrow derived macrophages were co-cultured as 3D spheroids. Different fluorophores were expressed in the cells to investigate the assembly of TAMs and N-HCC25 cells. In contrast to other co-culture models, direct cell-cell interactions and substrate exchange are possible in this approach. Supernatant was analyzed by ELISA and flow cytometry was performed to analyze macrophage polarization markers. Spheroid formation was analyzed in terms of volume, diameter and circularity.

We were able to establish a 3D multicellular spheroid model that allows the investigation of direct cell-cell interactions and the tumor immune microenvironment. Fluorophore marked cells allowed the comprehension of TAM distribution within the tumor spheroid. Our pilot study with Nrf2-KO and Keap1-KO macrophages showed differences with respect to the selected parameters.

Our new multicellular spheroid set-up is a great opportunity for more realistic in vitro approaches to study direct interactions between different cell types depending on Nrf2 activity.

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

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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.
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Stereological and Functional Characterization in a Hyperoxic Preterm Rabbit Model of Bronchopulmonary Dysplasia

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Bronchopulmonary dysplasia (BPD) is a chronic lung disease associated with preterm birth. For comparability of animal models, unbiased investigations are required. In this study, a widely used hyperoxic preterm rabbit model for BPD was investigated by design-based stereology as the gold-standard for quantitative morphometric analyses of the lung and by lung function testing.

Rabbits were delivered by caesarean section on 28th day of gestational age and housed in normoxic (21% O2, control) or hyperoxic (95% O2, intervention) conditions for seven days. Then, lung function testing was performed and the ratio of pulmonary arterial acceleration to ejection time (PAAT/PAET) was obtained. After euthanization and histological preparation, lungs were stereologically analyzed.

In lung function testing, hyperoxia-treated rabbits showed a reduced inspiratory capacity and static compliance, accompanied with higher tissue resistance and tissue elastance compared to normoxia-treated controls. The airway resistance, however, did not differ between the two groups. Lung and parenchymal volume were decreased in hyperoxia-treated rabbits. Furthermore, the alveolar number, total alveolar airspace volume and the septal surface area were reduced in this group, whereas the mean volume per alveolus showed no difference between the two groups. For lung vessels larger than 25 µm in diameter, vessel walls and perivascular tissue were thicker in hyperoxia-treated lungs, while the mean vessel diameter was decreased. Additionally, hyperoxia-treated rabbits showed a decreased PAAT/PAET-ratio, suggesting pulmonary hypertension in this group.

In conclusion, typical functional and morphological characteristics of BPD were represented in this rabbit model, verifying its suitability as BPD model for future research studies.

Adult rat proximal femoral head epiphysis cartilage as ex vivo and in vitro model: a novel methodological approach

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Isolation of articular chondrocytes from adult rodent joints is challenging due to the very thin joint cartilage layer. Adult rodents are lacking epiphysis closure. A novel method was established to gain a higher amount of hyaline cartilage from rat femoral heads following the 3R (reduce, replace, refine) principle.

The proximal femoral epiphysis of adult male Sprague Dawley rats undergoing a knee OA model was post mortem separated without special dissection technique: After medial exarticulation of the femoral head preserving the integrity the ligamentum teres, the entire femoral head cartilage layer remains attached to the ligament within the acetabulum whereas the bony metaphysis is separately released. The latter was used for histology, RNA extraction, explant cultures, chondrocyte isolation and immunolabeling.

In 87.88% of the rats (male, mean weight 558.84 g, age 70-153 days, n=33) the technique was successful. The mean proximal femoral epiphysis weight was 0.033±0.005 g. 110.48±87 ng/µL (in 30 µL) RNA could be extracted. Epiphyses were released by breakage within the hyaline cartilaginous epiphyseal plate. They consisted of hyaline cartilage with typical joint cartilage zonality. Hypertrophic calcified cartilage representing the early ossification centre was detectable, but blood vessels remained barely detectable. Epiphyses (5 mm diameter) explants showed many viable cells, even after 1 week of explant culture. After this, a mean of 301,000 vital chondrocytes/ epiphysis were isolated expressing cartilage-specific collagen type II, proteoglycans and the chondrogenic transcription factor SOX9.

This technique allows to gain additional cartilage from animals used for knee OA models, thereby reducing animal numbers.

Challenges in anterior cruciate ligament (ACL) tissue engineering

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Objective: Due to their poor healing capacity ruptured ACLs need appropriate reconstruction. Donor site morbidity and limited availability of autografts commonly used for this purpose encourage the development of alternatives including tissue engineered grafts.
The aim of the cooperating research teams was to develop an approach for tissue engineering an ACL graft using embroidered and functionalized polymer scaffolds.

Methods: Embroidered poly(L-lactide-*co*-ε-caprolactone)/ polylactic acid ACL scaffolds including also variants with zonal structure to mimic the enthesis were developed at the IPF (Dresden, Germany, Dr. A. Breier/Dr. J. Hahn) and functionalized using a novel gas phase fluorination strategy and collagen foams cross-linked with hexamethylene diisocyanate at the FILK (Freiberg Institute gGmbH, Germany, Dr. M. Meyer/Dr. M. Schröpfer). In addition, a custom-made mechanostimulator (PD Dr. B Hoffmann/J. Konrad, FZ-Jülich, Germany) was used to analyse the mechanoresponse of ACL fibroblasts. Versatile directed seeding strategies were established for co-culturing ligamento- and chondrogenic cells on the enthesis part of the scaffolds. Scaffold variants with/without functionalization were seeded with lapine or human ACL fibroblasts under dynamical conditions. Ligamentogenesis was analyzed *in vitro* and using a dynamic nude mice xenograft model.

Results: Mechanostimulation stabilized the ligament phenotype of ACL fibroblasts. Scaffold functionalization increased cell growth and neo-tissue formation. Spheroid- and cell sheet-based techniques allowed co-cultures on zonal enthesis scaffolds.

Conclusion: Functionalization of embroidered scaffolds with fluorinated cross-linked collagen foam provides a promising approach for ACL tissue engineering.

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VIP-receptor type 1 regulates inflammation and oxLDL-uptake in human M1/M2-macrophages

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Previous studies show that PACAP (pituitary adenylate cyclase-activating polypeptide) deficiency was proatherogenic and increased triglyceridemia in hypercholesterolemic mice. In vitro PACAP38 inhibited the formation of macrophage (MΦ)-derived foam cells after oxidized-(ox)LDL treatment. This effect was probably mediated by the PACAP receptor VPAC1 (VIP-receptor type-1). The aim of this study was to examine the regulatory effects of VPAC1 and PACAP38 under proinflammatory conditions induced by oxLDL in M1/M2-MΦ.

PMA-differentiated THP-1-MΦ were polarized into M1/M2-MΦ and incubated for 24h with oxLDL, PACAP38, VPAC1-agonist [Ala11,22,28]VIP and/or VPAC1-antagonist [Acetyl-His1,D-Phe2,Lys15,Arg16]Vip(3-7)GRF(8-27)-NH2. CCR7, CCL17, CD36, LOX-1, SR-A mRNA expressions were proved by qRT-PCR and IL-6, IL-10, TNF-α release were analysed by ELISA. Viability, oxLDL-uptake, as well as cholesterol and triglyceride measurements were performed.

M1-MΦ expressed an increased CCR7 mRNA level and TNF-α secretion, whereas M2-MΦ expressed an enhanced CCL17 mRNA level and IL-10 secretion.
PACAP38 and VPAC1-agonist decreased the oxLDL-induced viability in M1/M2-MΦ. In M1-MΦ, PACAP38 and VPAC1-agonist decreased the proinflammatory oxLDL-induced IL-6 release. VPAC1-agonist increased the anti-inflammatory IL-10 release in M2-MΦ independent of oxLDL.
An increased oxLDL-uptake was found in M1/M2-MΦ when treated with VPAC1-antagonist, however without effect on CD36, LOX-1 and SR-A mRNA expression. Additionally, VPAC1-antagonist enhanced the oxLDL-mediated cholesterol storage in M1-MΦ. Furthermore, PACAP38 and VPAC1-agonist reduced the oxLDL-mediated triglyceride accumulation in M2-MΦ.

Our present study demonstrates that VPAC1 plays an important regulatory role in inflammation and foam cell formation in M1/M2-MΦ. Therefore, VPAC1-agonists or PACAP38 itself may be suggested as a novel class of atheroprotective therapeutics.

Structural development and network integration of postnatally born hippocampal granule cells

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Neurogenesis of hippocampal granule cells starts in the late embryonic phase, peaks postnatally and continues during adulthood in the germinative subgranular zone. Increasing evidence points to functionally relevant hippocampal adult neurogenesis in mammals including humans. Postnatally born granule cells could serve as a valuable tool to study mechanisms of adult neurogenesis.

Entorhino-hippocampal organotypic slice cultures (OTCs) of neonatal rats were prepared at postnatal day 5. A GFP-retrovirus to label postnatally born granule cells and/or an td-tomato-adenoassociated virus to label maturated granule cells were injected locally in the dentate gyrus. In addition, a td-tomato-AAV-Channelrhodopsin2 virus was injected in the dentate gyrus for local optical stimulation. Live-imaging in OTCs was followed by fixation and histological examination.

Postnatally born granule cells exhibited dendritic growth starting at 7 days post injection (dpi) and reached their mature total dendritic length at 28 dpi. Structural development of the dendritic trees was well comparable to data from adultborn granule cells in vivo. Timelapse imaging revealed an unexpected rate of dendritic elongation and pruning of individual segments and branches at the time scale of days and even hours. Dendritic spines could be observed on proximal and distal segments from 14dpi on. The influence of chronic local optical stimulation was studied.

Retroviral labelling of postnatally born granule cells in OTCs reveals as a useful model for studying hippocampal neurogenesis under live imaging conditions.

Introduction to normal enthesis anatomy & dedication to Mike Benjamin

Hannah Shaw (Cardiff)

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.

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

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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.

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

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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.

Studying healthy tendons using single cell technologies

Sarah Snelling (NDORMS, University of Oxford, Oxford), The Tendon Seed Network CZI

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.

Tracing small extracellular vesicle uptake in equine mesenchymal stem cells by CFSE labeling

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Osteoarthritis (OA) is a degenerative multifactorial joint disease and one of the leading causes of lameness in recreational and sport horses. The use of mesenchymal stem cells (MSCs) from bone marrow or adipose tissue has increasingly become the focus of clinical interest in recent years, especially as an opportunity in osteoarthritis therapy. The therapeutic effects are mainly based on the efficacy of trophic factors synthesized by the MSCs and by small extracellular vesicles (sEVs) released by the cells stimulating regeneration.

sEV from equine donors (n=3) were purified, ultrafiltrated and then incubated with 5-(and-6)-Carboxyfluorescein Diacetate Succinimidyl Ester (CFSE) dye for 2 h. Afterwards, the labeled sEVs were separated from free CFSE dye by obtaining the relevant fractions using size exclusion chromatography. These were transferred to stem cells previously seeded on glass slides and incubated for additional 24 h. After fixation of these cells, Hoechst staining of the nuclei was performed to counterstain MSCs and sEVs were detected within cells by fluorescence microscopy.

After application of sEVs, small green fluorescent dots can be detected within the target cells. These dots are distributed over the entire cell body and a brighter signal appears to be near the nucleus. Outside the cells no fluorescent signal can be detected.

Using CFSE staining, purified sEVs can be localized within the cell. Size exclusion chromatography avoids artifacts and preserves the biological function of sEVs. This method is also suitable for other modalities to track sEVs, such as flow cytometry and could be used among different laboratories.

Cleavage of desmoglein 2 by catalytic autoantibodies as possible underlying pathomechanism in arrhythmogenic cardiomyopathy

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Recently, autoantibodies targeting intercalated disc (ICD) and the desmosomal cadherin desmoglein (DSG) 2 were reported in arrhythmogenic cardiomyopathy (AC) patients, which would be highly relevant for diagnosis, especially for patients without underlying pathogenic mutation. Here, we further demonstrate that the autoantibodies against ICD proteins exhibit a catalytic activity to cleave DSG2 and some of which reduce cardiomyocyte adhesion.

IgG fractions were purified from 16 AC patients, 1 healthy relative (HR), a borderline (BL) AC patient, and 4 healthy controls (pooled together). Dissociation assays, immunostainings, AFM, in vitro DSG2 cleavage assay, transient transfections with human Dsg2 protein-coding plasmid, and western blot analysis were performed utilizing HL-1 cells or DLD1 cells, and mouse or human cardiac tissue.

Immunostaining using human cardiac tissue revealed the presence of anti-intercalated disc antibodies in all 16 AC patients, HR and BL, but not in healthy controls. Dissociation assays revealed that 6 out of 16 AC patient IgGs impaired cardiomyocyte cohesion. AFM experiments demonstrated a reduced interaction of DSG2 after IgG incubation for all samples. In cleavage assays 12 out of 16 AC patient IgGs cleaved DSG2, which was inhibited by protease inhibitors. Among the 6 patient IgGs that impaired cardiomyocyte adhesion, 4 IgGs induced p38MAPK activation, which is known to impair desmosome adhesion.

Our study demonstrates that autoantibodies against ICD proteins were commonly detected in AC patients and some of them impaired cardiomyocyte cohesion and DSG2 binding. Most autoantibodies, which caused impaired cardiomyocyte cohesion, exhibited catalytic activity to cleave DSG2 and induced signaling involved in the loss of desmosome binding.

Neocortical microcircuits for information processing

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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.

SARS-CoV-2 spike protein variants interact differently with human receptor ACE2

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The SARS-CoV-2 pandemic has changed everyday life around the world. SARS-CoV-2 expresses the spike protein, which specifically binds with its receptor-binding domain (RBD) to the human receptor ACE2 to gain cell entry and to initiate infection. In emerging viral variants, mutations in the RBD are often at the interface to ACE2. However, the molecular consequences of these mutations on this interface were unknown.

All-atom molecular dynamics (MD) simulations were performed to characterize the interaction between the human ACE2 and the RBD of the spike protein from the wild type or from emerging SARS-CoV-2 variants.

Our results revealed changed structural RBD:ACE2 interfaces due to the RBD mutations. The N501Y mutation, firstly observed in the alpha variant, induces a local conformational rearrangement around Gln498 of the spike protein. In the beta and gamma variant, the E484K mutation introduces an additional salt bridge to ACE2, whereas the K417N/T mutation results in a salt bridge loss to ACE2. Moreover, our simulations of the delta and omicron variants indicate that residue 493 is a key residue for the RBD:ACE2 interaction. The mutation Q493R in the omicron variant even leads to the formation of a new salt bridge between Arg493 and Asp30 or Arg493 and Glu35/Asp38.

All in all, our studies identified RBD residues for different SARS-CoV-2 variants that are important for binding to the human receptor. This knowledge could be used to develop broadly effective antiviral strategies (e.g. neutralizing antibodies or peptides).

Topographic distribution of propriosensors in the distal human penis

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Although about ten times more studies address innervation for the external male than the female genitalia, implying good knowledge of the penis, it is still insufficiently considered with contradictory statements. Furthermore, the investigations have a narrow focus, mainly on circumcision and phimosis. Therefore, the presented morphological study aims to map the propriosensory elements in the distal penis, which perceive this organ's spatial and mechanical condition.

We analyzed specimens from 10 body donations qualitatively and quantitatively for the presence of propriosensory elements using light-microscopical methods to depict a schematical topographical representation of the innervated areas that allows functional interpretation.

Numerous Ruffini corpuscles with particular size distribution appear in some subcutaneous regions and the front of the tunica albuginea. The typical elastin distribution suggests functional systematics. Contrary to the current opinion in the scientific literature, we did not find a single Vater-Paccini corpuscle or Golgi-Mazzoni corpuscle.

The so far missing description for the anatomical extension of the propriosensory system should be considered not only for the current discussion on circumcision but also for sensory-preserving surgery and in the context of pathologies. In addition, the clarified distribution of sensory feedback provides new aspects for neuronal and functional understanding.

The orobasal organ (of Ackerknecht) in mammals – evolutionary considerations about a neglected organ

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Vestigial anatomical structures are considered to have lost much or all of their function through evolution. These structures (e.g., leg bones in whales, rugae palatinae in humans) can give insights into the phylogenetic history of species. Additionally, vestigial structures can be of clinical importance, since these structures might be confused with pathologies (e.g., juxtaoral organ of Chievitz and carcinomas). The orobasal organ was discovered by and named after the veterinary anatomist Eberhard Ackerknecht. In 1912, he described morphologically highly variable epithelial invaginations behind the lower medial incisors in different mammalian species. The orobasal organ is considered to be a rudimentary structure without physiological function, but the evolutionary history of the orobasal organ remains unknown, so far.

In this study, we systematically reviewed the occurrence of the orobasal organ in different mammalian species including humans.

The orobasal organ is found in all subclasses of mammals (protheria, metatheria and eutheria). Additionally, we were able to describe a novel case of an orobasal organ in a human body donor.

The orobasal organ seems to be an evolutionary conserved structure in mammals including humans. We hope that this study will increase awareness of this anatomical structure, and thereby decrease the risk of confusion with a pathological condition like oral cancer.

Human cancer cells utilize various pro-adhesive ligands for endothelial adhesion in vitro

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One critical step of metastasis formation is the escape of circulating tumor cells from the adverse conditions within the bloodstream. This process requires the adhesion of tumor cells (TCs) to endothelial cells (ECs) via carbohydrate ligands binding to E-selectin. Our study aimed to characterize these ligands more precisely.

The endothelial adhesion of nine human TC lines was analyzed using a laminar flow adhesion assay on human umbilical vein ECs (HUVECs). The TC lines were grouped into three subsets according to their canonical E-selectin ligand status (sialyl-Lewis A and X (sLeA/X) +/+, -/+, -/-) and their adhesiveness was compared after enzymatic, pharmacologic, chemical or antibody blockade treatment of the TCs or ECs, respectively.

Endothelial adhesion did not exclusively require the presence of sLeA and/or sLeX. Other (non-canonical) ligands must exist since all TCs were able to adhere on human ECs, including sLeA/X-negative TCs. However, two of the three sLeA/X -/- TCs additionally or fully depended on VCAM-1 for endothelial adhesion. Nearly all tested cell lines adhered via terminal α-2,3-sialic acid. The significance of O-GalNAc- and N-glycans varied among the cell lines tested. The sLeA/X +/+ subset showed glycoprotein-independent adhesion, so carbohydrate ligands on glycolipids must be considered as well.

The EC-coated flow chambers provided a more physiologic adhesion assay than our previous studies with recombinant selectins. E-selectin and α-2,3-sialic acid largely determine endothelial adhesion of human TCs. Nevertheless, the interaction between endothelium and TCs remains a complex process that requires future studies for a better understanding.

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

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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.

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

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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.

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

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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.

3D-printed replicates help raise unique specimens to higher power

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Prosections of wet specimens utilised hands-on in the dissection lab often deteriorate after 2 to 3 years of use. When mounted in glass cuvettes, size is limited,, and potentially hazardous conservation fluids are involved, thus limiting any hands-on learning. This is particularly disadvantageous for rare variations or unique pathologies. Our objective was to develop a standard protocol for scanning and multicolor 3D-printing high quality copies of such specimens.

As exemplary cases we choose a severe Mönckeberg's aortic valve sclerosis and a (TAVI)-treated aortic valve. A Skyscan 1173 micro-computed tomograph was used at a voxel resolution of 30 micrometers. Calcifications, soft tissues and the TAVI's wires were segmented separately, using the Slicer and Medtool software packages. For 3D-printing, a Prusa MK3S printer or SL1 resin printer was employed.

The time expense for scanning was 40 minutes. Data processing plus segmentation took 16 hours, and 3D-printing took between five hours (single-color resin print, scale 1:1) to four days (triple-color filament print, scale 3:1) per model. We generated models ranging from real size, opaque surface models to magnified semitransparent versions showing calcifications and the TAVI.

Taken together, after a substantial time expenditure for data processing, hands-on replica for teaching can be realistically be created at various scales and colors without the limitation of specimen durability or chemical hazards. Higher power is achieved by the ability to visualize internal structures in situ, making laborious, traditional whole mount-staining and -clearing dispensable.

The Trunk of Henle and its Variants

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In colon, pancreatic and hepatobiliary surgical research, an increasing interest developed in the Gastrocolic Trunk of Henle (GTH) due to its broad variability and its importance especially for right hemicolectomy with complete mesocolic excision.
The purpose of this study was to further corroborate details of the anatomical variants of the Gastrocolic Trunk, with respect to pancreaticoduodenal veins and the jejunal drainage into the superior mesenteric vein.

In 15 cadaveric bodies the venous drainage into the superior mesenteric vein was dissected first in a ventral, in situ approach as well as by a subsequent dorsal approach following careful abdominal exenteration.

The most frequent configuration was the tripodal gastropancreaticocolic trunk (GPCT) with an occurrence of 54.5%, followed by the gastropancreatic trunk (GPT) with 36.4%. The right gastroepiploic vein was a constant structure draining into the trunk of Henle in every case. During the dissection of the pancreatic drainage, a previously undescribed, middle anterior pancreatic vein, was found in 46.67% of the specimens, which drained in 85.71% into the GTH.

The trunk of Henle drains blood from several abdominal organs and bundles them into a short and vulnerable structure. The most common variant is a tripodal trunk, collecting veins from the pancreas, right colon, and the stomach. Dependent on the surgical approach, the right gastroepiploic vein or the superior right colic vein may serve as guiding structures towards the trunk of Henle. In addition, the finding of a middle anterior pancreatic vein prompts for further studies regarding venous drainage of the pancreatic head.

T

Effect of LIPUS on osteoblast function: 3D in vitro study

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Low-intensity pulsed ultrasound (LIPUS) has been shown to accelerate the time to fracture healing and stimulate osteogenesis under 2D culture condition. Nuclear factor erythroid 2-related factor 2 (Nrf2) has been identified as a key factor regulating the expression of anti-oxidant/detoxifying enzymes, which influence bone regeneration and fracture healing.
In this work, we investigate whether LIPUS enhances osteogenic differentiation in 3D culture and whether it is involved in the regulation of ARE.

Murine MC3T3-E1 pre-osteoblasts are used to produce 3D spheroids, which are treated with LIPUS q.d. Evaluation is carried out by CellTiter-Glo Viability and CyQuant Cell Proliferation Assay, µCT analysis, and histology. Cells are transduced with the SIN-lenti-ARE/-NFkB construct to execute Nano-Glo Luciferase Assay. Differentiation and mineralization are evaluated after 28 days by ELISA, ALP, and Calcium Assay.

In terms of viability, proliferation, and morphology no significant difference could be found. LIPUS treatment did not induce a direct anti-oxidant effect, but an inhibitory inflammatory effect. After 28 days the calcium content and ALP activity of the LIPUS-treated spheroids increased significantly. VEGF release increased significantly in all groups during the first three days.

Long-term use of LIPUS can induce mineralization in osteoblasts through mechanical stimulation between cells under 3D culture condition. We assume that demanding mineralization and increase of ALP can have good effects on bone formation.

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

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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.

Artificial neural network model for sex estimation based on mandibular measurements

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Sex estimation based on bones and bone fragments is a binary classification problem, which can be solved by different classification methods. Artificial neural networks (ANN) are a family of machine learning algorithms widely used for classification tasks and providing high performing models. The present study aims to train an ANN model for sex estimation based on mandibular measurements.

Computed tomography (CT) images of the head obtained with a Toshiba Aquilion 64 CT scanner were used in the study. The sample included 239 adult individuals (116 males and 123 females). The CT images were utilized for generation of polygonal surface models (stl-format) of the skulls via segmentation of the bone tissue in InVesalius. The 3D coordinates of 45 anatomical landmarks of the mandible were recorded from the surface models in MeshLab. Based on the 3D coordinates, fifty-one mandibular measurements were calculated. These measurements were assigned as attributes for training an ANN classifier by Weka's multilayer perceptron function. The architecture of ANN included two hidden layers. The classification accuracy of the ANN model was evaluated by means of 10-fold cross-validation.

The classification accuracy achieved by ANN trained on the full dataset of 51 mandibular measurements was 90.5% for males and 91.9% for females. The sex bias was only 1.4%.

The ANN classifier trained in our study provides an overall accuracy rate of 91.2% and can be considered a useful approach for sex estimation based on mandibular measurements.

Evaluation of correlation of articular cartilage staining for CILP-2 and DDR2 with histological tissue damage in patients with knee osteoarthritis: a pilot morphological study

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The aim of the study was to correlate the immunohistochemical expression of cartilage intermediate layer protein 2 (CILP-2) and discoidin domain receptor 2 (DDR2) and the ultrastructural changes in the cartilage with the degree of articular cartilage damage in osteoarthritis (OA) patients.

Cartilage samples from the tibial medial and lateral condyle were obtained from twenty patients aged from 46 to 68 years undergoing total knee arthroplasty in the Tartu University Hospital, Estonia. OARSI histopathology assessment system (2006) was applied to determine the degree of tissue damage. CILP-2 and DDR2 expression was detected by immunohistochemistry. Ultrastructural changes in the articular cartilage were studied by transmission electron microscopy

OARSI histopathology grades varied from 0.5 to 5.5. TEM studies demonstrated strong damage of chondrocytes, the organelles were often diminished or focally aggregated. The pericellular matrix (PCM) was frequently expanded. In samples of all patients CILP-2 immunohistochemical staining was seen in the articular cartilage, strong staining was found in the middle layer and in PCM in the deep zone. However, in the case of advanced cartilage damage, strong CILP-2 staining was noted in the superficial, middle and upper parts of the deep zone.

Comparison of CILP-2 expression with the degree of cartilage damage revealed that CILP-2 staining intensity was positively correlated with the OARSI histopathology grade. Similarly, DDR2 expression was seen in the samples of all patients but the staining intensity was not seen to correlate with the degree of cartilage damage.

FUNCTION FOLLOWS FORM - MICROLARYNGOLOGY

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Generally, the study of the non-pathologic larynx is split in two main directions of interest: the microscopic morphological study of the intrinsic laryngeal muscles and nerves and the functional, mechanical, and aerodynamic study of vocal fold posturing and vibration during speech and non-verbal sound production. We aim to add to the larger picture formed by the current theories of phonation by identifying an anatomical substrate for the finer particularities of muscular contraction as described in the models.

The study consists of a comprehensive literature review of both areas of previously mentioned. The studies are chosen based on the relevance for the subject and their importance, as advised by the corresponding author with experience in the field. We analysed mostly data regarding muscle fibre disposition, synapse disposition and morphological peculiarities observed and created a visual overlay of axial and coronal sections of the larynx. We then correlated the map obtained with the areas with the most impact on the properties of the vocal air column.

We observed a common distribution between motor-neuron unit location and the muscular areas with the most impact in the mathematical equations proposed by other authors.

Although a significant number of intrinsic laryngeal muscles play a role in vocal fold posturing, some have more of an effect on the mathematical model imposed by visual observation of the larynx during activation. This can be corelated with the frequency in distribution of synapses across intrinsic laryngeal muscles. However, accessory muscles also play a role in limiting and fine-tuning vocal fold stiffness probably as a reflex response more than a voluntary contraction.

Increased Homer long isoforms expression and subcellular compartmentalization during myogenesis in vitro

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The main objective of the present study is to investigate the spatial and temporal expression of the Homer protein isoform expression pattern during myogenic development in vitro and to analyze its role in postsynaptic signaling complexes.

The Homer short and long isoform expression pattern was investigated in differentiating C2C12 myotubes every 2 days interval, from day 0 to day 14 by RT-PCR. Homer protein expression and subcellular localization was analyzed by Western blot in native experimental conditions. Moreover, Homer protein subcellular localization was further explored by immunohistochemistry and 3D image acquisition with the Leica TCS SP8 confocal microscope.

During differentiation, a sustained increase in the Homer1b transcript was observed, while Homer2 appears to be induced transiently. In contrast, the Homer1a transcript remained constantly low over the analyzed time. In line with the increased Homer transcripts, there was a progressive increase of Homer protein expression during myotube differentiation. The comparison between cytosolic and membranous fraction showed an increase of Homer protein dimer and multimer in the membranous fraction. By confocal microscopy analysis, we observed a diffuse punctate Homer immunostaining pattern during the first few days of differentiation. Approximately, after the first week of differentiation, Homer immunostaining became progressively ordered in a cross-striated pattern, consistent with a longitudinal sarcoplasmic reticulum (LSR) cisternae/Z-line-/costamere-like structures subcellular localization.

Homer long isoforms transcripts and proteins are expressed early on the myogenic differentiation program. Later, dimerization and multimerization occurred and Homer isoforms are progressively localized to subcellular compartments where they fulfill the functions of scaffolds and possibly acting as Ca2+ sensors promoting protein cross-talk.

Aldolase C in the brain emerges as a protein that may connect early life stress to depression

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Early life adversity is a risk factor for the development of psychiatric disorders. Growing evidence suggest that the cerebral spinal fluid proteome may reflect the brain health status.

We withdrew cerebral spinal fluid (CSF) from mice exposed to early life stress and conducted an unbiased proteomic analysis.

We identified 30 differentially expressed proteins from which 25 were up- and 5 down-regulated proteins. Canonical pathways involved in glycolysis and acute phase response signaling were particularly implicated. We then performed western blot analysis on the brain hippocampal and frontal areas, brain regions known to be particularly vulnerable to the effects of ELS, to assess whether the differentially expressed CSF proteins would be reflected in brain tissues. From these analyses, aldolase C, an enzyme of the glycolysis cycle, was consistently affected, both in the CSF and in these candidate brain regions from mice exposed to ELS. Several lines of research point to the involvement of early life adversity and the emergence of major depression disorder. Hence, we then performed an immunostaining against aldolase C on hippocampal tissue from patients affected by major depression and found that aldolase C is downregulated in the hippocampal CA1 region of patients affected by major depression disorders.

In summary, we found that cerebral aldolase C is affected in an animal model for early life stress. Moreover, the hippocampal decrease in the number of aldolase C-positive cells in the post-mortem brains of patients diagnosed with major depression suggests an impaired aldolase C function in the brain may be a critical event linking adverse early life events to the emergence of major depression later in life.

Gephyrin modulates the turnover of connexin 43 in non-neuronal cells

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Gephyrin is a key organizer of the postsynaptic cytoskeleton in glycinergic and GABAergic neurons. By contrast, non-neuronal cells express gephyrin in the cytosol as part of a multiprotein complex. This complex may be implicated in the biosynthesis of molybdenum cofactor or it may serve another, yet unknown, function. Here we examined the effect of knocking down gephyrin in cultured 3T3 cells on the turnover of connexin 43.

3T3 cells were transfected with shRNA vectors that do or do not knock down gephyrin transcripts. G418-mediated selection of stably transfected cells was followed by screenings for the alterations in the expressions of cytoskeletal components and membrane proteins. Double fluorescence experiments on multiple coverslips were used to quantify the apparent alteration of connexin 43 expression in knockdown cells. Finally, scrape loading dye transfer studies were employed to measure the connectivity of knockdown and control 3T3 cells, respectively.

Immunoblot and immunofluorescence analyses revealed that the knockdown of gephyrin in 3T3 cells resulted in the increased expression of connexin 43, but not of other cytoskeletal or membrane proteins, when compared with control transfections. This increased expression was detected primarily in the vicinity of ZO-1 positive areas of cell membranes. In scrape loading dye transfer studies, knockdown cells displayed enhanced connectivity when compared with control-transfected cells.

These results provide evidence for a novel function of gephyrin in the turnover of connexin 43 in non-neuronal cells. Future experiments shall examine the precise role of gephyrin in the transport of connexin 43, its endocytosis or its phosphorylation status.

EFEM Lecture: The Life of an ear drum: development, defects and repair

Abigail Tucker (Centre for Craniofacial and Regenerative biology, King's College London, London)

Hearing as one of the five human senses is important not only for communication but also for our quality of life and integration into society. We are able to hear due to the unique and complex hearing organ that is the ear. In between the external and middle ear sits the eardrum, a thin transparent membrane that converts sound waves into vibrations. This membrane is very susceptible to damage caused by ear infections, pressure changes, or head trauma, yet has an impressive ability to repair. In this talk we follow the life of an ear drum as it forms in the embryo, and how it is able to repair itself in adult life.

For this, we use mouse and human embryos to understand how the three layers of the drum communicate so that they come together to create a thin membrane. Additionally, we use transgenic mouse models to follow the contribution of putative stem cell populations and investigate the impact of manipulation of key signalling pathways during repair in adults.

We show that the Fgf pathway plays an important role in guiding the ectodermal layer of the eardrum in the embryo, with defects leading to failure in ear drum formation. In the adult, we show how the different cell populations in the drum respond to injury and the critical role of the Wnt pathway in repair.

Such knowledge is crucial for understanding the mechanisms underlying eardrum defects and suggesting new avenues for therapeutics in the future.

Dosing transcranial magnetic stimulation of the primary motor and dorsolateral prefrontal cortices with multi-scale modeling

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Transcranial magnetic stimulation (TMS) is a clinically employed non-invasive brain stimulation technique that depolarizes cortical neurons through the intact skin and skull. The characteristics of the induced electric field (E-field) have a major impact on specific outcomes of TMS. Using multi-scale computational modeling, we explored whether the stimulation parameters derived from the primary motor cortex (M1) induce comparable macroscopic E-field strengths and subcellular/cellular responses in the dorsolateral prefrontal cortex (DLPFC).

To this aim, we calculated the TMS-induced E-field in 16 anatomically realistic head models and simulated the changes in membrane voltage and intracellular calcium levels of morphologically and biophysically realistic human pyramidal cells in the M1 and DLPFC.

We found that the conventional intensity selection methods (i.e., motor threshold and fixed intensities) produce variable macroscopic E-fields. Consequently, it was challenging to produce comparable subcellular/cellular responses across cortical regions with distinct folding characteristics.

Prospectively personalized stimulation intensity selection could standardize the E-fields and the subcellular/cellular responses to repetitive TMS across cortical regions and individuals. The suggested computational approach points at the shortcomings of the conventional intensity selection methods used in clinical settings that do not account for interindividual differences in anatomy. We propose that multi-scale modeling has the potential to overcome some of these limitations and broaden our understanding of the neuronal mechanisms for TMS.

Glial secreted factors regulate neuronal gene expression and function

Paul Turko (Integrated Neuroanatomy, Charite Uni. Medicine, Berlin)

To investigate how glial cells regulate neuronal gene expression and function through the secretion of proteins.

This is done using a combination of imaging, electrophysiology, mass spectrometry and RNA sequencing.

Through a series of neuron-glia coculture experiments, we find that neurons grown in the absence of glia have poor growth, survival and fail to establish functional excitatory connections. Coculture with glia improves all these conditions, through the positive regulation of gene expression. We have identified both protein and gene candidates which may mediate these effects.

Glial secreted factors are essential for neuron function, regulating critical disease associated genes. Disrupted glia-neuron crosstalk may be responsible for a variety of neurological disorders.

U

Investigation of lactic acid as a non-toxic substitute to formaldehyde for tissue fixation

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Despite its carcinogenic and embryotoxic effects, formaldehyde is the current gold standard for tissue fixation – since no adequate alternative is present to date.
In search for surrogates, we focused on food preservatives, which are well known for their antimicrobial and preservative properties and inquired about their suitability for tissue fixation.

When we initially tested various different food additives for their preservative effects, lactic acid (LA) resulted in promising outcomes. In further systematic investigations, we studied the microscopic effects of fixation with several concentrations of LA in comparison with formaldehyde in various murine tissues. For that purpose, specimens were fixed, paraffin-embedded and cut into 5 µm sections for subsequent histochemical and immunohistochemical staining. For evaluating the structural fixation, a scoring system – based on microanatomical and cytological preservation – was applied. Antibodies against various cellular proteins in different compartments of the cell were chosen for precipitations-based immunohistochemistry to analyze antigen retention. Computer-assisted digital image analysis was used for evaluation of the staining results.

Lactic acid fixation allowed very good morphological preservation despite minor artifacts due to shrinkage. Furthermore, lactic acid outperformed formalin fixation in immunohistochemical staining by showing a better signal-to-noise ratio.

To this date, an adequate alternative to formalin fixation is lacking. In this study, we have shown that lactic acid as a non-toxic fixative has the immense potential to replace formaldehyde in histochemistry and immunohistochemistry.

V

Unraveling the potential of a disintegrin and metalloproteinases (ADAM) in retinoblastoma

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Sheddases of a disintegrin and metalloproteinase (ADAM) family are important factors in numerous pathologies including cancer and have been suggested as promising therapeutic targets. The study presented focuses on the involvement of ADAM10 and ADAM17 in retinoblastoma (RB), the most common malignant intraocular childhood tumor.

The effects of lentiviral ADAM10 and ADAM17 knockdown (KD) on RB cell viability, proliferation and apoptosis in vitro were investigated by BrdU and DAPI stains, growth curve analyses, and soft agarose as well as WST-1 assays. Tumor formation and migration capacity in vivo was investigated in chicken chorioallantoic membrane (CAM) assays by inoculation of ADAM depleted RB cells on the extraembryonic CAM membrane or intravenous injection of GFP-labelled RB cells and subsequent real-time PCR analyses. Cell signaling pathways and regulatory mechanisms of ADAM expression by micro RNAs were studied by Western Blot analyses and real-time PCRs.

Lentiviral ADAM10 and ADAM17 single or ADAM10/17 double KD induced caspase dependent apoptosis and reduced cell viability, proliferation, growth, and colony formation capacity of RB cells, with most prominent effects seen after ADAM17 KD. The serine/threonine kinase AKT was differentially phosphorylated following ADAM17 KD in RB cells. CAM assays revealed that ADAM17 and ADAM10/17 depletion decreases the tumorigenic and migration potential of RB cells in vivo. Furthermore, micro RNAs miR-145, miR-365 and miR-152 proofed to be crucial for the regulation of ADAM10 and ADAM17 expression in RB cells and RB patient tumor samples.

Summarizing, we propose ADAM17 as a potential novel target for future therapeutic RB approaches.

Regional heterogeneity in human meninges

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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.

Inhibition of TGFβ signaling leads to enhanced phagocytosis of amyloid species

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Transforming growth factor β (TGFβ) plays a pivotal role in regulating microglia maturation and activation under physiological and pathological circumstances. As the resident macrophages of the CNS, microglia promote or inhibit inflammatory processes by phagocytosing pathogenic factors and expression of various kinds of cytokines. The role of microglia-driven neuroinflammation for neurodegeneration in pathologies such as Alzheimer's disease (AD) is only partially understood. TGFβ and its receptors have been described to be downregulated in AD patients and an increase of amyloid beta deposition in combination with activation of glial cells was observed. Here, we investigated microglial TGFβ signaling in aged APP/PS1 mice and further analyzed the effect of TGFβ signaling inhibition on the phagocytosis of oligomeric Aβ in vitro.

Wildtype microglia were treated with fluorescein-coupled Aβ oligomers and TGFβ-receptor I inhibitor. The phagocytic capacity was assessed by immunohistochemical staining. Aged WT and APP/PS1 mice were characterized by analyzing plaque load, glia activation, and microglial TGFβ signaling.

Aged APP/PS1 mice showed decreased microglia ramification and reduced nuclear accumulation of Smads. Interestingly, phagocytosis of Aβ species by microglia was increased after inhibition of TGFβ signaling in vitro.

Here, we show that inhibition of TGFβ signaling significantly increased the uptake of oligomeric Aβ species. In aged APP/PS1 mice, TGFβ signaling was decreased in Aβ plaque-associated microglia. Together, these data suggest that decreased TGFβ signaling in microglia from APP/PS1 mice could contribute to reduce the plaque load by microglial Aβ phagocytosis.

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

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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.

Characterization of glutamate-induced calcium transients in glioblastoma

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This study aimed at dissecting molecular pathways of glutamate-induced calcium transients in glioblastoma cells.

In vitro co-cultures of neurons and patient-derived glioblastoma cells (PDGBC) from two cell lines (S24 and BG5) and monocultures of glioblastoma were used. Calcium transients were induced by puffing glutamate with a Picospritzer onto the glioblastoma cells. The latter were stably transduced with a genetically encoded calcium indicator (GCamp7b). After baseline stimulations, pharmacological agents were washed in ACSF and afterwards washed out under stimulation. Calcium transients were analyzed using an event-based algorithm and subsequent custom-written R scripts.

Glutamate puffing induces calcium events in glioblastoma cells in co-cultures with neurons, but not in monoculture.
The analysis of glutamate-induced calcium events showed five classes of calcium events encompassing subcellular calcium microdomains and multicellular events.
Further experimental data showed, that IP3-signaling is involved in PDGBC S24, but not in the PDGBC BG5.
AMPA-receptor inhibition significantly reduced Ca2+ event frequency in both PDGBC S24 and BG5.

We present here a novel framework for analyzing glutamatergic synaptic neuron-glioma communication with a combination of glutamate puffing and subsequent whole-cell calcium imaging. This approach revealed heterogeneous subcellular and multicellular glutamate-induced calcium events increasing the complexity of neuron-glioma communication patterns. These glutamate-induced events were further pharmacologically analyzed revealing a dependence on AMPA receptors. Downstream signaling differed between the PDGBC analyzed here necessitating further investigation.

Langer's Axillary Arch: a large scale dissection study

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Originating from the latissimus dorsi muscle and crossing the axilla, Langer's Axillary Arch can cause a Thoracic Outlet Syndrom (TOS). This study aims at researching the frequency, tissue composition and dimensions of such Axillary Arches and at evaluating their capacity of compressing the axillar neurovascular bundle.

200 axillae from 100 non-embalmed body donors were carefully investigated using traditional anatomical dissection. Axillary Arches separated from surrounding tissue and their topology, tissue composition and relation to the neurovascular bundle was documented. A caliper gauge was used to measure length, thickness and width. In a last step passive elevation and external rotation were performed and the relation to the neurovascular bundle was again recorded.

In 8 body donors (8%) an Axillary Arch was identified, 4 bilateral. Thus, examination of 200 axillae revealed 12 axillary arches (6%). 8 solely comprised muscular tissue, 1 solely connective tissue and 3 both. The average length was 78.5mm (67.75-89.5), -thickness 2mm (1-3.75), and width 6.5mm (4.25-10.75). Arches composed of ($p=0.08$), or containing connective tissue ($P=0.029$) were significantly smaller than arches solely composed of muscle tissue. Compression of the neurovascular bundle was observed in 9 cases (75%).

8 % of the examined individuals, but only 6 % of their axillae had an Axillary Arch. Alternative studies, based on examining axillae, but not individuals therefore do suggest significantly smaller frequencies for this variation in the overall population. Furthermore, results from the passive manipulations on non-embalmed individuals demonstrated that axillary arches might be causal for TOS.

Differential temporal development of alveoli and capillaries during bulk alveolarization of the rat lung

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Understanding normal and pathological lung development requires quantitative data of lung structure at various developmental stages. For example, aberrant lung development or bronchopulmonary dysplasia cause deficits in alveolarization and microvascular maturation, however, the temporal relationship between these processes is still not clear. We hypothesized that alveolar and capillary development show a differential time pattern.

Therefore, the lungs of postnatal rats aged 3, 7, 14, 21 days or 3 months (n = 8-10 each) were used for microscopic analysis. After vascular perfusion fixation, the right lungs were processed for light microscopy. Design-based stereological analysis included estimation of number, surface area and volume of alveoli, septal capillaries and alveolar septa.

The total number and the total volume of alveoli increased progressively during postnatal development. Interestingly, the numerical density of capillary loops was significantly higher in 14 and 21 day old rats than before or after this age, causing a duplication of the total number of capillary loops between 1 and 2 weeks of age. The mean thickness of alveolar septa started to decline slightly at the age of 14 days and more pronounced at later stages. While the septal epithelial surface area increased in proportion to lung volume during the first 3 weeks, the capillary endothelial surface area increased largely between 3 and 7 days and remained constant until 21 days.

In summary, the comparative analysis of alveolarization and microvascular maturation has shown that the enlargement of the alveolar capillary network slightly precedes the alveolar development during the phase of bulk alveolarization.

A novel approach for cell type-specific and systematic analysis of transcription factors in vivo

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Transcription factors (TFs) regulate gene expression by direct DNA binding together with cofactors and in chromatin remodeling complexes. Their function is thus regulated in a spatiotemporally and cell type-specific manner. To analyze the functions of TFs in a cell type-specific context, genome-wide DNA binding as well as identification of the interacting proteins is required.

We use an in vivo approach (iGONAD) in mice to genetically modify TFs by adding reporter and affinity tags that can be exploited for enrichment of target cells, chromatin immunoprecipitation, and pull-down assays.

Using this approach, we show functional and cell-type specific modification of Bcl11 TFs in newborn mice.

iGONAD is a highly efficient procedure to modify TF coding genes by integration of small insertions, such as reporter and affinity tags. The novel Bcl11 strains described here, can be used to better understand Bcl11 function in neurodevelopment and disease.

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

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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.

Analyzing metabolic alterations in the mouse CNS using matrix-assisted laser desorption/ionization-mass spectrometry imaging

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Microglia are the resident immune cells of the central nervous system and participate in physiological and pathological processes. However, metabolic changes and lipid turnover of microglia are only partially understood. To characterize metabolic shifts in more detail, we combine immunohistochemistry with matrix-assisted laser desorption/ionization-mass spectrometry imaging (MALDI-MSI). We employed this specific technical approach in brain sections of wild-type and mice with microglia-specific Smad4-deficiency.

Fresh-frozen sagittal brain slices of wild-type and tamoxifen-induced Cx3cr1CreERT/Smad4fl/fl mutant mice were analyzed utilizing MALDI-MSI. Slides were covered with a NEDC (N-(1-napthyl) ethylenediamine dihydrochloride) matrix. Hence, different types of small molecules, endogenous cell metabolites and lipids can be detected on a single tissue section with a lateral solution of 50 µm. In addition, the spatial distribution of different metabolites is evaluated and mapped at the pixel level by combining laser microscopy and mass spectrometry on the computer.

Data from MALDI imaging experiments of adult wild type and Smad4 deficient animals were combined with immunohistochemical stainings to identify different brain regions and the microglial distribution. By this, we were able to showcase metabolic changes in brains from mice with microglia-specific knockout of Smad4.

The MALDI-MSI technique is a powerful tool to detect a broad spectrum of analytes in the mouse CNS. Furthermore, alterations of metabolic molecules seem to be present when microglial TGFβ signaling is impaired. Collectively, we identified microglial metabolic components in brain slices utilizing MALDI-MSI, which has not been demonstrated yet. Thus, leading to new insights into the functionality of microglia in health and disease.

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

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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.

The inositol-1,4,5-trisphosphate-3-kinase-A is a putative regulator of social behavior and motor function

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The Inositol-1,4,5-trisphosphate-3-kinase-A (Itpka) is a neuron-specific actin binding protein that regulates dendritic calcium transients and controls actin dynamics in dendritic spines. It is highly expressed in the murine cortex, hippocampus and cerebellum. Previous studies showed defects in memory performances and differences in emotional behavior in Itpka deficient mice.
In this study we analyzed the functional role of Itpka in social interaction and motor function by performing a set of behavioral experiments in transgenic mice deficient for Itpka.

We assessed male and female wildtype and knock-out mice in a battery of behavioral tests with a focus on motor function and social interaction. After behavioral experiments, we analyzed the morphology of the cerebellum by quantifying Purkinje cell densities in several lobular subregions as well as their output to deep cerebellar nuclei using immunfluorescence labelling with the marker calbindin.

Itpka deficient mice showed covered a longer distance in open field and a decreased social exploration in the resident intruder test in comparison to wild-type mice. Morphological investigations of the cerebellum revealed genotype- and gender-specific differences in Purkinje cell densities and calbindin intensities in the deep cerebellar nuclei.

Motor function, but also social behavior is associated with cerebellar functions. Itpka deficiency modulates both behavioral domains and induces morphological changes in the cerebellum. This may suggest a potential novel role for Itpka in the complex network of motor control and social behavior, relevant for health and disease.

Human neuro-mesodermal assembloids recapitulate aspects of peripheral nervous system development in vitro

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Within the last decade, numerous human stem cell-based 3D cell culture models were explored, mimicking the development of different organs and tissues. These tissue models are termed organoids. To increase tissue complexity and allow multi-lineage interaction, different organoids were combined forming assembloids. The aim of the presented work is the establishment of a novel neuro-mesodermal assembloid model which recapitulates aspects of peripheral nervous system (PNS) development.

Assembloids are produced by co-culturing neural and mesodermal spheroids which are separately generated from human induced pluripotent stem cells.

Neuro-mesodermal assembloids recapitulate aspects of peripheral nervous system development such as neural crest cell (NCC) induction, delamination, migration, and sensory as well as sympathetic ganglion formation. The ganglia send neuronal projections to the mesodermal as well as the neural compartment. Axons in the mesodermal part are associated with Schwann cells. In addition, peripheral ganglia, as well as nerve fibers, interact with the co-developing vascular plexus, forming a neurovascular niche. Finally, developing sensory ganglia show a response to capsaicin treatment indicating their functionality.

The presented assembloid model could help to uncover mechanisms of NCC delamination, migration, and PNS development in the human tissue context. Moreover, the model could be used for toxicity screenings or drug testing. The co-development of mesodermal and neuroectodermal tissues and of a well-organized vascular plexus along with a peripheral nervous system allows for investigating the crosstalk between neuroectoderm and mesoderm and between peripheral neurons/neuroblasts and endothelial cells. Such interactions influence NCC delamination and migration, sensory neuron differentiation, and rearrangement of the primitive vascular plexus in the embryo.

Large intestine anatomy - modern clinical applications

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In view of present-day clinical needs, we attempt here to standardize colon anatomy and terminology to meet all necessary needs of anatomists and clinicians.

To this end, important clinical and anatomical features are summarized and the associated anatomical terminology is analyzed. From a clinical point of view, ultrasound examinations of the fetal and adult mesentery of the sigmoid colon in pregnant women and in adults, the flexure between the descending colon and the sigmoid colon, and the number of "acute" (no more than 90°) colonic flexures are included and analyzed using AI methods to develop a terminology and parameters that meet the needs of modern colonoscopy.

We propose a simplification of the terminology for the curvatures of the colon, namely the use of the terms flexures for the colon and invaginations and curvatures for the rectum. The mesentery of the sigmoid colon can be visualized from the end of the first trimester. The flexure between the descending colon and sigmoid colon is two fingerbreadths from the left superior iliac spine anteriorly and one handbreadth from the median plane. A problematic colonoscopy course is expected in 14.9% of women and 6.1% of men. The right colon can be used for esophageal reconstruction. Our predictions suggest that children under 30 kg and adult women are at higher risk of having incomplete colonoscopy.

Our results and the simplified anatomic terminology of the colon will be a useful tool for the applied today clinic of the colon.

Pubic symphysis during singular and twin pregnancies. A pilot study.

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We evaluated the pubic symphysis parameters with ultrasound during singular and twin pregnancies

There were collected: the width on different levels on the entrance at three levels and at the half of the symphysis, its length (in the longitudinal distance) and intertubercular (between the pubic tubercles) distance. The typical anthropometric data were collected. We divided the female patients into clusters according to the foetal weights. In the twins pregnancy the weight of foetuses was established as 1500 g, and the groups were established respectively. In singular pregnancies the cluster were established under 1000 g (1st group), 1001-2000 g (2nd group), 2001-3000 g (3rd group), and more than 3000 g (4th group).

In the twins pregnancies (every foetus under 1500 g vs more than 1500) we established the symphysis entrance width parameters as 8.37 mm; 5.77 mm, and 3,38 mm vs 10.3 mm; 5.92 mm; 4.02 mm, respectively. The width was 5.96 mm vs 5.64 mm and the length 16.2 mm vs 14.6 mm, respectively. In the singular pregnancies the entrance width was: 1st group 8.51 mm, 4.7 mm and 2.65 mm vs 2nd group 8.55 mm, 5.54 mm, and 3.03 mm vs 3rd group 10.3 mm, 6.26 mm and 3.3 vs 4th group 10.8 mm, 7.4 mm and 4.24 mm, respectively. In the symphysis half the parameters were 4.56 mm vs 5.51 mm vs 6.4 mm vs 7.33 mm, respectively. The length was 13.9 mm vs 13.6 mm vs 14.8 mm vs 15.6 mm, respectively.

We think the symphysis parameters to be useful parameter in the pregnancy development evaluation.

The Actions of TGFβ1 in HMC3

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Microglia are resident cells of the central nervous system (CNS) and play critical roles during development, homeostasis, and neuropathologies. Pre- and postnatal differentiation and maturation of microglia are influenced by endogenous factors such as TGFβ1. Since recent studies were mainly conducted with rodent microglia we intended to analyze TGFβ1-related effects in a human microglia in vitro model.

The expression of TGFβ signaling-related proteins (SMADs) has been analyzed in a human microglia cell line (HMC3) using qPCR and phosphorylation of SMADs was detected by Western Blotting. TGFβ1-induced effects on effector targets were analyzed by ELISA, immunocytochemistry as well as RNA sequencing.

HMC3 treatment with TGFβ1 for 2h, 4h and 6h resulted in SMAD2 but not in SMAD1/5/8 phosphorylation. Overall downstream targets of TGFβ1 were analyzed using relative qPCR and RNA sequencing. TGFβ1 treatment results in moderate changes in the HMC3 gene expression including upregulation of 60 and downregulation of 176 genes. However, the microglia maturation markers P2RY12, TMEM119, OLFML3 and GPR34 and the expression of interleukins remain unaffected by TGFβ1 treatment.

HMC3 cells mechanistically fulfil prerequisites of TGFβ signaling. However, TGFβ1 target genes are strikingly different compared to primary mouse microglia. Moreover, homeostasis, activation and maturation of HMC3 cells were hardly affected by TGFβ1 indicating that HMC3 cells are not a suitable model system to analyze TGFβ1 effects on human microglia. In summary, human models such as iPSC-derived human-microglia-like cells may be more suitable to assess the role of TGFβ1 for human microglia.

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Blood vessel reactive autoantibodies in encephalitis patients – target identification and functional implications

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To study the autoantibody repertoire to brain blood vessels in patients with autoimmune encephalitis

149 human monoclonal IgG antibodies from the cerebrospinal fluid of patients with different forms of autoimmune encephalitis were tested on murine brain sections for reactivity to blood vessels using immunofluorescence. Positive candidates were further characterized and tested for effects on transendothelial electrical resistance (TEER) and expression of tight junction proteins as well as gene regulation using human brain microvascular endothelial hCMEC/D3 cells as an in vitro blood-brain barrier model. Additionally, antibodies were intrathecally injected into mice to study in vivo binding and effects on tight junction proteins such as Occludin. Target protein identification was addressed using transfected HEK293 cells.

Six antibodies reacted with brain blood vessels. Reactive monoclonal antibodies (mAbs) can be roughly divided into reactivity to mid- to large size vessels and reactivity to vessels of all sizes including capillaries. One antibody from a NMDAR encephalitis patient, mAb 011-138, also reacted with cerebellar Purkinje cells. Treatment of hCMEC/D3 cells with patient antibody resulted in decreased TEER values, reduced Occludin expression and mRNA levels. Functional relevance in vivo was confirmed as Occludin downregulation was observed in mAb 011-138-infused animals. Unconventional Myosin-X was identified as novel autoimmune target for this antibody.

Autoantibodies to blood vessels occur in autoimmune encephalitis patients and show a distinctive binding pattern. Following this prototype pipeline antibody targets on vessel structures can be identified and functional impact on blood vessel integrity assessed.

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A new 3D ex vivo eyelid slice culture model reveals the influence of melanocortins in meibomian glands

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The meibomian glands (MG) produce a lipid-rich secretion called meibum, which forms the outer layer of the tear film. Dysfunction of the meibomian gland (MGD) is the main cause of the increasingly common dry eye disease (DED). An immortalized human meibomian gland epithelial cell line is primarily used in MGD research, but is limited in its ability to display the in vivo situation. We therefore established a new 3D ex vivo slice culture of MG and investigated the effects of melanocortins.

The MGs were isolated and cut into slices via vibratome. Viability, functionality as well as morphological changes were monitored via viability assay, transmission electron microscopy and live-death, lipid as well as (immune-)histological staining for 21 days. The effects on lipid production and gene expression were examined after stimulation with MCR agonists, α- and β-melanocyte-stimulating hormone and/or a MCR antagonist, JNJ-10229570.

Our results demonstrated that the viability, physiological function, morphology, and expression of cell markers were maintained for at least 7 days in our new model. Stimulation with the MCR agonists induced lipid production in a dose-dependent way, whereas this effect was tapered with the simultaneously incubation of the MCR antagonist. In addition, the expression of markers for lipid synthesis were increased after stimulation.

Our new 3D slice culture model is a promising approach to study the (patho)physiological properties of MG. Therefore, it may accelerate the search for new treatments for MGD/DED, such as melanocortins, which likely stimulate meibum production.

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

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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.

Stimulation of tracheal epithelial brush cells with denatonium influences cAMP-dependent transepithelial ion transport and cholinergic signalling

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The epithelial ion and fluid transport represents an important part of mucociliary clearance. We have recently found that denatonium, a bitter substance that activates tracheal epithelial brush cells (BC), which express members of the bitter taste signalling cascade, e.g., the transient receptor potential melastatin 5 (Trpm5) channel, can influence transepithelial ion transport processes. However, the exact mechanism and which ion channels are involved remains unknown.

We measured transepithelial short circuit current (ISC) of freshly isolated tracheae of wild-type and Trpm5-/- mice, and of Trpm5-tauGFP mice treated with resiniferatoxin (RTX) to ablate sensory neurons. Successful depletion of sensory neurons was investigated by immunohistochemistry in tracheal whole mount preparations.

Application of denatonium decreased ISC. This effect was reduced in Trpm5-/- mice. Inhibition of cholinergic signalling via mecamylamine (nicotinic ACh receptor antagonist) reduced the denatonium-effect in wild-type but not in Trpm5-/- mice, while atropine (muscarinic ACh receptor blocker) had no effect. Cholinergic signalling affects the epithelial sodium channel (ENaC). Indeed, the denatonium-effect was reduced in the presence of amiloride (ENaC antagonist). Similarly, the amiloride-effect was reduced in the presence of denatonium. We next investigated the signalling responsible for the residual denatonium-effect in Trpm5-/- mice. Stimulation of adenylate cyclases with forskolin reversed the denatonium-induced current changes. Application of the chloride channel inhibitor NPPB reduced the denatonium-effect. Ablation of sensory nerves with RTX did not influence the denatonium-effect.

BC-stimulation influences ISC via two pathways: Trpm5-dependent cholinergic inhibition of ENaC as well as cAMP-dependent chloride transport, thereby contributing to airway surface liquid homeostasis.

Progression of lung injury during low volume ventilation in pre-injured lungs

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Research on protective mechanical ventilation has brought new insight in the past decades. However, mechanical ventilation can still cause severe lung injuries via ventilation-induced lung injury. Changes in alveolar micromechanics due to edema, inflammation or deactivation of surfactant in a pre-injured lung often result in heterogeneous ventilation. Not only primarily damaged alveolar septa, but also neighbouring alveoli can be affected by mechanical stress. Based on the hypothesis that small regions of collapse (microatelectases) function as “stress concentrators” we investigated the impact of low volume invasive mechanical ventilation on injury progression.

One day after bleomycin challenge no signs of injury were
found by means of oxygenation and lung mechanics. The rats were then mechanically ventilated for 2, 4, or 6 hours (n=15 per group, PEEP = 1 cmH2O, tidal volume 10 ml/kg BW). Repetitive measurements of lung mechanics were used to track disease development at the organ scale.

Whereas a healthy lung tolerates
that mode of mechanical ventilation, the bleomycin treated animals showed signs of lung injury in their lung mechanical readout parameters. The longer the ventilation, the worse the bleomycin treated lungs performed in terms of tissue elastance (H), tissue dampening (G), quasi-static compliance (Cst) and inspiratory capacity (IC). This resulted in lowered oxygenation and eventually culminated in respiratory failure in 1/3 of animals in particular between 4 and 6 hours of ventilation.

While at the start alveolar micromechanics were characterized by permanent alveolar derecruitment, increase in the peak-inspiratory pressure suggests progressive alveolar instability with intratidal recruitment and derecruitment after 4 hours of mechanical ventilation.

Sox9 deficiency induced by Nrf2 KO impairs chondrogenesis and promotes gender-specific osteoarthrosis

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Nuclear Factor Erythroid 2-Related Factor 2 (Nrf2), the universal cellular detector of oxidative stress, which influences SRY-Box Transcription Factor 9 (SOX9) regulation, a key initiator of chondrogenesis, chondrocyte development and maturation. We aim to examine the influence of this compound on cartilage degradation and the following osteoarthritis, using a coordinated mouse model.

Nrf2 knockout mice (KO) and wild-type control mice (WT) were divided into groups according to age (12W/ 90W) and sex. The Sox9 expression was investigated by immunohistochemistry. The femoral part of the knee joint and the growth plate was divided into areas according to the anatomical position. Area, total chondrocyte count, and positive cells were determined and put into relation.

Overall old mice show fewer Sox9+ chondrocytes. In young WT mice, significantly more positive cells were detected in articular cartilage and amplified in the growth plate compared to KO mice of the same age. Surprisingly, a significantly higher chondrocyte number was detected in the cartilage of young KO mice- this phenomenon was even more pronounced in the female individuals.

Due to the major influence of Sox9 on chondrogenesis during endochondral ossification, particularly little was detected in the KO growth plate. The gender difference is highlighted by the increased number of chondrocytes in the cartilage of female KO mice. The negative effects of oxidative stress on cartilage formation already in the early developmental phase seem clear. The relationship between the two transcription factors (i.e. Nrf2 and Sox9) could be a starting point for prophylactic therapies against osteoarthritis.