Programme of the 115th Annual Meeting

September 21 – 23, 2021 | Innsbruck, Austria

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

FIRST AUTHOR NUMBER:	LECTURE (L) POSTER (P)
Abdel Wadood N.	L59
Ahrens G.	P66
Albrecht A.	P23
Anikeva I.	P14
Anstötz M.	L20
Antipova V.	P36
Arnold P.	L14
Arumugam G.	L62
Ascheid D.	P16
Bauer D.	L8
Behrens V.	L30
Bilella A.	P12
Bub S.	P56
Burhmann C.	P43
Cambridge S.	L33
Carrero-Rojas G.	L19
Casaril A.	P41
Catanese A.	L15
Cheng L.	P69
Clarner T.	P31
Dahlke E.	P45
Dillinger A.E.	P35
Eichler A.	P29
Einhäupl L.	P32
Evers S.	L27
Fedotchenko A.	L29
Ferdinand A.	P54
Ferner F.V.	L57
Fragoulis A.	L32
Freiberger I.	L41
Friederich M.	P3
Frintrop L.	L35
Fuchs M.	L46
Fuchssteiner C.	P2
Galanis C.	L21
Gasterich N.	L32
Getwan M.	L66
Geyer S.H.	L61
Gögele C.L.	P67
Gruber G.M.	P13
Hachmann H.	L54
Hamarsheh D.	L24
Heinze T.	L9
Hoang N.A.	P65
Huchzermeier R.	P48
Jahr H.	L28
Joost S.	L36
Kamp A.M.	P4
Keiler J.	P10
Kleefeldt F.	L53

Kleine A.D.	P39
Koblenzer M.	L56
Kogel V.	L55
Kremer C.	P27
Krömer J.	L58
Kruse P.	P28
Kubo Y.	P62
Kubo Y.	P63
Kulow C.	L5
Kumar P.	L26
Kunt T.	L49
Kvitka D.	P19
Lenz M.	L12
Li L.Y.	P40
Li W.	P57
Lückstädt W.	P49
Mahmoud W.	L23
Maier E.	L67
Maurer-Gesek B.	P6
Maxeiner S.	P25
Mayadli Ü.S.	L18
Mehlhorn J.	P24
Meister A.L.	P50
Melzer L.	P18
Miroschnikov N.	L17
Mohren L.	P44
Muellerleile J.	P17
Nicolaus H.	P47
Nökel K.	P15
Pearcy Q.	L37
Perniss A.	L25
Pichler R.	P68
Poiliot A.	L40
Pretterklieber B.	L3
Pretterklieber B.	L4
Pretterklieber B.	L6
Preuß F.	P26
Pruidze P.	P7
Pu Q.	L64
Quabs J.	P22
Ranceviene D.	P30
Reissig R.	L60
Reuss B.	L68
Rihani L.	L48
Rockel A.	P59
Runge J.	P11
Sandrock M.	L43
Saudenova M.	P8
Scheer A.	P61
Schinner C.	L52
Schmidt S.	L63
Schmitt T.	L45

Schneider M.	P34
Schomburg S.	P58
Schön M.	L11
Schöning T.L.	P38
Schotten A.L.	P21
Schulz A.	P60
Schweizer F.	P53
Sigmund A.	L47
Siwetz M.	L39
Socher E.	L22
Steinert L.	P46
Stelz V.	P33
Stofferin H.	L44
Tao H.	P71
Terzis R.	P9
Toedtling M.	L7
Tomlinson J.	L38
Topka M.	P5
Treccani G.	L16
Trinh S.	L34
Urban S.	L1
Van Meenen D.F.	P20
Venghaus A.	P55
Voelz C.	L31
Walther I.L.	P37
Wang Y.	L65
Wanuske M.T.	P51
Welling C.	P64
Winkelmann A.	L42
Witte M.	L13
Wöhner B.	P42
Wolf-Vollenbröker M.	P1
Yeruva S.	L51
Zahn I.	L50
~~Zanon-Rodriguez L.~~	~~P52~~
Zhou P.	P70
Zwirner J.	L2

Vortrag 1:

Title:
The anterolateral ligament of the knee – Phantom of anatomy within the anterolateral complex

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Abstract:
Concerning the ongoing controversy about the existence and nature of the anterolateral ligament (ALL) of the knee joint, we reinvestigated the formation of the anterolateral part of its fibrous capsule in anatomic specimens.

Forty paired embalmed lower extremities taken from 20 human body donators (15 men and five women) underwent exact macroscopic dissection.

In all specimens, the anterolateral part of the knee joint fibrous capsule was established by the iliotibial tract and the anterior arm of the aponeurosis of the biceps femoris muscle. According to their close connection and the fact that the anterolateral part of the fibrous capsule is exclusively assembled by these two aponeuroses, they do not leave any space for a distinct ALL connecting the lateral femoral epicondyle and the lateral tibial condyle.

Our results show that there is no evidence for the existence of an ALL in human knee joints. It is represented either by the iliotibial tract or – most likely – by the anterior arm of the short head of the biceps femoris muscle.

Vortrag 2:

Title:
Traction or compression? - A histological analysis of heel spurs considering the reason for their development

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Abstract:
It is unclear whether plantar and posterior heel spurs are truly pathological findings and whether they are stimulated by traction or compression forces. Previous histological investigations focused on either one of the two spur locations, thereby potentially overlooking common features that refer to a uniform developmental mechanism.

In this study, 19 feet from 16 cadavers were X-ray scanned to preselect calcanei with either plantar or posterior spurs. Subsequently, seven plantar and posterior spurs were histologically assessed using Giemsa and silver stain. Five spur-free Achilles tendon and three plantar fascia entheses served as controls.

Plantar spurs were located either intra- or supra-fascial whereas all Achilles spurs were intra-fascial. Both spur types consistently presented a trabecular architecture without a particular pattern, fibrocartilage at the tendinous entheses and the orientation of the spur tips was in line with the course of the attached soft tissues. Spurs of both entities revealed tapered areas close to their bases with bulky tips.

Achilles and plantar heel spurs seem to be non-pathological calcaneal exostoses, which are likely results of traction forces. Both spur types revealed commonalities
such as their trabecular architecture or the tip direction in relation to the attached soft tissues. Morphologically, heel spurs seem poorly adapted to compressive loads.

Vortrag 3:

Title:
Pectineus muscle's double innervation – fact or fiction?

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Abstract:
It is generally accepted that the pectineus receives double innervation by the pectineal branch of the femoral (PB) and the anterior branch of the obturator (AO) nerve. The PB courses close to the femoral vessels, where it may be harmed during surgical procedures. The aims of this study were to examine the course of the PB, and to evaluate which part of the pectineus is innervated by the PB and the AO.

The level of origin, exact course, ramification, and level of entrance of the PB were determined in 50 Formalin-embalmed legs. Double-innervated muscles underwent Sihler's technique and qualitative analysis of the intramuscular nerve branches.

The PB branched from the femoral nerve caudally to the inguinal ligament in 52% of cases, cranially in 42%, at its level in 6%. In about 50%, two to three branches were observed. They consistently coursed deep to the femoral vessels, cranially and/or caudally close to the medial femoral circumflex artery. Mostly, they entered the middle third of the pectineus. In six cases, a small branch from the AO entered its deep surface. After intramuscular evaluation, in only two of them, a small medial portion of the muscle was innervated by the AO.

As the PB has to be preserved during surgical procedures on the femoral artery, exact knowledge about its proximity to these structures is mandatory. The generally accepted assumption of the pectineus' constant double-innervation seems to be obsolete. These first results presented here need to be confirmed in further case series.

Vortrag 4:

Title:
Is the anatomy of the rat hip and thigh muscles comparable to human?

Authors:
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Abstract:
Rodents are commonly used as animal models to assess the effects of pathologies or drug treatment. However, anatomical descriptions on rodent morphology, especially of the rat musculoskeletal apparatus lack in sufficient detail to conclude adequately to humans. The aims of this study were to provide a detailed and accurate description of all muscles of the hip and thigh of the rat, and to compare these muscles with the morphology of human hip muscles.

Sixty hindlimbs of thirty adult male albino rats were dissected in a formaldehyde-fixed condition. The nerve branches for some of the more superficial muscles were marked with coloured yarn for exact identification. To determine the exact bony attachments, plain X-rays (a.p. and lateral aspect) of each hip muscle marked with pins were obtained.

A detailed systematic and topographical description for each muscle group of the rat hip and thigh muscles and a detailed dissection guide were elaborated. In general, rat hip anatomy is similar with the human anatomy. Particular differences were found for the gluteals, adductors and hamstrings, which is likely related to the different functions of these muscles when comparing the squat position with bipedal humans.

Given the rat is an established animal model to extrapolate findings to humans, it is vital to understand spatial and functional differences to the human musculoskeletal system. This study provides detailed comparative anatomy on the hip and thigh regions to fill this gap in literature.

Vortrag 5:

Title:
Muscles levator scapulae and rhomboid minor: a morphological unit ?

Authors:
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Abstract:
Clinically, pain over the superior angle of the scapula is common, with pain often radiating to the neck, head, and shoulder. The etiologies can be numerous, and some may also be idiopathic in nature.

To explore the fascial connections of this region, 22 unembalmed, 2 Thiel, and 2 alcohol cadavers were studied by dissection, histological probes, and plastination.

A large prominent triangular area of white connectives is revealed, varying in mass, and located beneath Trapezius, its descending and transversal parts. A subdivision of these connectives can be further dissected to prove that the Rhomboid minor and Levator scapulae are interconnected and enclosed by connectives creating a morphological unit. Between these muscles a fatty triangular fascia is observed, ending cranially at the surface of the Splenius, and the median line, containing vessels and nerves, as supported by histology and plastination. This unit is separate from the Rhomboid major but overlaps the latter dorsally. It connects to the Superior angle of the scapula and its medial borders' upper part, respectively, cranially to the root of the spine of the scapula.

Beneath the morphological unit of Levator scapulae and Rhomboid minor described here, the Serratus posterior superior and possibly Serratus anterior form a hypermochlion or fulcrum at the Superior angle.
Any tension on this morphological unit by other muscles or fascia can throw this fulcrum out of balance. Knowledge of a fascial unit for these two muscles may help to explain the often idiopathic nature of superior scapular pain.

Vortrag 6:

Title:
When and why was in German textbooks of anatomy the phrenicoabdominal branch of the left phrenic nerve placed into the eosophageal hiatus? An anatomical study on 400 specimens re-evaluating its course through the diaphragm

Authors:
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Abstract:
The left phrenicoabdominal branch passes through the costal part of the diaphragm. In most German anatomy textbooks, however, its passage has been described through the oesophageal hiatus. The aim of this study was to re-evaluate its topography in relation to the diaphragm and to retrospectively investigate the origin of its description.

In 400 embalmed body donors, distances between the passage of the left phrenicoabdominal branch relative to three topographical landmarks was measured. German anatomy textbooks published between 1700 and 2018 were reviewed for their description of the nerve's pathway.

In none of the investigated body donors, the left phrenicoabdominal branch passed through the oesophageal hiatus. In 99.5%, the nerve pierced the costal part of the diaphragm dorsal to or at the same level as the apex of the pericardium. The mean distances were 3.4 (±1.5) cm to the apex of the pericardium, and 5.8 (±2.2) cm to the oesophageal hiatus. The first description of the passage of the left phrenicoabdominal branch through the eosophageal hiatus was given in 1791 provided by Sömmering. Ever since, a variable description of its passage persisted in German anatomy textbooks.

Detailed knowledge about the course of the left phrenicoabdominal branch in relation to the diaphragm help to improve topographical knowledge and to prevent inadvertent nerve injury during surgical interventions. Further, the results presented herein may form a solid basis to adopt the correct description of the passage of the left phrenicoabdominal branch to anatomical textbook knowledge.

Vortrag 7:

Title:
Course and branching pattern of the dorsal within the deep muscles of back rami are more complex as usually described

Authors:
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Abstract:
There are few and largely insufficient descriptions of the topographical relationship between the autochthone muscles and their nerve branches. However, for a nerve-sparing preparation during spinal surgery it is important to know their exact course. The aim of this study was to provide a precise description of the dorsal rami, to determine the number of branches emerging from them, and to give an overview of their innervation areas.

The course of the dorsal rami was visualized by stratigraphic dissection in 6 formalin-embalmed and 12 unembalmed anatomic specimens. Thereby, the musculature has been exposed from dorsal to ventral in order to obtain a uniform image of the dorsal branches of the spinal nerves, their ramifications, and relations to the deep muscles of the back.

The dorsal rami divided into two or three main branches often forming plexus with those from other segments. The change in skin innervation from the medial to the lateral branches took place at the level of the seventh (nine bodies, 18 sides) or eighth (three bodies, six sides) branch, rarely (one body, both sides) cranial to them. The topographic relationship between the nerve branches and the autochthone muscles differ from cranial to caudal, and have been exactly described.

This study adds detailed information concerning the course of the dorsal branches of the spinal nerves. Extensive knowledge of nerve plexus formation within the deep muscles of the back may assist to avoid nerve injuries during surgery.

Vortrag 8:

Title:
Formation of the azygos and hemiazygos vein in humans and their passage through the diaphragm

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Abstract:
Anatomical textbooks often present a linear connection between the ascending lumbar and the (hemi) azygos veins. As this is incongruent with reality, we aimed to show the exact course and source of the azygos and hemiazygos veins.

The azygos and hemiazygos veins were examined by careful macroscopic dissection in 20 formalin-fixed human body donators with special emphasis on their source and course and passage through the diaphragm.

The azygos vein constantly arose from a dorsal and ventral root. The hemiazygos vein also originated mainly (85%) from a dorsal and ventral root. In rare cases, it was formed only (10%) by a ventral and even more seldom solely from a dorsal root (5%). The dorsal root was always (100 %) established by the confluence of the ascending lumbar and subcostal veins. The ventral root, the lumbar (hemi)azygos vein, showed a variable origin. It passed the diaphragm either exclusively dorsal to the diaphramatic crus or the aortic hiatus or - being bipartite - parallel through the diaphramatic crus and the aortic hiatus. The mean anterioposterior distance between the level of the (hemi)azygos and ascending lumbar veins was 3,8 and 4,1 cm, respectively

The azygos and hemiazygos veins arise almost exclusively from a dorsal and ventral root. The results clearly show the different anatomical layers in which the right and left ascending lumbar veins course compared to the position of the azygos and hemiazygos veins. A direct continuation of the veins into each other is not possible.

Vortrag 9:

Title:
The middle rectal artery - a forgotten but relevant blood vessel for both surgery and lateral lymphatic spread of rectal carcinoma

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Abstract:
The reported prevalence of the middle rectal artery (MRA) ranges from 12% to 97%. Inspite its relevance for lateral rectal tumor spread and risk of intraoperative bleeding, data on the origin, course and size of the MRA are highly divergent. Therefore, the aim of this study was to systematically reevaluate the topographic anatomy.

Pelvic specimens of formalin fixed body donors (n=14) were subjected to a stepwise macroscopic dissection to expose the MRA, adjacent pelvic organs and nerves. The findings were photodocumented, and several morphometric parameters were recorded. Selected areas were processed for histological study. One specimen was used for 3D-reconstruction by 360° multiangle photogrammetry.

The MRA was present in 71.43%, originated as separate branch from the anterior division of the internal iliac artery (31.8%), internal pudendal artery (45.5%) or inferior gluteal artery (22.7%), and showed varying diamters (0.5-3.5 mm). 1-3 branches reached the rectum either by ventrolateral (35.7%), lateral (42.9%) or dorsolateral (21.4%) approach. The MRA was accompanied by podoplanin-immunreactive lymphatic vessels and gave off additional branches (81.8%) to urogenital pelvic organs. 3D-reconstruction illustrated the complex course of the MRA from the pelvic sidewall throughout the pelvic nerve plexus into the rectum via the lateral rectal ligaments.

The study emphasizes the rather high frequency of the MRA and offers reliable topographic 3D-orientation to prevent inadvertent injury and bleeding during rectal surgery. Moreover, the data provide an anatomical rationale for lateral lymphatic rectal tumor spread and an anatomical basis for nerve-preserving pelvic lymph node dissection.

Vortrag 10:

Title:
The anatomical basis for collecting nasopharyngeal material to challenge Covid-19 and respiratory diseases

Authors:
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Abstract:
This systematic anatomical study aims at defining a safe procedure and easily identifiable landmarks for reaching the nasopharynx mucosa when performing nasopharyngeal swabs, for diagnosing diseases affecting the respiratory system, such as Covid-19. In addition, it focuses on evaluating the success of entering the nasopharynx if relying on the external acoustic meatus as a landmark.

We simulated nasopharyngeal swabs bilaterally in head/neck specimens stemming from 157 body donors (314 simulations). Metal probes and commercial swabs were used. Important anatomical parameters were photo-documented and measured.

We provide information on angles and distances between prominent anatomical landmarks and particularly important positions the probe occupies during its advancement from the nares to the nasopharynx. Building on these results we suggest a simple, safe and always successful three-step procedure for advancing swabs into the nasopharynx. Landmarks and signs guiding the advancement from externally are provided. Evaluations of using the ostium of the external acoustic meatus as a landmark revealed a success rate of less than 50%.

The results of our study provide important information and guidance for safe and successful collection of nasopharyngeal material for detecting and characterizing pathogens, such as SARS-CoV-2.

Vortrag 11:

Title:
Longitudinal SARS-CoV-2 infection during the gross anatomy course

Authors:
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Abstract:
The gross anatomy course at Ulm University was performed in face-to-face teaching during the past winter semester. Our approach was to conduct an infection epidemiological study in addition to a comprehensive hygiene and information concept.

The hygiene concept included infection control measures and involved in particular organizational, environment-related, and individual measures. The infection study consisted of two swabs with RT-PCR, two rapid antigen tests, and a quantitative SARS-CoV-2 antibody test with questionnaires at the beginning and end of the course. Students from other semesters were also invited (in total n=868).

Direct pathogen detection identified two asymptomatically infected individuals at the beginning of the semester (in total n=568). After the Christmas break, all 429 swabs from anatomy course participants and staff were negative. More than 6% of the students were antibody positive at semester start. Infection-related sero-conversions increased minimally towards the end of the semester (n=9 of 671 participants present at both testing times). Only two students participating in the dissection course showed seroconversion. Antibody titers were stable over the three-month period, and none became seronegative. Infections occurring during the study period were not associated with classroom activities.

Although an above-average number, compared to the German population, had a past infection at the beginning of the semester, there was only a slight increase in infections until the end of the semester, in contrast to the population-wide trend. We therefore suspect a preventive effect by 1) the hygiene concept, 2) continuous information, and 3) the test study itself.

Vortrag 12:

Title:
All-trans retinoic acid induces synaptic plasticity in the human neocortex

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Abstract:
The central nervous system is capable of adjusting the structural and functional properties of its synaptic contacts in response to appropriate stimuli. Although this neuronal feature, i.e., the expression of synaptic plasticity, has been widely studied in various animal models, direct experimental evidence for coordinated structural and functional synaptic plasticity in the adult human cortex is currently missing. Here, we used all-trans retinoic acid (atRA), which has been recently suggested as a potential medication for the treatment of neuropsychiatric and neurodegenerative disease, to probe and study synaptic plasticity in human cortical neurons.

Acute human cortical slices were prepared from neurosurgical access material. Slices were treated with atRA (1 µM) for 6 – 10 h, and synaptic changes of superficial (layer 2/3) pyramidal neurons were assessed using whole-cell patch-clamp recordings, immunohistochemistry and ultrastructural analysis.

Our experiments reveal that atRA facilitates excitatory neurotransmission onto human cortical neurons. These functional changes are accompanied by structural plasticity of dendritic spines and spine apparatus organelles. Moreover, we show that atRA-mediated synaptic plasticity requires mRNA-translation.

We conclude that atRA is a potent mediator of synaptic plasticity in the adult human cortex.

Vortrag 13:

Title:
GABAB-receptor mediated modulation of inhibitory neurotransmission on Martinotti cells in the Barrel Cortex

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Abstract:
Martinotti cells (MC), a subset of somatostatin-expressing cortical GABAergic interneurons (IN), are integrated into the network of local pyramidal cells. Moreover, MC are also a major component of disinhibitory circuits, as vasoactive intestinal polypeptide (VIP)- and parvalbumin (PV)-expressing IN are targeting MC in Layer (L) 2/3 and L5. Currently, little is known about the properties of these two inhibitory projections and their possible modulation.

We performed whole cell patch-clamp recordings of L2/3 and L5 MC in the primary somatosensory (barrel) cortex of juvenile transgenic mice in combination with pharmacological modulation of induced postsynaptic inhibitory currents (IPSC). In addition, we investigated the expression of metabotropic GABAB-receptors in different IN subgroups by fluorescence in situ hybridisation (FISH) and immunohistochemistry.

Inhibitory currents (IPSC) in MC of L2/3 and L5 are mainly mediated by postsynaptic GABAA-receptors. Activation of presynaptic GABAB-receptors at the terminals of inhibitory axons derived from PV- and VIP-cells leads to a reduction in eIPSC amplitudes. These inhibitory synapses exhibit depressing short-term plasticity and showing modulation by GABAB-receptor agonist only on L2/3 MC, as compared to L5 MC. Using FISH and antibody staining against the GABAB(2)-receptor , we detected the receptor in almost all subtypes of IN.

Based on our data, we could clearly demonstrate that presynaptic GABAB-receptors can modulate inhibitory transmission on MC in the barrel cortex. In which situation these receptors are activated is still unknown, but self-inhibition via GABA spillover at terminals of inhibitory axons can be excluded.

Vortrag 14:

Title:
Exploration of LIMP-2 as an expression platform for Parkinson's disease associated GCase variants

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Abstract:
Mutations within the gene encoding for the lysosomal glucocerebrosidase (GCase) is the most common genetic risk factor to develop Parkinson's disease (PD) with 5-20% of patients' being affected. To reach the lysosome GCase requires a transport protein the lysosomal-integral-membrane-protein-2 (LIMP-2). These two protein form a complex in the ER and then travel through the secretory system, where they are sorted towards the lysosomal pathway. We exploited LIMP-2 as a platform to co-express with GCase and PD associated GCase variants for cell biological characterization.

Using site directed mutagenesis, we produced a non-binding variant of LIMP-2 that lacks the capacity to transport GCase to the lysosome. When expressing differently tagged GCase variants into the supernatant, we also measured lower activity for His- or STREP-tagged variants. Thus, expression and purification of tagged and additionally mutated GCase might produce unreliable results. Expressing GCase variants together with both LIMP-2 variants, binding and non-binding, and measuring GCase activity in lysate and enriched lysosomes, helps to determine transport and activity of respective GCase variants.

We were able to reconfirm the positive effect of LIMP-2 on GCase activity and stability, not only for wt GCase, but also for E326K GCase, a PD associated variant. Using our cell culture system, we could show that LIMP-2 increases the transport of wt and E326K GCase to the lysosome. Here we found an increased enzymatic activity for wt and E326K GCase. This makes the E326K variant targetable for interventional studie, that increase the lysosomal transport and/or stabilize enzymatic activity in the lysosome.

To date activity measurements on GCase are mainly done using recombinant expressed proteins that carry different tags. Here, we propose a cell system that uses the endogenous interactor of GCase and measure GCase activity in the lysate and lysosome directly. With our system, we can directly compare transport capacity to and activity in the lysosome and test activators of GCase activity in a cellular system.

Vortrag 15:

Title:
Multi-omics analysis of hiPSC-derived motoneurons highlights synaptic dysregulation as a driving pathomechanism in ALS

Authors:
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Abstract:
Amyotrophic lateral sclerosis (ALS) is a progressive fatal neurodegenerative disease, which mainly affects the spinal motoneurons. To date, despite the extensive efforts of the scientific community, the specific molecular pathomechanisms leading to the loss of this specific neuronal population are still poorly understood. The heterogeneity of genetic causes, together with the lack of reliable and coherent animal models has been a major obstacle for the clear understanding of the dynamics characterizing the disease progression. Indeed, an effective treatment for this neurodegenerative disorder is still missing.

We differentiated a broad and heterogeneous cohort of ALS patient-related hiPSC lines into spinal motoneurons to perform a variety of "omic" analyses. With this approach we aimed at covering the epigenome (whole genome bisulfite sequencing), transcriptome (total RNAseq) and proteome (untargeted mass spectrometry) of ALS motoneurons to gain mechanistic insights into novel pathomechanisms.

Our results identified a profound and dramatic pathological signature converging toward synaptic impairments at all the levels investigated. Synaptic genes were indeed significantly hypermethylated in ALS cells and, accordingly, the expression of synaptic transcripts was also significantly reduced in mutant cells. Interestingly, proteomic analysis revealed a significant accumulation of synaptic proteins in ALS motoneurons, resulting from impaired autophagic degradation occurring centrally in the soma, as well as locally at the synapses.

Our novel and comprehensive approach highlighted a central role of synaptic disturbances in ALS pathology, and supports the notion that restoring synaptic physiology might represent a valid therapeutic strategy to rescue motoneuron degeneration.

Vortrag 16:

Title:
Early life stress targets the transcriptional signature and functional properties of voltage gated-sodium (Nav) channels in hippocampal NG2+ glia

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Abstract:
The detrimental effects of early life stress (ELS) on brain development and mental health are well established. While studies have identified myelination and oligodendrocytes as targets of ELS across species, knowledge about the precise molecular mechanisms and cell populations involved is still lacking. NG2+ cells are a particularly interesting subpopulation, comprising oligodendrocyte precursor cells with unique properties, as they form synapses with neurons and respond to stress hormones.

Using a mouse model of ELS, we performed molecular profiling in hippocampal NG2+ cells at early postnatal and adult stages. To further dissect the impact of glucocorticoids on ELS-induced transcriptional changes, we integrated our data with Chip-seq data on genomic binding sites of the glucocorticoid receptor (GR). The functional relevance of one candidate, Scn7a, was confirmed by electrophysiological recordings in hippocampal NG2+ cells.

ELS targeted the hippocampal NG2+ cell transcriptome, and the observed molecular changes correlated with the ELS-induced increase of corticosterone. The overlap analysis with Chip-seq data on genomic binding sites of the GR revealed nine overlapping genes. Amongst these, Scn7a, coding for a subunit of sodium channels, remained upregulated until adulthood in ELS animals. Upregulation of Scn7a was accompanied by an increase in the density of voltage-gated sodium channel activated currents in hippocampal NG2+ cells.

Our findings indicate that ELS specifically targets the transcriptional profile and electrophysiological properties of hippocampal NG2+ glia. Considering that voltage-gated sodium channels are important for NG2+ cell-to-neuron communication, our findings suggest novel insights into the pathophysiology of stress related mental disorders.

Vortrag 17:

Title:
Role of inositol polyphosphate 4-phosphatase type II (INPP4B) in chemotherapy-resistant retinoblastoma cells

Authors:
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Abstract:
Retinoblastoma (RB) is the most common malignant tumor of the infant retina. Eventhough the standard chemotherapy leads to healing success, many patients still suffer from relapses. During chemotherapy, surviving cancer cells metamorphose to more hazardous derivates with altered characteristics often induced by gene expression changes of tumor suppressor genes. Inositol polyphosphate 4-phosphatase type II (INPP4B) is a tumor suppressor and a negative regulatory factor of the PI3K/AKT pathway in different cancer entities. The study presented focused on investigating the role of INPP4B in chemotherapy-resistant RB cells.

Effects of lentiviral INPP4B overexpression on etoposide-resistant RB cell viability, proliferation, apoptosis and cell growth were revealed by WST-1 assays, BrdU and DAPI stains, growth curve analyses and caspase3/7 assays. Anchorage independent growth and tumorigenicity were analyzed using soft agarose and in vivo chorioallantoic membrane (CAM) assays.

Etoposide-resistant Y-79 and RB355 RB cells display significantly reduced INPP4B expression levels compared to their chemosensitive counterparts. Upon INPP4B overexpression cell viability and anchorage independent growth are significantly reduced in both etoposide-resistant RB cells, whereas caspase 3/7 mediated apoptosis is increased. Besides, INPP4B overexpression decreases cell growth and proliferation of RB355 etoposide-resistant cells. A general anti-tumorigenic effect of INPP4B overexpression leads to significantly reduced tumor weight, tumor size and/or tumor formation capacity of etoposide-resistant RB cells in vivo.

Etoposide-resistant RB cells display reduces INPP4B levels and potentially bare the risk for relapses. Therefore, an up-regulation of INPP4B resulting in tumor reducing effects in aggressive, chemoresistant RB cells is a promising novel therapeutic tool for prevention of retinoblastoma recurrence.

Vortrag 18:

Title:
Transmitter and ion channel profiles of neurons in the primate abducens and trochlear nuclei

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Abstract:
Extraocular motoneurons initiate dynamically different eye movements, including saccades, smooth pursuit and vestibulo-ocular reflexes. These motoneurons subdivide into two main types based on the structure of the neuro-muscular interface: motoneurons of singly-innervated (SIF), and motoneurons of multiply-innervated muscle fibers (MIF). SIF motoneurons are thought to provoke strong and brief/fast muscle contractions, whereas MIF motoneurons initiate prolonged, slow contractions. While relevant for adequate functionality, transmitter and ion channel profiles associated with the morpho-physiological differences between these motoneuron types, have not been elucidated so far.

This prompted us to investigate the expression of voltage-gated potassium, sodium and calcium ion channels (Kv1.1, Kv3.1b, Nav1.6, Cav3.1–3.3, KCC2), the transmitter profiles of their presynaptic terminals (vGlut1&2, GlyT2 and GAD) and transmitter receptors (GluR2/3, NMDAR1, GlyR1α) using immunohistochemical analyses of abducens and trochlear motoneurons and of abducens internuclear neurons (INTs) in macaque monkeys.

The main findings were: (1) MIF and SIF motoneurons express unique voltage-gated ion channel profiles, respectively, likely accounting for differences in intrinsic membrane properties. (2) Presynaptic glutamatergic synapses utilize vGlut2, but not vGlut1. (3) Trochlear motoneurons receive GABAergic inputs, abducens neurons receive both GABAergic and glycinergic inputs. (4) Synaptic densities differ between MIF and SIF motoneurons, with MIF motoneurons receiving fewer terminals. (5) Glutamatergic receptor subtypes differ between MIF and SIF motoneurons. While NMDAR1 is intensely expressed in INTs, MIF motoneurons lack this receptor subtype entirely.

The obtained cell-type-specific transmitter and conductance profiles illuminate the structural substrates responsible for differential contributions of neurons in the abducens and trochlear nuclei to eye movements.

Vortrag 19:

Title:
Are palisade endings candidates to provide eye position signals?

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Abstract:
Proprioception from extraocular muscles (EOMs) is important for proper visually-guided behavior. Because classical proprioceptors are absent in the EOMs of most mammals and palisade endings have been found in virtually all species analyzed so far, they are alternative candidates for EOM proprioception. We have analyzed the molecular profile and the central connection of palisade endings.

Whole mount of cat EOMs were triple labeled with neuronal markers, markers for exocytosis proteins, and markers for cholinergic receptors. In anterograde tracing experiments, neuronal tracer was injected into the EOM motor nuclei.

Palisade endings were formed by nerve fibers that, coming from the muscle, extended into the tendon where they made a u-shaped turn to approach the muscle tendon junction. At the tip of single muscle fibers, recurrent nerves formed a terminal nerve specialization (palisade endings) consisting of axonal branches and terminal varicosities. Terminals varicosities were found at the level of the tendon and around the muscle fiber tips. Molecular analyses showed that palisade endings express ChAT and key proteins (SNAP25, synaptobrevin, synaptotagmin, complexin, and syntaxin) involved in neurotransmitter release. However, no receptors for cholinergic transmission were associated with palisade endings as demonstrated by the absence of alpha-bungarotoxin signals. Following neuronal tracer injection in the EOM motor nuclei, palisade endings were labelled in the EOMs.

Our findings show that palisade endings are cholinergic, have an exocytosis machinery for neurotransmitter (acetylcholine) release and originate from the EOM motor nuclei. Because of these clear motor features, it is questionable if palisade endings are candidates for EOM proprioception.

Vortrag 20:

Title:
Neural circuits of the subiculum and their involvement in epileptiform activity

Authors:
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Abstract:
The activity of local excitatory circuits of the subiculum has been suggested to be involved in the initiation of pathological activity in epileptic patients and experimental animal models of temporal lobe epilepsy (TLE). A proposed key event in epileptogenesis is the decrease expression of the KCC2 membrane transporter in subicular neurons. As result altered chloride homeostasis and thereby GABAergic synaptic input might contribute to TLE. The morpho-functional study of subicular networks and their pharmacological modulation are a goal to shed light into pathomechanisms of TLE.

We have taken advantage of multimodal techniques to classify subicular cells (principal- and interneurons) in distinct subclasses and have investigated their morphofunctional properties and connectivity in vitro. In a second step, we pharmacologically modulated the investigated network by blocking the KCC2 transporter. Using patch-clamp electrophysiology, optogenetic stimulation and inhibition, as well as calcium imaging in vitro we analyzed emerging epileptiform activity.

Our results indicate that local subicular excitatory and inhibitory circuits are connected in a cell type-specific fashion. We show that local excitatory circuits, isolated from extrasubicular inputs and pharmacologically disinhibited, are sufficient to initiate synchronous epileptiform activity. Furthermore, blocking the KCC2 transporter was sufficient to induce epileptiform, inter-ictal like network activity, driven by (especially) by Parvalbumin expressing interneurons. Optogenetic modulation of those interneurons confirmed these findings. Furthermore, calcium imaging indicates a spatially large hypersynchronous network activity.

In conclusion, this work provides a high-resolution description of local excitatory and inhibitory circuits of the subiculum and highlights their mechanistic involvement in the generation of pathological network activity.

Vortrag 21:

Title:
Repetitive magnetic stimulation induces synaptic plasticity through cooperative pre- and postsynaptic activation

Authors:
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Abstract:
Repetitive transcranial magnetic stimulation (rTMS) is a non-invasive brain stimulation technique used to stimulate the brain through the intact skin and skull. It induces long-lasting changes in cortical excitability. Despite its current use in diagnostics and therapy, the cellular and molecular mechanisms of rTMS-induced plasticity remain poorly understood. Here, we combined experimental approaches with multi-scale modeling to learn more about the mechanisms of rTMS-induced plasticity.

We performed whole-cell patch-clamp and local field potential recordings in acute slices and organotypic tissue cultures. Recorded neuronal morphologies were implemented into a newly developed multi-scale modeling toolbox (Nemo-TMS) to dissect physical and physiological effects of repetitive magnetic stimulation (rMS) in neurons. Calcium imaging, genetic and pharmacological approaches were used to assess the relevance of calcium signaling pathways in rMS-induced plasticity.

Confirming our previous work, we showed that 10 Hz rMS induces long-term potentiation (LTP) of excitatory neurotransmission onto hippocampal CA1 pyramidal neurons. Calcium imaging revealed that cooperative activation of pre- and postsynaptic neurons is observed during rMS. 10 Hz optogenetic activation of CA3 neurons only, induced long-term depression (LTD), while activation of CA3 and CA1 neurons transformed LTD into LTP. Multi-scale modeling and inhibition of voltage-gated calcium channels and intracellular calcium stores during rMS confirms the relevance of calcium signaling pathways; suggesting that a metaplastic transformation of 10 Hz LTD into LTP underlies rMS-induced plasticity.

These results provide new important insight on how rTMS induces lasting changes in synaptic transmission and network excitability. Prospectively, the multi-scale modeling approach may help devising more effective rTMS protocols in clinical settings.

Vortrag 22:

Title:
SARS-CoV-2 Spike Protein: Molecular Dynamics Simulations of Sequence Variants Compared to the Wild Type Protein

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Abstract:
The ongoing severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic has changed everyday life around the world. The trimeric spike glycoprotein of SARS-CoV-2 expressed at the viral surface specifically binds with its receptor-binding domains (RBDs) to the host cell receptor angiotensin-converting enzyme 2 (ACE2) to gain entry into a cell to initiate infection. In emerging viral variants, mutations within this trimeric SARS-CoV-2 spike protein affect inter-monomeric contact sites within the trimer as well as the RBD interacting with the human ACE2-receptor. However, the molecular consequences of mutations within the variants on spike protein dynamics and stability or ACE2 binding were largely unknown.

In this research project, we performed all-atom molecular dynamics (MD) simulations in order to compute the 3-dimensional motions in the SARS-CoV-2 wild type spike protein and compared them with the different variants.

At the atomic level, our investigations revealed regions within the spike protein trimer with an altered structural flexibility, rearranged contacts between the three monomers building the trimeric spike and changed structural RBD–ACE2 interfaces. In addition, we observed changes in the binding affinity to ACE2 due to mutations in the RBD.

In our study, we identified residues across different viral variants that are important for viral binding to the host cell. As such residues cannot be replaced without diminishing infectivity of the virus, these residues represent primary targets for intervention, for example by neutralizing antibodies. Moreover, our findings can be starting points for further experimental characterization of the emerging virus variants.

Vortrag 23:

Title:
CXCL13 defines a neuroendocrine cell phenotype in the murine trachea and lung

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Abstract:
Rare airway epithelial cell types include ionocytes, cholinergic chemosensory cells, solitary and clustered neuroendocrine cells. Airway neuroendocrine cells have been implicated in diverse functions including mechanosensation, chemosensation, regeneration and immunoregulation. We assessed the expression of the chemokine CXCL13 (B-cell attracting chemokine) by these cells in the lower airway epithelium in homeostatic condition.

We assessed CXCL13 expression in C57BL/6 J mice by immunohistochemistry, RT-PCR, immunoelectron microscopy, and in-silico-analysis of publicly available data sets.

Unbiased in silico-analysis of published sequencing data of murine tracheal epithelium revealed Cxcl13-mRNA in 68% (36/53) of neuroendocrine cells whilst expression in other cell types was negligible. However, unbiased analysis did not yield subclusters of neuroendocrine cells. Immunolabeling (n=5 whole-mounts, 2254 cells) also defined 2 phenotypes of tracheal neuroendocrine cells: solitary PGP9.5+/CXCL13+ (69%) and solitary PGP9.5+/CXCL13- (31%). In the lung (n=5 lungs, 1548 cells), 4 phenotypes were identified: solitary neuroendocrine cells (5%), among which 7% of them were CXCL13+ and 93% were CXCL13-, and neuroepithelial bodies (95%) in which 5.5% of cells were CXCL13+ and 94.5% were CXCL13-. Ultrastructural immunohistochemistry validated CXCL13-immunoreactive cells as neuroendocrine cells by the presence of numerous cytoplasmic secretory granules.

We identified a phenotype of neuroendocrine cells producing the chemokine CXCL13 in the naïve mouse. CXCL13 is predominantly expressed in tracheal solitary neuroendocrine cells and to a lesser extent in solitary cells and neuroepithelial bodies of the lung. Our observation demonstrates phenotypic heterogeneity in airway neuroendocrine cells and points towards a potential immunoregulatory role in bronchial-associated lymphoid tissue formation and B cell homeostasis.

Vortrag 24:

Title:
Solitary chemosensory cells in the respiratory tract of man

Authors:
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Abstract:
Specialized sensory epithelial cells sense noxious substances and initiate protective reflexes. They 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) (synonyms: brush or tuft cells; markers: GNAT3, PLCβ2, TRPM5). In humans, neuroendocrine cells are present but the existence of SCCC remains unknown.

Single- and multiple-labelling immunofluorescence with antibodies against established marker proteins was performed on specimens of human papillae vallatae (positive control) and respiratory tract (nose, trachea, lung) obtained from anatomy body donors and pathology; TRPM5-eGFP reporter and C57BL6/J mice served as references. Publicly available single cell RNA sequencing (scRNAseq) data were analyzed in silico.

PLCβ2 antisera labelled cells in human taste buds, but not in the respiratory mucosa; TRPM5 and GNAT3-positive cells were not found. Accordingly, in silico-analysis revealed only minimal expression of these markers in human respiratory epithelial cells, in contrast to mice. Instead, scRNAseq data pointed to the endoplasmic reticulum protein LRMP (lymphoid-restricted membrane protein) as a human brush cells marker. LRMP-antibodies labelled rare (0.07-0.7 cells/mm basement membrane), slender epithelial cells along the entire airways reaching deep into the lung. Double labelling showed that they form an independent population, separate from ciliated, secretory, neuroendocrine cells and ionocytes. In mice, LRMP was also localized specifically to SCCC cells, which were restricted to extrapulmonary airways.

These data identify chemosensory cells in human airways. Their distribution along the airway tree and expression of signaling pathway proteins differ from mice.

Vortrag 25:

Title:
Development of epithelial cholinergic chemosensory cells of the urethra and trachea of mice

Authors:
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Abstract:
Cholinergic chemosensory cells (CCC) are infrequent epithelial cells with Immunosensor function, positioned in mucosal epithelia preferentially near body entry sites in mammals including man. We here addressed the time points of their initial emergence as well as
their postnatal development in the urethra and trachea of mice.

Immunostainings of the trachea and urethra of choline acetyltransferase (ChAT)-eGFP reporter mice, mice with genetic deletion of either MyD88, or toll-like receptor-2 (TLR2), or TLR4 and of TLR2/TLR4 as well as germ-free mice were performed.

CCC of the trachea emerge during embryonic development at E18 and expand further after birth, reaching a maximum number of ~4000 cells per trachea in adulthood. Urethral CCC show gender diversity and appear first at P6-P10 in male and at P11-P20 in female mice, reaching a total number of about 650 cells per urethra in adulthood. Urethrae and tracheae of MyD88- and TLR-deficient mice showed significantly fewer CCC in all four investigated deficient strains, with the effect being most prominent in the urethra. In germ-free mice, however, CCC numbers were not reduced, indicating that TLR2/4-MyD88 signalling, but not vita-PAMPs, governs CCC development.

Our data show a marked postnatal expansion of CCC populations with distinct organ specific features, including the relative impact of TLR2/4-MyD88 signalling. Strong dependency on this pathway (urethra) correlates with absence of CCC at birth and gender-specific initial development and expansion dynamics, whereas moderate dependency (trachea) coincides with presence of first CCC at E18 and sex-independent further development.

Vortrag 26:

Title:
The bitter taste receptor agonist denatonium influences mouse tracheal epithelial ion transport

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Abstract:
The epithelial ion and fluid secretion as part of mucociliary clearance protects the pulmonary system from infections. In murine tracheal epithelium, we have previously shown that denatonium-induced activation of brush cells (BC) evokes PLC□2- and Trpm5-dependent release of acetylcholine. Yet, the impact of BC on transepithelial ion transport remains unknown.

Transepithelial short circuit currents (ISC) of freshly isolated tracheae of Trpm5+/+, Trpm5-/- or Trpm5-DTR mice were recorded using a modified Ussing chamber. BC-depletion in Trpm5-DTR mice was confirmed by whole-mount immunohistochemistry. Changes in [Ca2+]i were studied in HEK293 cells transfected with the taste receptor type 2 (Tas2R) 108 or Tas2R105.

Application of the Tas2R agonist denatonium increased ISC dose-dependently (EC50=397µM) in Trpm5+/+ and Trpm5-/- mice (EC50=40µM). Denatonium (1mM) increased [Ca2+]i in Tas2R108- or Tas2R105-expressing cells. Inhibition of the bitter taste signaling cascade with gallein (G□□ antagonist), U73122 (PLC□□□inhibitor), 2-APB (IP3-recceptor antagonist) and TPPO (Trpm5 inhibitor) significantly decreased the denatonium-effect. In Trpm5-/- and Trpm5-DTR mice, in which BC were depleted by diphtheria toxin, the denatonium-effect was reduced compared to Trpm5+/+ mice. Inhibition of cholinergic signaling with mecamylamine and atropine did not influence the denatonium-effect. Interestingly, application of denatonium led to ATP-release from BC as inhibition of the ATP-release channel pannexin1 (probenecid), ATP-dependent K+-channels (glibenclamide), and ATP-receptors (suramin) decreased the denatonium-effect. Additionally, the ENaC antagonist amiloride reduced the denatonium-effect.

Denatonium evokes ATP-release from BC via the canonical bitter taste signalling cascade. ATP acts on ENaC-mediated transepithelial ion transport. Thus, activation of BC results in a decreased absorption of the airway surface liquid.

Vortrag 27:

Title:
Activation of tracheal brush cells promotes Trpm5-dependent innate immune responses in Pseudomonas aeruginosa infection

Authors:
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Abstract:
Previously, we have shown that transient receptor potential channel M5 (Trpm5) expressing tracheal brush cells (BCs) are cholinergic chemosensors that elicit respiratory reflexes triggered by Pseudomonas aeruginosa quorum-sensing-associated metabolites (QSM). Here, we investigate the impact of Trpm5 function on the disease outcome in P. aeruginosa (PA) pneumonia.

Changes in [Ca2+]i levels in BCs in response to the PA product PQS (3,4-dihydroxy-2-heptylquinoline) were studied on tracheal explants of Trpm5-GCaMP3-mice. Plasma extravasation and neutrophil recruitment was studied 30 minutes after Evans Blue (EB) application followed by inhalation of either PQS or supernatants of various PA strains. An experimental infection model was established using the mucoid NH57388A strain isolated from a cystic fibrosis patient to inoculate Trpm5+/+ and Trpm5-/- mice.

In BCs PQS increased the [Ca2+]i level. Inhalation of either PQS or bacterial supernatants induced a significant increase of EB extravasation and enhanced recruitment of neutrophils to the tracheal extraepithelial space. Interestingly, also supernatants of a QSM-deficient strain (D8A6) led to EB extravasation but this effect was significantly reduced compared to QSM-competent strains (PA103 and NH57388A). Additionally, an increased number of neutrophils was observed in the lung after stimulation with PQS or NH57388A supernatant. Infection with NH57388A led to significantly higher weight loss and mortality in Trpm5-/- than in Trpm5+/+ mice. Lungs of Trpm5-/- mice exhibited more damaged areas compared to healthy lungs.

Our results provide evidence for a BC-dependent activation of neurogenic inflammation in order to rapidly eliminate inhaled pathogens via neutrophil recruitment ameliorating infection outcome.

Vortrag 28:

Title:
Physiological biocompatibility screening of novel absorbable metallic implants ex vivo

Authors:
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Abstract:
Treating large bone defects is still a clinical challenge. From the currently available bone substituting biomaterials only metallic implants are mechanically suitable for fully load-bearing applications in humans. Recent 3D printing technologies now enable additive manufacturing (AM) of personalized porous implants even from absorbable metals, like magnesium (Mg) or zinc (Zn) alloys. These corrode progressively inside the human body to completely dissolve and allow for fully natural bone remodeling. Proper biocompatibility testing of such implants is still challenging, though. Our objective was to develop bioreactor systems to better characterize the biodegradation of AM metal scaffolds under dynamic physiological conditions ex vivo.

We used laser powder bed fused standardized absorbable implants, and identical Ti64 controls, in a custom-built perfusion bioreactor with different cell types to study bioabsorption and cytocompatibility under in vivo mimetic conditions. We used commercially available hardware and kits and a modified ISO 10993 protocol, EDS and XRD, micro-CT, FACS, fluorescence imaging and SEM analyses.

Biocorrosion under physiological dynamic cultures was much faster as compared to standard static in vitro conditions. Cytocompatibility of Ti64 controls was excellent, while metabolic activity of MG-63 and L929 cells on iron was compromised, but significantly increased specifically under dynamic conditions and for L929 cells. This big impact of dynamic culture conditions was also confirmed by direct cytocompatibility assessment.

Our findings suggest that choice of cell type(s) and culture condition(s) considerably influence cytocompatibility of absorbable implants and both should be carefully considered to improve (pre-)clinical success rates of novel implants.

Vortrag 29:

Title:
Some aspects of morphogenesis of the marginal transitional zone of the rat hip joint during the postnatal period.

Authors:
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Abstract:
To conduct a comparative analysis of the marginal transitional zone morphogenesis in relation to the rest (large) part of the joint capsule.

hip joint fragments of Wistar rats were fixed, decalcified and dehydrated. Paraffin-embedded tissue sections were processed with Mallory's and Hart's elastin stains. Cells and components of the extracellular matrix (ECM) of the marginal synovium and synovial subintima of the rest (large) part of the joint capsule were counted by means of the intersection-point counting method using the light microscopy with oil immersion (X100 magnification) and a square lattice graticule in the ocular of microscope. The obtained data were processed using the statistical methods (Student's t-test, Satterthwaite approximation, Greenhouse-Geisser correction).

Formation of the marginal transitional zone in addition to the rest (large) part of the joint capsule from birth to the 90th day was accompanied by a gradual decrease in the number of cells together with a gradual increase in the number of collagen and elastic fibers. In the marginal synovium, the number of cells and vessels was always increased; the number of elastic fibers was always reduced; the number of collagen fibers was increased in newborns and decreased on the 14th, 60th and 90th days; the amount of ground substance was less in newborns and on the 14th day compared to the rest (large) part of the joint capsule.

These phenomena indicate certain morphological differences between the studied parts, emphasizing the anatomical and physiological specificity of the marginal transitional zone.

Vortrag 30:

Title:
Lipocalin 2 modulates local inflammatory processes in the spinal cord and is a possible component of organ cross-talk after spinal cord injury

Authors:
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Abstract:
Lipocalin 2 (LCN2) is an immunomodulator with various biological functions in different organs, including iron transport and defense against bacterial infection. In different murine stroke models, LCN2 was shown to support glial activation and the production of proinflammatory cytokines in the brain. Further, in a spinal cord injury (SCI) mouse model, LCN2-deficiency leads to a decreased expression of proinflammatory cytokines in the spinal cord. In SCI, LCN2 may also have detrimental effects on the functional outcome but the extent of its involvement in SCI is not yet fully known.

Using a SCI contusion mouse model, the expression patterns of LCN2 in different parts of the CNS (spinal cord and brain), blood serum and the liver were investigated by quantitative real-time PCR, Western Blot, immunohistochemical staining and ELISA. Additionally, by including mice with a general LCN2-deficiency (LCN2-/-), the effect of LCN2 on astrogliosis and astrocyte polarization was studied on the gene level.

We found that SCI induces LCN2 production throughout the whole spinal cord, in the brain, liver and in blood serum. This demonstrates that LCN2 is spread via the blood circulation and potentially modulates pathological processes in peripheral organs post SCI. Further, gene expression of the gliosis marker GFAP was significantly reduced in LCN2-/- mice after SCI.

Taken together, first valuable hints are provided, suggesting that LCN2 is involved in the local and systemic effects of SCI. The specific role of LCN2 in the impairment of peripheral organs after injury is the subject of further research.

Vortrag 31:

Title:
The influence of LCN2 on NLRP3 inflammasome after spinal cord injury

Authors:
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Abstract:
Spinal cord injury (SCI) is often caused by traumatic injuries to the spine and leads to massive impairment of life quality. After initial injury and local cell death, SCI is accompanied by neuroinflammation and production of pro-inflammatory cytokines due to inflammasome activation.

The influence of Lipocalin-2 (LCN2) on SCI was studied using a LCN2-/- mouse strain. Traumatic injury was performed with a contusion at T10. After 24 and 72 h, animals were finalized and RNA, miRNA and protein studies were conducted focusing on inflammatory targets.

LCN2 is increased in WT mice with a peak at 24 h on the gene and at 72 h on the protein level. SCI temporally increased the expression of the NLRP3 inflammasome complex including NLRP3, and ASC in WT mice. SCI-induced inflammasome components were significantly reduced in LCN2-/- mice. Similarly, we found mir-223-3p, which is known to directly target NLRP3, reduced expression levels in LCN2-/- mice after 24 h following SCI.

The role of LCN2 is not yet clearly defined and seems to be versatile in the CNS. It conveys tissue protection during inflammation in the brain, whereas in the spinal cord its role appears more detrimental. Our study supports the latter finding, since we observed lower inflammatory responses in LCN2-/- animals.

Vortrag 32:

Title:
Astrocytic lipocalin 2 regulates the blood-brain barrier integrity in MS lesion development

Authors:
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Abstract:
Astrocytes are closely associated with endothelial cells as part of the blood-brain barrier (BBB). Using mouse models for multiple sclerosis (MS), we identified an astrocyte subpopulation in the vicinity of inflammatory lesions characterized by high lipocalin 2 (LCN2) expression. As secreted protein, we hypothesize that astrocytic LCN2 targets LCN2 receptors on endothelial cells. LCN2 appears to stabilize the integrity of the BBB by restoring endothelial permeability under inflammatory conditions which might be involved in the initiation and progression of inflammatory brain lesions.

The combinatory neurodegenerative and autoimmune MS animal model (Cup/EAE) was conducted with wild-type and LCN2-deficient mice. We analyzed the histopathological characteristics regarding myelination and immune cell infiltration. Additionally, we focused on the processes at the BBB using transmission electron microscopy to evaluate LCN2 secretion towards endothelial cells. Further, endothelial cells in vitro were used to investigate endothelial permeability in inflammation and the influence of LCN2.

In the Cup/EAE model, LCN2-deficient mice showed an increased number of inflammatory infiltrates and a severe myelin loss compared to wild-type. LCN2-expressing astrocytes were found in the vicinity of inflamed blood vessels, where endothelial cells perceive LCN2 via cell surface receptors. Endothelial permeability in vitro could be improved by LCN2 treatment.

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

Vortrag 33:

Title:
Single-dose ethanol intoxication causes acute and lasting neuronal changes in the brain

Authors:
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Abstract:
Alcohol intoxication at early ages is a risk factor for development of addictive behavior, therefore it is important to uncover neuronal correlates of acute ethanol intoxication.

We used stable-isotope labeled SILAC mice combined with quantitative mass spectrometry to screen over 2000 synaptic hippocampal proteins. Longitudinal two-photon in vivo imaging in mice was performed to reveal neuronal correlates of single dose ethanol exposure.

72 synaptic proteins changed synaptic abundance up to two-fold after ethanol exposure. Among those were mitochondrial proteins and proteins important for neuronal morphology, including MAP6 and Ankyrin-G. Based on these candidate proteins, we found acute and lasting molecular, cellular, and behavioral changes following a single intoxication in alcohol-naïve mice. Two-photon imaging showed increased synaptic dynamics and mitochondrial trafficking in axons. Immunofluorescence analysis revealed a shortening of axon initial segments. Knockdown of mitochondrial trafficking in dopaminergic neurons abolished conditioned alcohol preference in Drosophila.

This introduces mitochondrial trafficking as a process implicated in reward learning, and highlights the potential of high-resolution proteomics to identify cellular mechanisms relevant for addictive behavior.

Vortrag 34:

Title:
Leaky gut?: Morphological alterations of the intestinal tract in an animal model of anorexia nervosa

Authors:
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Abstract:
Gut barrier dysfunctions due to chronic food restriction may contribute to low-grade inflammation and an increased risk of autoimmune diseases in patients with anorexia nervosa. Using the translational activity-based anorexia model, morphological alterations of the intestinal tract that possibly contribute to a leaky gut were analyzed.

Adolescent female rats were divided into 4 groups: limited food with running-wheel, limited food, controls with and without running-wheel. Food intake was reduced until a body weight reduction of 25% was reached and stabilized for two weeks to imitate chronic starvation. Tissue slices of the intestinal tract were morphologically analyzed. The expression of tight-junction proteins, permeability and inflammatory markers were measured.

The thickness of the gut wall, height of the villi in the small intestine, and depth of the crypts in the colon were decreased in food-deprived rats. The protein expression of the tight-junction protein occludin was decreased in food-restricted rats, while the levels of the gut permeability marker zonulin and the inflammasome NLRC4 were increased compared to controls.

These alterations in the intestinal tract after chronic food restriction could contribute to increased gut permeability. A so-called leaky gut could facilitate microbial components or metabolites passing through the gut wall, thus entering blood circulation and potentially influencing immunologic processes and inflammation.

Vortrag 35:

Title:
Glia cell degeneration in an activity-based anorexia model

Authors:
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Abstract:
Anorexia nervosa (AN) is associated with a disturbed awareness of the own body and brain atrophy. In the past, we have established a novel AN animal model, the so-called activity-based anorexia (ABA) model. This model mimics the core features of the disease including body weight reduction, amenorrhea and hyperactivity. Since brain atrophy is observed in AN patients, we asked in this study whether this neuropathological feature can be mimicked in ABA animals and what cellular changes underlie this atrophy.

To analyze brain volume changes, we performed a longitudinal MRI study and post-mortem histological investigations. Immunohistochemical stainings were used to quantify the density of astrocytes and neurons. Novel object recognition tasks were performed before and after starvation and compared with normally fed controls.

Longitudinal animal MRI and post-mortem brain sections confirmed a reduction in the brain volumes of ABA animals compared to controls. After chronic starvation, we showed that the GFAP positive astrocyte cell density was reduced in the gray and white matter, while the number of neurons was unchanged. After refeeding, the starvation-induced effects were mostly reversible. The recognition memory function after starvation was impaired compared to controls.

As the causes for reductions in astrocyte density are currently unknown, we aim to analyze the underlying pathophysiology of astrocytes in future studies. The investigation of glia cell alterations can identify new targets for the treatment of AN, and thereby contribute to the understanding of pathologic processes in the brain of patients with AN.

Vortrag 36:

Title:
Morphological and functional characterization of a glia limitans perichoroidalis

Authors:
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Abstract:
The choroid plexus is discussed as a site for immune surveillance and an entry route for activated immune cells into the central nervous system under pathological conditions. While most authors suggest that immune cells can travel from the choroid plexus into the ventricular lumen and from there into periventricular areas, a direct invasion from the choroid plexus stroma into the brain parenchyma at its attachment point has, so far, not been experimentally addressed. We hypothesize that peripheral immune cell migration is regulated at this site via glial structures.

For analyzing the attachment site we mapped the morphology of the murine ventricular system and choroid plexus by micro CT imaging and three-dimensional reconstruction. Glial border structures and basal laminae at the attachment point were analyzed by immunohistochemical labeling and transmission electron microscopy. Furthermore, peripheral immune cell recruitment was analyzed in two different mouse models of multiple sclerosis.

The attachment region of the choroid plexus at the roof of the third ventricle in close proximity to the subfornical organ was characterized by the increased expression of astrocytic and microglial marker proteins. Ultrastructural analysis showed complex interlinked structures of astrocytic processes ensheathed by a continuous basal lamina at the attachment regions. Peripheral immune cells, in particular CD3+ lymphocytes, accumulated at these sites but eventually migrated through the glial borders.

From these findings, we deduce the existence of a to date not described glia limitans perichoroidalis that potentially plays an important role in immune cell migration into the brain parenchyma.

Vortrag 37:

Title:
Biomechanical properties of the human dura mater differ in relation to traversing vessels

Authors:
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Abstract:
Lifelike biomechanical properties of the human dura mater are required to realistically simulate the tissue in computational head models. This study aimed to determine whether the biomechanical properties of vascular and avascular areas of the human dura mater differ and how this relates to morphological characteristics of the dura mater.

A total of 324 sub-samples were subjected to quasi-static uniaxial tensile tests. The subsamples were grouped into three groups: i) avascular, ii) transverse stretching of the sample related to the vessels and iii) longitudinal stretching of the sample related to the vessel. Additionally, 36 dura mater samples were histologically analysed using the Van Gieson's stain.

The elastic modulus and ultimate tensile strength of the transverse group (106 +/- 7 MPa; 10 +/- 1 MPa; median +/- standard error) is significantly lower compared to the longitudinal (142 +/- 9 MPa; 14 +/- 1 MPa; $p < 0.001$) and avascular group (127 +/- 11 MPa, $p = 0.041$; 15 +/- 1 MPa; $p < 0.001$). The meningeal layer is significantly thicker than the periosteal layer around vessels ($p \leq 0.019$) but dura samples were non-different in thickness between the tested groups ($p \geq 0.071$).

The elasticity and strength of the dura correlate with the course of traversing vessels and likely depend on collagen orientation rather than thickness.

Vortrag 38:

Title:
The central aspect of the human hip capsule likely has a minimal role in hip neuromechanics

Authors:
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Abstract:
The incidences of hip joint osteoarthritis and total hip arthroplasty (THA) have increased across the world in recent years. Many replacements require revision within the first year, with dislocation being one of the most common reasons. In order to reduce dislocation rates, THA with capsular repair (CR) has evolved. One hypothesis presented in the literature of its success is that CR allows for the preservation of mechanoreceptors within the hip capsular complex (HCC). These, however, have not been extensively mapped or studied with immunohistochemical techniques, to date. This study aims to determine if mechanoreceptors are present across the HCC and if regional or demographic differences in mechanoreceptor density exist.

33 anterior, medial and lateral operative zones of the human HCC were obtained from 29 cadavers with an age range of 2-83 years (43.8 ± 23.8; mean ± standard deviation, post-mortem interval ≤ 139 hours). Consecutive sections from each HCC region were histologically stained with hematoxylin and eosin, and immunohistochemically stained with anti-S100, anti-protein gene product 9.5, anti-neurofilament, and anti-von Willebrand factor antibodies. Sections were assessed quantitatively.

2,673 sets of five consecutive sections from 33 HCCs were assessed. These represented 160µm each. Preliminary analysis showed no type I-III mechanoreceptors in any of the samples. No regional and demographic differences were present.

The HCC appears to likely play no relevant role in proprioception alone. This indicates that other factors, such as surrounding tissue preservation, restoration of HCC biomechanical properties and atmospheric pressure may contribute to the success of CR during THA.

Vortrag 39:

Title:
Determining a nerve-free zone for surgical exposures to the anterior glenoid

Authors -->
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Abstract:
The aim of this study was to anatomically describe a nerve free zone in which a split can safely be performed.

In 85 cadaveric specimen the brachial plexus and its branches were dissected. Distance from the myotendinous junction to the entrance points of the subscapular nerves was measured with the arm abducted 60° and rotated externally in 84 specimens. Thereof, in 16 specimens further measurements were taken in neutral and internal rotation as well as 90° abduction and external rotation. Sides, sexes and joint positions were compared using ANOVA with statistical significance set at $p \leq 0.05$.

In 88.2% two distinct subscapular nerves were observed. In 11.8% an accessory nerve could be observed. The average distance was 42.9 +- 7.5 mm for the upper subscapular nerve and 37.9 +- 7.9 mm for the lower subscapular nerve with the arm rotated externally and abducted 60°. No side difference was observed. Sex difference was only observed for the lower subscapular nerve. Comparing the different positions, it showed that internal rotation decreased the distance to 33.4 +- 6.8 mm and 30.5 +- 7.4 mm while external rotation increased it to up to 48.1 +- 6.2 mm and 41.5 +- 6.7 mm in maximum external rotation and 90° abduction.

The subscapular nerves insert into the muscle in proximity of the surgical field when approaching the glenoid from anterior. A safe zone of 2 cm medial to the myotendinous junction with the arm rotated externally, seems to provide sufficient protection from nerve injury.

Vortrag 40:

Title:
Computed tomography osteoabsorptiometry-based investigation on subchondral bone plate alterations in sacroiliac joint dysfunction

Authors: -->
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Abstract:
Sacroiliac joint dysfunction (SIJD) is an underappreciated source of back pain. Mineralization patterns of the sacroiliac (SIJ) subchondral bone plate (SCB) may reflect long-term adaptations to the loading of the joint.

Mineralization densitograms of 27 SIJD patients and 39 controls, were obtained using CT osteoabsorptiometry. Hounsfield unit (HU) values of the SCB mineralization of superior, anterior and inferior regions on the iliac and sacral auricular surfaces were derived and statistically compared between SIJD-affected and control cohorts.

Healthy controls showed higher HU values in the iliac; 868 ± 211 (superior), 825 ± 121 (anterior), 509 ± 114 (inferior), than in the sacral side; 541 ± 136 (superior), 618 ± 159 (anterior), 447 ± 91 (inferior), of all regions ($p < 0.01$). This was similar in SIJD; ilium 908 ± 170 (superior), 799 ± 166 (anterior), 560 ± 135 (inferior), sacrum 518 ± 150 (superior), 667 ± 151 (anterior), 524 ± 94 (inferior). In SIJD, no significant HU differences were found when comparing inferior sacral and iliac regions. Furthermore, HU values in the inferior sacral region were significantly higher when compared to the same region of the healthy controls (524 ± 94 vs. 447 ± 91, $p < 0.01$). Region mineralization correlated negatively with age ($p < 0.01$).

SIJD-affected joints reflect a high mineralization of the sacral inferior region, suggesting increased SIJD-related mechanical stresses. Age-related SCB demineralization is present in all individuals, regardless of dysfunction.

Vortrag 41:

Title:
Hermann Stieve distorted biographical information in his published case descriptions to partly conceal the circumstances of his tissue acquisition from Plötzensee execution victims

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Abstract:
Berlin anatomist Hermann Stieve (1886-1952) focused his research on the female reproductive system. His "research material" included the bodies of execution victims from Berlin Plötzensee prison. We examine case descriptions from Stieve's publications to compare them with historically verifiable information.

Stieve's publications of two exemplary periods (1942-1943 and 1950-1952) and 990 histological drawings from the estate of Stieve's son Friedrich-Ernst were analysed. Inscriptions on the reverse of the drawings were transcribed and 45 individual names could be identified. These names were followed up using published and archival sources to challenge biographical details published by Stieve.

A total of 181 cases were identified, of which 101 cases could be linked to 39 historical individuals. In 8 cases (9%) from 1942-1943 and in 24 cases (25%) from 1950-1952, Stieve's case descriptions depart substantially from the historically verifiable biographical information. This concerns, among others, the causes of death (e.g. death by air raid instead of capital punishment) or the reason for the conviction of the imprisoned women (e.g. murder instead of plundering). Clinical information given in the case descriptions, like information on the menstrual cycle, is more difficult to verify historically.

In biographical information given in published case descriptions, Stieve often departed from the historical truth. His main motivation will have been to cover up the true circumstances of the acquisition of researched tissues and to defend himself against the post-war accusation of using "political" victims. How much his misinformation affected his scientific conclusions is more difficult to judge.

Vortrag 42:

Title:
The use of bodies of the executed for histological research - an ongoing practice in Berlin after 1945

Authors:
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Abstract:
It is well known that during the Nazi period, Berlin anatomist Hermann Stieve (1886-1952) used the bodies of execution victims, including resistance fighters, for his research. His ongoing use of this source of "research material" after 1945, however, has been largely neglected.

This analysis is based on well-known and on newly surfaced historical sources, partly provided by Stieve's grandchildren.

Until the abolishment of capital punishment in West Germany and West Berlin in 1949, 11 men and 2 women were executed in the Western sectors of Berlin. Of these, 4 appear in the body register ("Leichenbuch") of the Anatomical Institute headed by Stieve. In at least 4 additional cases, Stieve was allowed to dissect the body on the premises of the execution site. While in most cases, the offence was "common" murder, the 2 women were sentenced to death by a Berlin court in 1946 for involvement in "child euthanasia" in a psychiatric institution. The exact number of executions in the Soviet sector of Berlin is unclear. Only 3 executed men from East Germany appear in the body register in 1950. As with cases before 1945, research based on organs from these individuals was openly published by Hermann Stieve and others from his institute.

The allied authorities did not see research on tissues of executed individuals as something Nazi-specific and allowed the practice to continue after 1945. Strangely, Stieve benefitted not only from the death of Nazi victims, but also from the death of Nazi perpetrators.

Vortrag 43:

Title:
Transforming the anatomy lab into a creative space

Authors:
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Abstract:
Our aim is to creat opportunities for medical students to learn in a supportive and creative way, in an effort to develop kinaesthetic technics, team work, communication, improve exam and presentation skills, palpation and empathy - all skills that are transferable to their future careers.

Arts-based pedagogic approaches such as body painting, drawing and sculpting, 3D modelling, chain-linking, storytelling, palpation and meditation/hypnosis were introduced to medical students in the 2nd and 4th semesters at the University of Leipzig through an elective. A bilingual (German /English) elective running over 12 weeks alongside the scheduled second semester dissection course, 30 hours, 18 students, 2 tutors and 2 teaching staff, an artist/MD/hypnotherapist and a British osteopath/researcher. Questionnaires were used for evaluation.

Using the different art mediums brought an alternative method to learning difficult subjects and reducing fear of these topics. The students overall reported improved learning together, sharing the methods used in class with other peer groups. Meditation at the beginning of the lesson brought relaxation and focus during the lecture, which resulted in effective learning and improvements in oral examinations. The students also reported the use of a bilingual programme to be very beneficial and that overall the course made them step out of their comfort zones.

Kinaesthetic-tactile learning combined with visual and auditory study techniques produces a powerful multi-sensory learning technique, promoting knowledge retention. It’s exciting and fun, and brings a new perspective to a student’s engagement in their anatomical studies as well as improving their well-being.

Vortrag 44:

Title:
Generation Z in Medicine: No longer just a Boys Club

Authors:
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Abstract:
We do not know enough about the choice and motivation towards certain specialties within generation Z to counteract the shortage of recruits in different specialties in the future. Therefore, we aimed to survey the choice in speciality in medical students dedicated to generation Z and sought to identify gender differences that influence this life-changing decision.

We invited 384 students from one semester to complete a six-item questionnaire. The anonymized questionnaire included, among others, the following items:
Item 4 “If you were to start work tomorrow, what specialty would you choose?” comprised of single choice for all 50 medical specialties accredited by the Austrian Medical Chamber. Item 5 “What speaks FOR your chosen specialty?” inquired with 31 short statements (14 with a positive annotation, 14 with a negative annotation) as multiple-choice the influencing factors for the specialty chosen in item 4.
Item 6 “What speaks AGAINST your chosen specialty?” used the same multiple-choice options as item 5. Statistically significant differences in individual responses between genders were tested using a chi-squared test ($p < 0.05$).

There were significant gender differences in the choice of specialty (especially gynecology and obstetrics, pediatrics). Among others, women cited "Lots of patient contact" and "Lots of patient communication," while men cited "Good financial income" significantly more often as reasons to choose a specialty.

There are significant gender differences in the choice of and the reasons for choosing a medical specialty. The results of our study guide disciplines with staff shortages to make them more gender-specific attractive.

Vortrag 45:

Title:
Desmoglein 1 is a signaling hub controlling keratinocyte Ca2+ influx in pemphigus

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Abstract:
Objectives: Characterization of the Ca2+ flux pathway and delineate its importance for pemphigus pathogenesis and clinical phenotypes caused by different antibody profiles.

Methods: Immunoprecipitation, Ca2+ flux analysis, Western-blotting, Immunofluorescence staining, dissociation assays, human skin ex vivo model.

Results: PV-IgG and PF-IgG but neither DSG3-specific monoclonal antibody (AK23) nor mPV-IgG caused Ca2+ influx in primary human keratinocytes. Phosphatidyl inositol 4 kinase-α (PI4K) interacts with DSG1 but not DSG3. Its downstream target Phospholipase-C-γ1 (PLC) was activated by PV-IgG and PF-IgG but not AK23 nor mPV-IgG. PLC releases Inositol-1,4,5-trisphosphate (IP3) causing IP3-receptor (IP3R) activation and Ca2+ flux from the endoplasmic reticulum into the cytosol, which stimulates Ca2+-release-activated-channels (CRAC)-mediated Ca2+ influx. Inhibitors against PLC, IP3R and CRAC effectively blocked PV-IgG and PF-IgG-induced Ca2+ influx, ameliorated alterations of DSG1 and DSG3 localization, reorganization of keratin and actin filaments and inhibited loss of cell adhesion in vitro. Finally, inhibiting PLC or IP3R was protective against PV-IgG-induced blister formation and redistribution of DSG1 and DSG3 in human skin ex vivo.

Conclusions: Ca2+-mediated signalling is important for epidermal blistering and dependent on the autoantibody profile, which indicates different roles for signalling complexes organized by DSG1 and DSG3. Interfering with PLC and Ca2+ signalling may be a promising approach to treat epidermal manifestations of pemphigus.

Vortrag 46:

Title:
Desmoglein 2 is upregulated in pemphigus and can undergo Ca 2+-dependent interactions with both desmosomal and classical cadherins including E-cadherin and N-cadherin

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Abstract:
Desmoglein (Dsg) 2 is a desmosomal cadherin present in all cell types forming desmosomes including epithelial cells and cardiomyocytes. In lesions of patients suffering from pemphigus vulgaris, an autoimmune blistering skin disease caused by autoantibodies directed against Dsg1 and Dsg3, Dsg2 is upregulated. Thus, we here characterized and investigated the impact of homo- and heterophilic Dsg2 interactions.

Ex-vivo pemphigus model, immunofluorescence, AFM

We observed that Dsg2 interacts with Dsg3, which may be a compensatory mechanism to preserve keratinocyte adhesion. Interestingly, besides homophilic Dsg2-Dsg2 and heterophilic interactions to the Dsg3 and Dsc2, heterophilic interactions with the classical cadherins E-cadherin and N-cadherin were detectable which may be relevant for cross-talk between desmosomes and adherens junctions in epithelia and cardiomyocytes. All interaction types were Ca 2+-dependent and show comparable binding forces and bond lifetimes. We used tryptophan, which is known to be critical for cadherin trans-interaction, and a tandem-peptide (TP) designed to cross-link Dsg isoforms to further characterize the mode of interactions. TP prevented tryptophan-induced loss of Dsg2 interaction with the desmosomal cadherins Dsg2 and Dsc2 but not with E-cadherin and N-cadherin indicating that the interaction mode of Dsg2 with desmosomal and classical cadherins differs. On living enterocytes, TP also rescued tryptophan-induced loss of Dsg2 binding suggesting that interaction with desmosomal cadherins may be more relevant.

Taken together, the data demonstrate that the ubiquitous desmosomal cadherin Dsg2 can accomplish cross-talk with adherens junctions by interacting with multiple binding partners and is upregulated in diseases to rescue cell adhesion such as in pemphigus.

Vortrag 47:

Title:
Apremilast is protective against pemphigus autoantibodies in human skin

Authors:
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Abstract:
In the bullous autoimmune 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. At present, therapy is focused 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 analyze the phosphodiesterase 4 inhibitor Apremilast, a drug already used to ameliorate other skin diseases like psoriasis, for its effectiveness in PV.

Atomic force microscopy (AFM), cAMP ELISA, electron microscopy, ex-vivo pemphigus skin model, immunostaining, Keratinocyte dissociation assay, Western blot

Apremilast abrogated PV-IgG-induced acantholysis in human epidermis ex-vivo and loss of intercellular adhesion in keratinocytes. This was paralleled by inhibition of keratin filament retraction and desmosome splitting but affected neither desmoglein (Dsg) depletion nor Dsg3 single molecule binding properties. cAMP stabilizes cardiomyocyte adhesion via phosphorylation of plakoglobin (Pg) at S665. Thus, we analyzed skin samples and keratinocytes for Pg phosphorylation and established a keratinocyte cell line of a Pg-S665-phosphodeficient mouse model (PgS655A). Apremilast treatment induced Pg phosphorylation and PgS665A keratinocytes showed drastically altered organization of desmosomal proteins and keratin filaments and impaired cell cohesion.

These data demonstrate that Apremilast is effective to stabilize keratinocyte adhesion in human skin at least in part via Pg-S655 phosphorylation that is required for keratin filament organization. Thus, Apremilast may serve as treatment option during the acute phase in pemphigus.

Vortrag 48:

Title:
The regulatory effect of ALDH1a1 and ALDH1a3 paralogues in skeletal muscle differentiation

Authors:
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Abstract:
Skeletal muscle is a terminally differentiated organ system with satellite cells (SC), the stem cells, as its sole source of regenerative processes. The enzymatic function of Aldehyde Dehydrogenase 1 (ALDH1) correlates with myogenic properties of SCs and its paralogues ALDH1A1 and ALDH1A3 are co-localized in SCs of human skeletal muscle. ALDH1 is not only associated with retinoic acid signaling and differentiation, but also with cell-maintenance against oxidative stress products. However, the molecular mechanism of ALDH1 in SC activation and regulation of myogenesis has not yet been characterized.

ALDH1 paralogues 1A1 and 1A3 were investigated in myogenic human RH30 and murine C2C12 myoblast cell lines using Western Blot, Immunofluorescence and Aldefluor Assay. Both paralogues were either separately knocked out using CRISPR-Cas9 technique or recombinantly overexpressed using plasmidvectors. In addition, manipulation of retinoic acid pathway was performed by agonist and antagonist of retinoic acid receptors.

We show, that isoforms ALDH1A1 and ALDH1A3 are pivotal factors in the process of myogenic differentiation, since ALDH1A1 and ALDH1A3 knock-out, respectively, impaired differentiation potential. Recombinant re-expression of ALDH1A1 and ALDH1A3, respectively, in corresponding ALDH1-isoform knock-out cells recovered their differentiation potential. Interestingly, the chemical inhibition of enzymatic activity by disulfiram leads to ALDH1A1 and ALDH1A3 protein upregulation and subsequent myogenic differentiation. Chemical manipulation of retinoic acid receptors in ALDH1 paralogues knock out cell lines induced differentiative behavior and proves ALDH1-retinoic acid regulation.

These findings show that ALDH1A1 and ALDH1A3 proteins are pivotal factors for skeletal muscle cell differentiation and depict potentially essential activators and regulators of SCs.

Vortrag 49:

Title:
Adrenergic signaling modulates tenocyte intercellular contacts and collagen synthesis

Authors:
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Abstract:
Tenocytes synthesize extracellular matrix proteins in tendons, connective tissues forming the interface of muscles and bones. Although tendon injuries are a common clinical entity, little is known about the regulation of tenocyte function via their intercellular contacts. Particularly, the impact of adrenergic signaling on tenocyte regulation is largely unknown. Thus, we analyzed cAMP-dependent changes of tenocyte intercellular contacts, which were accompanied by modulation of collagen synthesis.

Immunofluorescence, atomic force microscopy (AFM), ELISA, scanning electron microscopy

Cultured human tenocytes express gap and adherens junction proteins but neither desmosomal nor tight junction molecules. Interestingly, they show broad laminar and small finger-like intercellular contacts with distinct N-Cadherin single molecule binding events as revealed by SEM or AFM, respectively. Moreover, Cx43 as well as N-Cadherin and ZO-1 are redistributed along these intercellular contacts when cAMP is elevated. Under these conditions, Cx43 phosphorylation at Ser368 is increased, indicating that gap junction function is modulated upon cAMP elevation. Similar changes were present in a human iliotibial tract slice model when specimen were incubated with cAMP-increasing mediators. Elevated cAMP levels affected N-Cadherin single molecule binding properties on tenocytes and decreased Young’s modulus in human iliotibial tract samples suggesting that cAMP signaling impacts biomechanical properties of tendons. In accordance, we detected impaired collagen 1 production in human tenocyte cell culture.

Taken together, these data indicate that adrenergic signaling modulates intercellular contacts of tenocytes, and alters the biomechanical properties of tendons by impaired collagen 1 synthesis.

Vortrag 50:

Title:
Impact of melanocortin receptor ligands on lipid accumulation in meibocytes

Authors:
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Abstract:
Meibomian gland dysfunction (MGD) is the main cause of dry eye disease and is characterized by terminal duct obstruction and/or alterations in the glandular secretion. Since meibocytes and sebocytes share great similarities and ligands of the melanocortin receptor (MCR) family influence sebocyte differentiation and sebum production, the expression of MCRs and their effects on meibum secretion and meibocyte differentiation should be investigated.

RT-PCR and immunohistochemistry were performed to verify MCR expression in human meibomian glands (hMG) and an immortalized human meibomian gland epithelial cell line (hMGECs). Additionally, hMGECs were stimulated with α-/β-melanocyte-stimulating hormone (α/β-MSH), selective MCR ligands, as well as with HS024, an MCR antagonist. The effect of α/β-MSH on lipid production was quantified by Oil Red O staining. The effects on the expression of MC5R and stearoyl-CoA desaturase (SCD), a central enzyme in lipid synthesis, were assessed by qRT-PCR and Western blot.

hMGs and (un-)differentiated hMGECs express MC1R and MC5R at the protein level and all five MCRs at the transcript level. Stimulation with α/β-MSH induces lipid production 1.83±0.05 -fold ($p<0.001$) in hMGECs and increase the expression rate of SCD 2.11±0.23 -fold ($p=0.035$), while HS024 inhibits MC5R expression 14.32±0.19 -fold ($p=0.001$) in hMGECs.

Our data show that human meibocytes and hMGECs express MCRs and that stimulation/inhibition of MCRs alter MC5R expression, meibocyte maturation and thus meibum genesis. It is therefore likely that melanocortins positively influence meibum production and should be investigated with regard to changes in the glandular secretion of MGD and possible treatments.

Vortrag 51:

Title:
Autoantibodies against intercalated disc proteins are frequent in Arrhythmogenic cardiomyopathy and are pathogenic in few patients

Authors:
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Abstract:
Arrhythmogenic Cardiomyopathy (AC) is a severe heart disease-causing sudden cardiac death in young individuals and in about half of the AC patients, mutations in desmosomal components of an intercalated disc are detectable. Recently, antibodies against the desmosomal cadherin desmoglein (Dsg) 2 and intercalated disc proteins were reported in AC patients but their role in AC pathogenesis is not completely understood. Therefore, in this study, we focused on whether there are autoantibodies in AC, if so are they pathogenic?

We recruited 14 AC patients, 1 healthy relative (HR), and a borderline (BL) AC patient from Germany. IgG fractions were purified. Dissociation assays, immunostainings, transient transfections, Western blots, Triton X-100 assays, utilizing either HL-1 cells or DLD1 cells, or human heart left ventricle, were performed.

Immunostainings revealed all 14 patients harboring pathogenic mutations in DP or Pkp2 genes, HR and BL have auto-antibodies targeting intercalated disc proteins but immunostainings in human Dsg2 transfected cells did not reveal the presence of autoantibodies against DSG2. Western blots utilizing human Dsg2-Fc protein as bait revealed false positives in the healthy IgG controls along with AC patients. Interestingly, dissociation assays revealed that only 5 out of 14 AC patient IgGs caused loss of cardiomyocyte adhesion, and among the six only 3 patient IgGs induced p38MAPK activation

Taken together, the data demonstrate that autoantibodies targeting intercalated disc proteins, but not against Dsg2, were common in AC patients. However, only some autoantibodies cause loss of cardiomyocyte adhesion and activation of p38MAPK and thus are pathogenic.

Vortrag 52:

Title:
The desmoglein-2 adhesive interface is essential for cardiac integrity: identifying mechanisms causing Arrhythmogenic Cardiomyopathy

Authors:
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Abstract:
Arrhythmogenic Cardiomyopathy (ACM) is characterized by fibrosis, ventricular dysfunction and arrhythmias. ACM is mainly caused by mutations in genes of the desmosomal cell-cell adhesion complex. Even though the pathological phenotype is known, the underlying mechanisms are not well understood. Here, we tested the hypothesis that impaired adhesive function of desmosomes is central for disease development and progression.

We mutated the binding site of desmoglein-2, a crucial desmosomal adhesion molecule in cardiomyocytes. Based on structural data, the exchange of tryptophan-2 to alanin (DSG2-W2A) abrogates a central binding mechanism of DSG2. Impaired adhesive function of DSG2-W2A was confirmed by cell-free and cell-based binding assays. To address the loss of cardiomyocyte adhesion in vivo, we generated a CRISPR/Cas9-based knock-in mouse model, which was characterized by echocardiography, histological and immunostaining, Western blot analysis, transmission electron microscopy, and cell-cell adhesion assays.

We mutated the binding site of desmoglein-2, a crucial desmosomal adhesion molecule in cardiomyocytes. Based on structural data, the exchange of tryptophan-2 to alanin (DSG2-W2A) abrogates a central binding mechanism of DSG2. Impaired adhesive function of DSG2-W2A was confirmed by cell-free and cell-based binding assays. To address the loss of cardiomyocyte adhesion in vivo, we generated a CRISPR/Cas9-based knock-in mouse model, which was characterized by echocardiography, histological and immunostaining, Western blot analysis, transmission electron microscopy, and cell-cell adhesion assays.

In summary, the adhesion-deficient DSG2-W2A mouse model fulfils the criteria to establish an ACM diagnosis. This shows that disruption of desmosomal adhesion is sufficient for ACM development. Further, the Dsg2-W2A mouse line represents a valuable model to study mechanisms of the disease and to identify novel treatments for ACM.

Vortrag 53:

Title:
TNF-α mediated adherence junction disassembly induces β-catenin-dependent CEACAM1 transcription in endothelial cells

Authors:
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Abstract:
Age is an independent risk factor for cardiovascular diseases like myocardial infarction and stroke. We previously showed increased endothelial expression of carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1) with progressive age that contributes to age-dependent pro-inflammatory signaling, endothelial barrier dysfunction and vascular fibrosis. Since our results suggested a critical role of TNF-α in these processes, we aimed to analyze the underling mechanisms.

The study was conducted using the human endothelial cell line EA.hy926. We employed pharmacological inhibition and analyzed the phosphorylation status of signaling molecules to dissect the mechanisms of TNF-α-induced CEACAM1 expression. Immunoprecipitation was used to study alterations in protein interaction, i.e. TNF-α-mediated adherens junction disassembly.

Our analyses revealed that TNF-α-dependent CEACAM1 expression is sensitive to pharmacological inhibition of β-catenin signaling (IWR-1, PNU74654). On the mRNA level, CEACAM1 is induced by TNF-α even when protein translation is inhibited by cycloheximide. TNF-α induces Src kinase activity with subsequent phosphorylation of adherens junction proteins and disassembly of these cell-cell contacts. Src kinase inhibition (SU6656) also attenuated TNF-α-dependent CEACAM1 expression.

TNF-α induces the release of β-catenin from adherens junction complexes via Src kinase-dependent phosphorylation. β-catenin then translocates into the nucleus and promotes expression of CEACAM1 in a TCF/LEF-dependent manner. Our results demonstrate a novel mechanism of TNF-α-induced CEACAM1 expression in endothelial cells. Since CEACAM1 in turn contributes to age-related TNF-α expression and TNF-α-mediated endothelial barrier breakdown, this mechanism might lead to a feedforward loop, which favors the transition from physiological aging to age-related vascular pathologies like atherosclerosis.

Vortrag 54:

Title:
The role of peroxisomes in Barth syndrome

Authors:
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Abstract:
Barth syndrome (BTHS) is a rare, X-linked inherited disease associated with cardiomyopathy and heart failure in the first years of life. It is caused by a loss-of-function mutation in the TAZ gene encoding the enzyme tafazzin. Tafazzin catalyses the last steps in cardiolipin (CL) synthesis, which is an essential phospholipid in the inner mitochondrial membrane for the formation of correct mitochondrial structures. TAZ mutation leads to a loss of CL and severe mitochondrial alterations like ROS-overproduction, oxidative stress, and altered lipid metabolism. Peroxisomes are ubiquitous organelles involved in several metabolic pathways, namely ROS-defence and lipid metabolism. The importance of peroxisomes in Barth syndrome is poorly understood. Therefore, our aim is to characterize the role of peroxisomes and their associated signaling mechanisms in response to mitochondrial dysfunction.

We used a well-established BTHS mouse model. Hearts of WT and TAZ knockdown were subjected to analyses of their peroxisomal compartments by using immunofluorescence, electron microscopy, qPCR and western blotting.

Our results show that the number of peroxisomes is increased in TAZ hearts before the development of the disease. Furthermore, upregulations of peroxisomal catalase and plasmalogen synthesizing enzymes (GNPAT and FAR1) were observed in TAZ hearts, maybe compensating for plasmalogen decrease in BTHS. However, peroxisomal number is unaltered in the disease state.

This suggests an important role of peroxisomes in the pathogenesis of BTHS-associated heart failure. As there is currently no cure for BTHS, this study addresses an unmet medical need for a therapeutic treatment plan that utilizes the compensatory metabolic potential of peroxisomes.

Vortrag 55:

Title:
How to starve astrocytes – A functional characterization of long-term starved astrocytes

Authors:
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Abstract:
Astrocytes in the brain are crucial to ensure the metabolic supply of neurons and are involved in neuroinflammatory reactions. Brain and astrocyte function are known to be impaired in diseases correlated with malnutrition, for example obesity. The eating disorder anorexia nervosa is associated with severe undernutrition as well as volume reductions of the cerebral cortex. This brain volume loss is probably caused by reduced astrocyte proliferation and cell count. While influence of overnutrition on astrocytes and their function was confirmed, a role of these cells in long-term undernutrition remains unclear.

Primary rat cerebral cortex astrocytes were used to investigate their response to chronic glucose semi-starvation. Glucose concentration in cell medium was reduced to 2mM for 15 days. Gene and protein levels were measured to characterize those astrocytes under chronic glucose semi-starvation.

A subset of pro-inflammatory genes was increased after long-term glucose starvation. The shift of primary astrocyte population to the pro-inflammatory A1-like phenotype and an altered morphology point towards an increased reactivity. Moreover, expression of genes and proteins involved in the unfolded protein response were elevated. Regulation of several miRNAs and of genes associated with the miRNA biosynthesis point towards a modulation of these stress responses by miRNAs in semi-starved astrocytes.

The results demonstrate that astrocytes respond to chronic glucose semi-starvation with multiple stress reactions. Astrocytes fulfill multiple functions in the brain. Therefore, a connection between elevated inflammatory responses as well as morphological and functional alterations found in the brain of patients suffering from undernutrition is conceivable.

Vortrag 56:

Title:
Spheroidal osteoblast progenitor cell culture for physiological biomineralization

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Abstract:
Our increasingly elderly population suffers from age-related diseases which, among others, affect the skeletal system. Drug development strategies targeting bone health rely mainly on 2D in vitro screenings but 3D-cell culture technologies more closely resembling the in vivo cell environment would accommodate much better precision to this end. The objective of this study was thus to develop a scaffold-free progenitor cell-based mineralization model for high-throughput screenings.

MC3T3-E1 pre-osteoblasts (250k) were seeded into concave non-adherent wells and centrifuged (300xg, 8min) to form uniformly sized spheroids, which were cultured for up to 28 days in medium with and without osteogenic supplements. Differentiation and mineralization stages were weekly examined by RT-qPCR from peqGold TriFAST RNA, measuring alkaline phosphatase (ALP) activity, histochemistry (Alizarin Red S/von Kossa staining), and micro-CT analyses (Imalytics software).

Under mineralization inducing conditions, geNorm-corrected expression of selected osteoblast differentiation markers (Alp, collagen type I, osteocalcin) was strongly upregulated from day 14 on, as was Alp activity and subsequent mineralization (Alizarin Red S, von Kossa staining) of the spheroid core. Mineralization dynamics was further quantified by micro-CT imaging. In contrast, expression of mineralization inhibitor osteopontin decreased over the culture period.

We established a versatile 3D culture model for physiological biomineralization that, to our mind, holds tremendous potential for improved manipulations, and thus understanding, of bone mineralization. Ongoing studies are characterizing in how far it specifically recapitulates intramembranous ossification in vitro and elucidate the role of osteopontin, the function of which in bone formation is still largely unknown.

Vortrag 57:

Title:
Bordetella pseudohinzii: a mouse specific pathogen as a new model to study respiratory infection

Authors:
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Abstract:
Bordetella pseudohinzii colonizes the respiratory tract and subsequently leads to histopathological changes, e.g. BALT (bronchus associated lymphoid tissue)-formation, interstitial pneumonia and infiltration of immune cells into the lungs. Current murine airway infection models use human specific pathogens which are highly adapted to their natural host. We here used B. pseudohinzii to colonize the respiratory tract of mice.

C57BL/6J mice were infected intranasally with 1100 CFU of B. pseudohinzii. Animals were sacrificed after 7 and 14 days. CFU in trachea, lung and blood were analyzed. H&E and immunostainings (B220: B-cells, CD3: T-cells) were performed for histopathological validation and classification of BALT-formation.

Administration of B. pseudohinzii resulted in high bacterial counts in trachea and lung after 7 days and 14 days (trachea: 10_6 CFU/100 mg; lung: 10_5-6 CFU/100 mg). Infiltration of immune cells into the tracheal epithelium and clustering of immune cells within the lamina propria could be observed at both time points, but to a greater degree after 14 days. Within the lung, signs of an acute pneumonia were present after 7 days which were pronounced after 14 days. After 7 days, beginning of BALT-formation indicated by infiltration of B- and T-cells could be observed. After 14 days, 60 % of the infected animals showed organized BALT with distinct B- and T-cell zones.

B. pseudohinzii allows experimental persistant infection of mice. Histopathological changes in the airways and lungs already occurred after 7 days. B. pseudohinzii represents a new model of a mouse specific pathogen to study airway infections and BALT-formation.

Vortrag 58:

Title:
Local anesthetics reduce tumor viability in tissue cultures of non-small cell lung cancer

Authors:
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Abstract:
Non-small cell lung cancer (NSCLC) remains the leading cause of cancer-related death, despite significant improvements in chemotherapy and surgery. The majority of patients dies as a result of metastasis. Several retrospective studies have shown a potential benefit regarding recurrence-free survival in patients who had received local anesthetics during the perioperative period. This observation might be due to the immune-modulatory function of these drugs, which could be shown in malignant cells in vitro as well.

Patient samples of NSCLC resections are cultured ex vivo and are treated with local anesthetics lidocaine (LID) and ropivacaine (ROP, 1 and 10µM, respectively) in absence or presence of cisplatin (3µM). After 72 h of treatment, NSCLC tissue cultures (TC) are processed and analyzed using immunohistochemistry, immunofluorescence, Western blot and qPCR. To focus on key players during the pathogenesis of metastasis we measure tumor proliferation (Ki67) and tumor apoptosis (cPARP) as well as Phosphoinositide-3-kinase, Intercellular-Adhesion-Molecule-1 (ICAM-1), caveolin-1 and the phenotype of tumor-associated macrophages (TAM) in TC.

TC of NSCLC responded dose-dependent to ROP but not to LID in individual specimens with decreased tumor proliferation. The expression of ICAM-1 was reduced upon supplementation of LID and ROP, independent of cisplatin administration. Investigating TAM, alterations in the expression of the CD163 macrophage marker are observed.

Local anesthetics might have the ability to influence tumor cells and their microenvironment ex vivo towards a less metastatic potential. If our findings can confirm previous cell culture studies, this promising effect might be crucial for clinical implementation in onco-anesthesiology.

Vortrag 59:

Title:
TRPM5 channel modulates immune response to Pseudomonas aeruginosa infection

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Abstract:
In the lower respiratory tract, the transient receptor potential subfamily melastatin 5 (TRPM5) channel is expressed only in specialized epithelial cell type called brush cells (BCs). We, and others, have recently observed that the detection of bacterial signaling molecules by BCs can enhance the mucociliary clearance. Here, we address the hypothesis that BCs could modulate the acute immune response against P. aeruginosa infections through TRPM5 channel activation.

Trpm5-/- and Trpm5+/+ mice were infected intratracheally with P. aeruginosa NH57388A, a stable mucoid cystic fibrosis isolate. The mice were sacrificed 4 hours post infection or sham and the immune cells recruitment was studied in bronchoalveolar lavages (BAL), tracheae, and lungs of Trpm5-/- and Trpm5+/+ mice . Blood serum and BAL cytokines were analyzed using ELISA.

The counts of neutrophils, monocytes and natural killer cells were significantly increased in BAL, tracheae and lung of infected Trpm5+/+ mice compared to non-infected Trpm5+/+. This increase was not observed in Trpm5-/- mice. Additionally, serum cytokines analysis revealed high concentrations of cytokines important for neutrophils recruitment and survival, IL-1α, IL-6, KC, G-CSF and MCP-1, in Trpm5+/+ mice but not in Trpm5-/- mice. Moreover, elevated levels of eotaxins, chemoattractants for eosinophils and neutrophils, were detected in Trpm5+/+ mice but not in Trpm5-/- mice. BAL samples obtained from infected Trpm5+/+ mice showed higher levels of IL-5, a cytokine involved in eosinophil maturation, and MCP-1 compared to infected Trpm5-/- mice.

TRPM5 channel activation in BCs is needed for induction of the early innate immune response against P. aeruginosa infections.

Vortrag 60:

Title:
Morphologic indicators for severe central nervous system defects in genetically engineered mouse embryos

Authors:
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Abstract:
In researching human central nervous system (CNS) disorders the identification of appropriate knockout (KO) mouse models is essential. As many KO-lines produce pre- or perinatally lethal homozygous offspring, their analysis rests upon phenotyping accessible embryonic stages. This is complicated by the fact that many mouse lines show highly variable penetrance of phenotypes, wherefore severe phenotypes, causing early lethality are easily missed and large numbers of embryos have to be bred and harvested. To facilitate reduction of numbers and to ensure that early lethal malformations are not missed, we aimed at identifying mild morphologic abnormalities that have the potential to serve as indicators for low penetrant CNS defects in genetically modified mouse lines.

Approximately 500 homozygous null mutant embryos of 81 single gene KO-lines were harvested at embryonic day 14.5 and digital volume data were created using High-resolution episcopic microscopy (HREM). Employing the data stacks and volume rendered computer models the phenotypes of the embryos were systematically analysed following a standardized protocol.

First analysis identified two promising indicator candidates. Hypoglossal nerve (HGN) abnormalities (absent, thin, and abnormal topology) and abnormal morphology and topology of head arteries. Both are frequently associated with the full spectrum of morphological CNS defects. Statistical analysis however confirmed only for HGN abnormalities a significant correlation with CNS defects.

These results demonstrate that KO-lines showing HGN abnormalities are also likely to produce CNS defects. Therefore the HGN can be used as indicator to identify KO-lines featuring low penetrant CNS malformations.

Vortrag 61:

Title:
Phenotype analysis of Zfp-/- and Zfp-/+ mouse embryos based on High resolution episcopic microscopy

Authors:
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Abstract:
In mice Zfp516 is known to regulate brown fat tissue formation and stemness in embryonic stem cells. Loss of its function leads to embryonic lethality. Yet, information whether morphogenesis and fetal anatomy is abnormal in homozygous and heterozygous mice is scarce and contradicting. The function of the gene is therefore still not fully characterised and it is not yet clear whether it fits as model for studying the function of the human orthologue ZNF516. Our study aims at providing comprehensive and high detail information on the morphological phenotype of homozygous and heterozygous harvested at embryonic day (E) 14.5.

High resolution episcopic microscopy (HREM) was used to create digital volume data with voxel sizes of 3x3x3 µm3 from 4 Zfp516-/- and 10 Zfp516-/+ E14.5 mouse embryos. A standardised protocol, involving scrolling through the images of all three body planes, virtual slicing, volume and surface rendering was followed to perform systematic phenotype analysis.

Both, Zfp-/- and Zfp-/+ E14.5 embryos showed a wide range of structural abnormalities, in particular of the cardiovascular and nervous systems. Four homozygous and two heterozygous embryos showed perimembranous or muscular ventricular septal defects. Four homozygous and two heterozygous embryos showed brain defects or cranial nerve abnormalities.

Our analysis demonstrate that Zfp516 plays an essential role in cardiovascular formation and the development of the nervous system.

Vortrag 62:

Title:
The lincRNA Pantr1 is a critical mediator of dendritic outgrowth in a mouse model of FOXG1 syndrome

Authors:
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Abstract:
This study aimed to identify whether and how non-coding RNAs (ncRNA) are involved in FOXG1-syndrome. NcRNAs impact brain development and their aberrant expression was observed in neurological diseases. We showed that reduced levels of FOXG1 alter miRNA biogenesis, but its impact on long(l) ncRNA was not explored. In hippocampus of Foxg1+/- mice, we observed decreased expression of the lncRNA Pantr1, which controls expression of transcription factors affecting brain development.

We used RNA immunoprecipitation followed by mass spectrometry to investigate Pantr1 interacting proteins in NPCs and hippocampus. RNA-seq was performed to detect putative target genes after shRNA-mediated knockdown of FOXG1, Pantr1 or PURB as potential interaction partner, respectively, in cultured hippocampal neurons. Alterations of neuronal morphology were determined by Sholl analysis. Chromatin-immunoprecipitation was used to resolve whether FOXG1 and PURB colocalised at the chromatin of putative target genes, potentially bridged by Pantr1.

FOXG1/PURB/Pantr1 form an interactive network, which is essential for dendritic outgrowth and neuronal differentiation in hippocampus. Overexpression of Pantr1 in Foxg1 loss-of-function condition rescues dendritic complexity. Pantr1 might mediate binding of FOXG1 and/or PURB to Htr7, which is a target of the network.

The interactive FOXG1/PURB/Pantr1 network impacts brain development by transcriptional regulation of genes affecting dendritic complexity. Aberrant expression of Pantr1 in the context of Foxg1 syndrome seems critically involved in impaired dendritic outgrowth. Therefore, Pantr1 could be evaluated as a target for therapies of FOXG1 syndrome.

Vortrag 63:

Title:
Investigations on the role of human Connexin43 and Connexin45 during vasculogenesis in blood perfused vascular organoids

Authors:
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Abstract:
Much progress has been made in establishing induced pluripotent stem cell (iPSC) derived organoids for studying developmental biology and for disease modelling. The aim of our research was to generate a perfused human organoid model for blood vessel development and utilize this model to investigate the role of connexins during blood vessel development.

Human iPSC derived mesodermal progenitor cells (MPC) were used to generate vascularized organoids, which were transplanted onto the chicken chorioallantoic membrane (CAM) to achieve blood perfusion. Moreover, Connexin43 and Connexin45 deficient iPS cell lines were generated.

Perfusion of blood vessel organoids resulted in increased vessel wall maturation indicated by mural cell recruitment and increased Smooth Muscle Actin (SMA) expression.
To test the validity of the organoid system, Connexin (Cx) deficient iPS cell lines were generated. Several connexin genes are expressed during vasculogenesis, among these Cx43 and Cx45. Mice deficient in either Cx43 or Cx45 show severe defects in blood vessel development, in particular deficiency in mural cell recruitment. We generated Cx43/Cx45-double deficient human blood vessel organoids and xenografted those onto the CAM. We demonstrate that connexin deficiency affects vessel wall maturation, indicated by poorly associated mural cells and reduced SMA expression, probably due to reduced TGFb signaling.

We present a cost-effective, simple approach for the generation of perfused human blood vessel organoids. Moreover, we demonstrate the validity of our model as tool for the investigation of human vasculogenesis by showing the involvement of connexins in human vessel wall maturation, similar to the situation in connexin deficient mice.

Vortrag 64:

Title:
Protein and mRNA relay for a long-range FGF8 concentration gradient

Authors:
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Abstract:
Morphogen gradients provide positional information for cell fate determination and are required for spatial patterning in many developmental systems of multicellular organisms. In addition to the synthesis-diffusion-clearance mechanism, morphogen gradients can also be manifested by mRNA gradients in the growing tissue. In this project, we want to address whether and how these two mechanisms integrate in shaping morphogen gradients.

The anterior-posterior diffusion within the neural tube and presomitic mesoderm was blocked by barrier implantation. FGF signalling was manipulated by electroporation of gene constructs into the presomitic mesoderm and neural tube. The fgf8 mRNA and FGF8 activity gradient were imaged by the in situ hybridisation chain reaction and the immunohistochemistry with the dual phosphorylated MAP-Kinase.

We detected anterior-posterior diffusion of FGF8 in the neural tube and presomitic mesoderm. Interruption of the diffusion impaired FGF8 activity gradient and fgf8 mRNA gradient. FGF8 protein stabilizes Fgf8-mRNA expression.

The FGF8 gradient along the presomitic mesoderm and neural tube is established by cooperation between the short diffusible FGF8 protein and graded fgf8 mRNA.

Vortrag 65:

Title:
Hox genes initiate the limb by permissive and instructive induction

Authors:
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Abstract:
Hox genes which are expressed in a spatial and temporal collinearity pattern specify structures along the longitudinal axis of the embryonic body. For instance, the position of the wing which is located at the interface between the neck and thorax should be determined through Hox genes. However, the wing position is not altered in many Hox gene mutants, which exhibit cervical-thoracic skeletal morphological transformations. Hence, it is not clear how Hox genes determine the axial position of the wing.

To up- and down-regulate Hox gene expression, Hox gene constructs were electroporated into the lateral plate mesoderm at the wing and neck level. After 1 and 2 days of reincubation, the formation of endogenous and ectopic wing bud was analyzed by in situ hybridization.

We show that inhibition of Hox4 genes delayed wing formation. The ectopic expression of Hox6/7 genes induced an ectopic budding in the neck region. While the ectopic budding had Lmx1 and Tbx5 transcripts, no Fgf10 and Fgf8 expressions were detected in the induced budding.

Within a wide permissive field induced by Hox4 genes, the exact position of the wing is instructively induced by Hox6/7 genes.

Vortrag 66:

Title:
Ttc30a affects tubulin modifications in a frog model for ciliary chondrodysplasia with polycystic kidney disease

Authors:
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Abstract:
Skeletal ciliopathies (e.g. Jeune syndrome, short rib polydactyly syndrome, Sensenbrenner syndrome) are frequently associated with nephronophthisis-like cystic kidney disease and other organ manifestations. Despite recent progress in genetic mapping of causative loci, a common molecular mechanism of cartilage defects and cystic kidneys has remained elusive.

Targeting two ciliary chondrodysplasia loci (ift80 and ift172) by CRISPR/Cas9 mutagenesis, we established novel models for skeletal ciliopathies in Xenopus tropicalis. Phenotypic analysis included immunostaining and in vivo excretion assays of embryos and micro-CT scans of froglets. A data-mining based in silico screen identified genes with similar characteristics to known disease associated loci, four of which were evaluated in vivo. Analysis of scRNA-Seq datasets revealed in which clusters ciliopathy genes were primarily expressed. Patient cohorts were screened for mutations in TTC30A and TTC30B.

CRISPR targeting of the disease genes ift80 and ift172 froglets led to severe limb deformities, polydactyly, and cystic kidneys, closely matching the phenotype of affected patients. Targeting the novel candidate ttc30a replicated limb malformations and renal cysts identical to the models of established disease genes. Loss of Ttc30a impaired embryonic renal excretion and ciliogenesis due to altered posttranslational tubulin acetylation, glycylation and defective axoneme compartmentalization. Ttc30a/b transcripts were enriched in chondrocytes and osteocytes of single cell RNA-sequenced embryonic mouse limbs.

We identify TTC30A/B as an essential node in the network of ciliary chondrodysplasia and nephronophthisis-like disease proteins and suggest that tubulin modifications and cilia segmentation contribute to skeletal and renal ciliopathy manifestations of ciliopathies in a cell type specific manner. These findings have implications for potential therapeutic strategies targeting tubulin modifications in ciliopathies.

Vortrag 67:

Title:
The histone methyltransferase DOT1L plays an important role in the proper differentiation and integration of GABAergic interneurons into the developing mouse cortex

Authors:
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Abstract:
Objectives
The histone methyltransferase DOT1L plays an important role in the expansion of the neural progenitor pool and the proper neuronal differentiation. We here investigate the effects of DOT1L knockout in the development of cortical GABAergic interneurons and their proper integration into networks in the cerebral cortex and hippocampus.

Methods
We employed multi-omics, single cell RNA sequencing, and histological stainings on three different DOT1L conditional knockout (DOT1L-cKO) mice, to reveal cell-autonomous and non-cell-autonomous effects on interneuron development.

Results
DOT1L-cKO in cortical glutamatergic progenitors resulted in dysregulated mRNA expression of GABAergic markers. We observed e.g. decreased expression of Sox6, Sst, Ascl1 and Nkx2.1. Non-cell-autonomous loss of DOT1L reduced the number of Sst- and Npy-positive cells in the cortical plate and in the piriform cortex/striatum. CGE-derived interneurons enriched in the cortical plate. Analysis of the transcriptome revealed that DOT1L-cKO affected particularly the maturation of GABAergic interneurons. Proteomic alterations upon DOT1L-cKO revealed mis-expression of proteins impacting interneuron migration within the cortical plate. Single cell transcriptomics showed accumulation of interneurons in the hippocampus. Cell-autonomous DOT1L-cKO in Nkx2.1-expressing cells also resulted in reduced interneurons in the cortical plate, where they accumulated in deeper layers. Transcriptional dysregulation of GABAergic markers was observed together with reduced number of cells expressing Lhx6, Nkx2.1, Nkx6.2, Nr2f2 and Sst.

Conclusions
We here show that DOT1L knockout has a critical impact on GABAergic interneuron development in a cell-autonomous and non-autonomous manner, which makes it into an important player controlling GABAergic differentiation, integration and maturation, mediated by altered gene expression.

Vortrag 68:

Title:
Maternal infections, molecular mimicry and fetal brain development: what could we learn from multiprotein-arrays?

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Abstract:
For an increasing number of neurological and neuropsychiatric diseases, underlying causal changes can be traced back to early stages of fetal brain development. Such a neurodevelopmental disorder is schizophrenia, for which obstetric complications, and prenatal maternal immune activation by infections are thought to trigger changes in brain development, structure, and functioning which later in life could contribute to disease outbreak. Whereas the roles of neurotropic viruses and eukaryotic parasites have been extensively studied in this context, more recently, also bacterial infections have come into the focus of scientific interest. Thus, maternal infections with Neisseria (N.) gonorrhoeae (Babulas et al., 2006; Sørensen et al., 2009) as well as Helicobacter (H.) pylori (Yilmaz et al., 2008), have been suggested to play a role in schizophrenia pathology.

We hypothesized an antibody mediated mechanism for this, and therefore have analyzed interactions of polyclonal antisera directed to these and other bacteria species with proteins of a multiprotein array of the first trimester human fetal brain (HexSelect, Engine, Berlin, Germany) by molecular mimicry.

By this we could identify several interactive schizophrenia candidates, including the synaptic proteins Synaptosomal-associated protein 23 (snap23) for N. gonorrhoeae, and Synaptotagmin5 (syt5) for H. pylori, impaired functions of which resulted in typical cellular changes which in the end could well contribute to the functional brain changes found in schizophrenic patients.

In my talk I will give an overview on some of our findings with a special focus on immune mediated functional changes in vitro and their significance for brain development and pathology in vivo.