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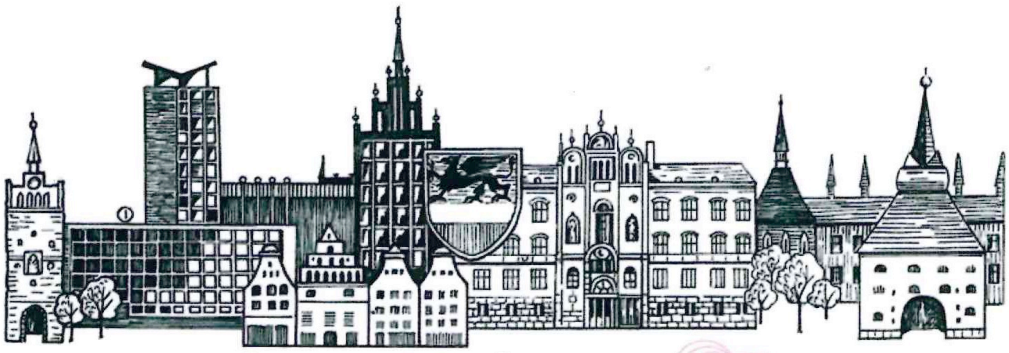

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Biomaterials

Poster 1

Titel:
Wound dressings affect vitality and adherence of juvenile and adult human fibroblasts in vitro in dependency of the test model

Autoren/Adressen:
Clemens Gögele (Paracelsus Medical University), Gundula Schulze-Tanzil (Paracelsus Medical University), Maria Kokozidou (Paracelsus Medical University), Julian Müller-Kühnle (Paracelsus Medical University), Moritz Billner (Paracelsus Medical University), Bert Reichert (Paracelsus Medical University), Christiane Gäbel (Leibnitz Institute of Polymer Research Dresden), Karl Bodenschatz (Nuremberg General Hospital, Paracelsus Medical University); clemens.goegele@pmu.ac.at

Abstract:
A successful wound care requires often covering biomaterials which protect and promote the reepithelialization. Fibroblasts, which are important for deep wound healing are often strongly impaired in their proliferation and growth. The aim of this study was to compare cell adherence, viability and morphology of murine fibroblasts (cell line L929) and human primary juvenile and adult fibroblasts on four different wound dressings.

Murine fibroblasts and freshly isolated juvenile and adult fibroblasts were cultured for 24 h – 7 d in statical and dynamical two-dimensional (2D) – and 3D culture settings on: Suprathel, Epicite, Cuticell and Biobrane. Cell viability was tested with the life-death assay and the ratio between viable and dead cells was calculated as well as the surface area of the materials covered with adhering cells using Image J software. Hematoxylin-Eosin and F-actin stainings were performed to monitor the overall cell organization in culture and depict the cell cytoskeleton in response to the biomaterials. Permeability of all four materials was tested using a previously established diffusion chamber.

Human fibroblasts showed an elongated and flatted cell shape with multiple focal adhesion points. Cell survival was not as satisfactory in medium-air interface culture compared withthe two other culture systems. Adult fibroblasts showed the earliest adherence on Biobrane. The rotatory culture system led to cell cluster formation on Cuticell. A homogeneous cell distribution could be found on Epicite in the rotatory culture with adult fibroblasts.

A high cytocompatibility was verified with murine and human fibroblasts on Cuticell, Suprathel, Epicite, Biobrane.

Poster 2

Titel:
Topographic mapping and structural characterization of neointimal lead encapsulations - a post mortem study in arrhythmia patients with implantable cardiac electronic devices

Autoren/Adressen:
Jonas Keiler (Rostock University Medical Center), Marko Schulze (Rostock University Medical Center), Sylvia Pfensig (), Pascal Dohmen (Rostock University Medical Center), Andreas Wree (Rostock University Medical Center); jonas.keiler@med.uni-rostock.de

Abstract:
Although leadless implantable cardiac electronic devices (ICEDs) are on the rise, artificial pacemakers and implantable cardioverter defibrillators with up to three leads are preferred systems for the treatment of cardiac arrhythmias. Since ICED leads are prone to fractures and infections their extraction is indicated in some cases. Despite advanced extraction techniques based on mechanical milling or laser cutting exist, lead adhesions due to neointimal fibrotic encapsulations are still challenging and extractions still constitute risky procedures for patients. To gain information for the optimization of ICEDs in terms of reduced lead adhesion and better extractability, we re-evaluated the topographic and histological patterns of lead ingrowths in arrhythmia patients with ICEDs post mortem.

Lead-bearing hearts and veins were dissected from corpses with ICEDs suffering from cardiac arrhythmia during lifetime. Lead encapsulations were mapped topographically using anatomical landmarks. Vascular tissue affected by lead-induced fibrosis was analyzed histologically and immunohistochemically.

Vascular tissues which have been affected by lead-induced mechanical irritation had developed a collagen-rich sheath covered luminally by neointimal endothelial cells. The inner contact zone between encapsulation and lead was almost acellular while the outer layers possessed smooth muscle cells positive for alpha-actin. Correlations between ingrowth characteristics and lead course, lead properties and implant duration are discussed.

Post mortem analysis of ICED leads help to understand both characteristics and formation of lead-induced neointimal fibrotic encapsulations. The combination of a selective facilitation of lead ingrowth based on drug-elution and surface-modification and a drug-based suppression of ingrowth might improve extractability due to a reduced overall ingrowth.

Cell Biology

Poster 3

Titel:
Immunolocalization of surfactant proteins SP-A, SP-B, SP-C, and SP-D in infantile labial glands and mucosa

Autoren/Adressen:
Mechthild Stoeckelhuber (Technical University of Munich), Friedrich Feuerhake (Hannover Medical School), Christoph Schmitz (Ludwig-Maximilians-University of Munich), Klaus-Dietrich Wolff (Technical University of Munich), Marco R. Kesting (Technical University of Munich); mechthild.stoeckelhuber@tum.de

Abstract:
Surfactant proteins in different glandular structures of the oral cavity display antimicrobial activity for protection of invading microorganisms. Moreover, they are involved in lowering liquid tension in fluids and facilitate secretion flows. In the oral cavity, minor salivary glands secrete continuously saliva for the maintenance of a healthy oral environment. The aim of the study was to examine surfactant proteins in infantile labial glands and mucosa.

The localization and distribution of surfactant-proteins SP-A, SP-B, SP-C, and SP-D in the labial glands and mucosa were studied by histochemical and immunohistochemical methods.

The labial glands were located in the lamina propria of the labial mucosa and showed the characteristic feature of a mixed salivary gland with serous and mucous endpieces. Serous glandular cells are grouped in the form of an acinus at the excretory duct that emptied into the oral cavity. For the first time, we could demonstrate that infantile labial glands show expression of the surfactant proteins SP-A, SP-B, SP-C, and SP-D in acinar cells and the duct system in different intensities. The stratified squamous epithelium of the oral mucosa revealed positive staining for surfactant proteins especially in the basal and intermediate cell layers.

The presence of all four surfactant proteins point on the one hand to functions like the reduction of surface tension in saliva, on the other hand to immune defense early in infant development.

Poster 4

Titel:
Impact of Psoriasin (S100A7) on VEGF-mediated Neovascularization of the Cornea

Autoren/Adressen:
Fabian Garreis (Friedrich-Alexander University Erlangen-Nürnberg (FAU)), Eva Beckenbauer (Friedrich-Alexander University Erlangen-Nürnberg (FAU)), Ulrike Schulte (Friedrich-Alexander University Erlangen-Nürnberg (FAU)), Martin Schicht (Friedrich-Alexander University Erlangen-Nürnberg (FAU)), Friedrich Paulsen (Friedrich-Alexander University Erlangen-Nürnberg (FAU)); fabian.garreis@fau.de

Abstract:
The antimicrobial peptide psoriasin is part of the innate immune response at the ocular surface. VEGF-induced psoriasin expression contributes to increased angiogenesis and proliferation in various tumor cells. Neovascularization of the cornea is associated with decreased vision and may lead to blindness. In the present study we analyzed psoriasin-dependent neovascularization as well as involved molecular factors.

Cell culture experiments were used to investigate the concentration and time-dependent impact of psoriasin, VEGF, soluble receptor for advanced glycation end products and H2O2 on a human corneal epithelial (HCE) cell line. Psoriasin-dependent factors were analyzed by qPCR, western blot as well as ELISA. Additionally, impact of psoriasin, VEGF and sRAGE to proliferation and the formation of reactive oxygen species (ROS) was determined by functional assays. Furthermore, we analyzed expression of psoriasin as well as similar antimicrobial S100 proteins in clinical biopsies of corneas associated with neovascularization.

Stimulation of cultivated HCE cells with VEGF and sRAGE increased psoriasin expression at mRNA level. Gene expression of VEGF was significantly increased whereas VEGF receptor-1 was reduced after application of 250 ng/ml recombinant psoriasin after 24 hours. Expression of the potential psoriasin receptor RAGE, was significantly increased after application of VEGF and in contrast reduced by psoriasin. HCE cells showed significantly increased cell viability as well as an increased ROS formation after stimulation with VEGF, psoriasin and low H2O2 concentrations.

Our results likely indicate an involvement of psoriasin in VEGF-mediated corneal neovascularization. These results may have therapeutical implications to the development of drugs to inhibit the VEGF-induced neovascularization.

Poster 5

Titel:
MTORC1 regulates renal proximal tubular megalin function

Autoren/Adressen:
Madlen Kunke (Christian-Albrechts-Universität), Luis Zanon Rodriguez (Christian-Albrechts-Universität), Gerburg Schwärzer (Christian-Albrechts-Universität), Prof. Dr. Franziska Theilig (Christian-Albrechts-Universität); f.theilig@anat.uni-kiel.de

Abstract:
Renal proximal tubular cells constantly recycle nutrients to ensure minimal loss of essential substrates into the urine. Thus, endocytosis is a hallmark of the proximal tubule. Protein uptake is mediated by the scavenger receptors megalin and cubilin, followed by internalization of the ligand-receptor complex via the clathrin-mediated pathway. The mTORC1 complex is a principle regulator of proximal tubular function, including endocytosis. However, the effect of mTORC1 on megalin function remains unknown

Tubular deletion of mTORC1 was created by crossbreeding Raptorfl/fl with Pax8rt-TA and TetOCre mice. Phosphoproteomics were performed to analyze the phosphorylation of megalin in the renal cortex. Endocytosis was detected in proximal tubules using Alexa555-labelled lactoglobulin. In vitro, we induced mTORC1 activity through transient transfection with Rheb, used megalin minireceptor 2 (MMR2) to introduce mutations in the respective phosphosite S4577A, S4577D, S4577E, and followed endocytosis through Alexa555-labelled albumin.

mTORC1-deficient mice showed normal megalin expression and distribution within proximal tubules compared to wildtype mice. Interestingly, receptor-mediated endocytosis of Alexa555-labelled lactoglobulin was blocked in mTORC1-deficient mice. Phosphoproteomics of mTORC1 deficient mice revealed a strongly reduced phosphorylation at S4577 of the C-terminus of megalin, which was confirmed by transient transfection of MDCKII cells with Rheb stimulating mTORC1 activity. Transfection of wildtype or mutated MMR2 in MDCKII cells caused no difference in megalin expression and its cellular distribution. However, endocytosis was increased in the presence of S4577D mutant compared to megalin wildtype.

mTORC1 complex is an important regulator of proximal tubular function and phosphorylates megalin to influence megalin function.

Poster 6

Titel:
CD68+ cells in myocardium, epicardial fat and subcutaneous fat in cardiovascular surgeryPATIENTS with metabolic disease

Autoren/Adressen:
Aneta Pierzynová (1st Faculty of Medicine Charles University), Jaromír Šrámek (1st Faculty of Medicine Charles University), Jana Kloučková (1st Faculty of Medicine Charles University, Institute for Clinical and Experimental Medicine), Jaroslav Lindner (1st Faculty of Medicine Charles University, General University Hospital), Michal Lipš (1st Faculty of Medicine Charles University, General University Hospital), Martin Haluzík (1st Faculty of Medicine Charles University, Institute for Clinical and Experimental Medicine), Tomáš Kučera (1st Faculty of Medicine Charles University); Aneta.Pierzynova@lf1.cuni.cz

Abstract:
Pathogenesis of chronic diseases of the cardiovascular system appears to involve subclinical inflammation not only in epicardial fat but also in myocardium. The role CD68+ cells in these pathological conditions is still incompletely understood. The goal of the study was stereological quantification of CD68+ cells in the right atrium, epicardial fat and subcutaneous fat in cardiovascular surgery patients with obesity (BMI>30), type 2 diabetes mellitus (DM2T) and coronary artery disease (CAD). The results were compared with clinical parameters.

We used the samples obtained from heart surgeries. A total of 31 right atrium myocardial samples, 44 epicardial fat samples, and 47 subcutaneous fat samples were divided into groups according to whether patients suffered from obesity, DM2T, CAD, or not. Immunohistochemistry was used to visualize CD68-PGM1. Histomorphometric measurement was performed using ImageJ.

CD68+ cells were significantly more abundant in epicardial fat, whereas their frequency was higher in obese patients. In subcutaneous fat, the quantification of CD68+ cells revealed their significantly higher density in CAD. The numbers of CD68+ cells in right atrium were also significantly higher in obese patients. The correlation analysis captured dependence between the abundant of CD68+ cells and BMI, waist-to-hip ratio, HbA1C and HDL.

Our finding supports hypothesis that increased amount of CD68+ cells accompany pathological conditions associated with local subclinical inflammation, but inflammation has little effect on the taxis of cells from epicardial fat to the adjacent myocardium.
Supported by Project AZV 15-26854A and PROGRES Q25.

Poster 7

Titel:
Distribution and density of melatonin receptors in human main pancreatic islet cell types

Autoren/Adressen:
Juliane Zibolka (Martin Luther University Halle-Wittenberg), Ivonne Bazwinsky-Wutschke (Martin Luther University Halle-Wittenberg), Eckhard Mühlbauer (Saxon Academy of Sciences Leipzig), Elmar Peschke (Saxon Academy of Sciences Leipzig); juliane.zibolka@medizin.uni-halle.de

Abstract:
Melatonin modulates hormone secretion of pancreatic islets via melatonin receptor types MT1 and MT2. Expression of both receptors has been proven in mouse, rat, and human pancreatic islets as well as in the BETA-, ALPHA-, and DELTA-cell lines INS-1, ALPHATC1.9, and QGP-1. However, detailed distribution and density of melatonin receptors on the main islet cell types in human pancreatic tissue is not yet clear.

Therefore, double immunofluorescence labeling in combination with confocal laser scanning microscopy was used to analyze receptor distribution in pancreatic tissue obtained from nondiabetic and type 2 diabetic patients. Islet hormone mRNA expression was measured by real-time RT-PCR. In addition, isolated human islets of a nondiabetic and a diabetic donor were incubated with different melatonin concentrations and somatostatin secretion was quantified by radioimmunoassay.

Immunohistochemical analyses demonstrated the presence of MT1 and MT2 in BETA-, ALPHA-, and DELTA-cells, but notably, with differences in receptor density. In general, the lowest MT1 and MT2 receptor density was measured in ALPHA-cells. In type 2 diabetic islets, MT1 and MT2 receptor density was increased in DELTA-cells. In addition, nondiabetic islets showed an increase of somatostatin secretion under melatonin treatment while in diabetic islets an inhibitory influence could be observed.

These data suggest a cell-type-specific density of MT1 and MT2 receptors in human pancreatic islets, with an influence of diabetes on the density in DELTA-cells as well as a differential impact of melatonin on somatostatin secretion of nondiabetic and type 2 diabetic islets, which should be considered in the context of islet functions.

Poster 8

Titel:
Endothelial barrier function is differentially regulated by CEACAM1-mediated signaling

Autoren/Adressen:
Uwe Rückschloß (Julius-Maximilians-Universität Würzburg), Florian Kleefeldt (Julius-Maximilians-Universität Würzburg), Sharang Ghavampour (Julius-Maximilians-Universität Würzburg), Heike Bömmel (Julius-Maximilians-Universität Würzburg), Kevin Volland (Julius-Maximilians-Universität Würzburg), Alexander Paus (Universitätsklinikum Essen), Andrea Horst (Universitätsklinikum Hamburg-Eppendorf), Verena Pfeiffer (Julius-Maximilians-Universität Würzburg), Stefan Hübner (Julius-Maximilians-Universität Würzburg), Nicole Wagner (Julius-Maximilians-Universität Würzburg), Süleyman Ergün (Julius-Maximilians-Universität Würzburg); uwe.rueckschloss@uni-wuerzburg.de

Abstract:
CEACAM1 is an important mediator of vascularization/ angiogenesis. However, its role in mature vessel homeostasis is largely unknown. Therefore, we analyzed the impact of CEACAM1 on an essential aspect of endothelial function.

We used aortic samples and WT or Cc1-/- endothelial cell lines (MyEnd cells). We analyzed leucocyte-endothelial interaction, protein tyrosine phosphorylation and protein-protein interaction (immunoprecipitation), quantified gene expression (Western Blot) and evaluated endothelial barrier function in situ (aortic dye deposition) and in vitro (trans-endothelial electrical resistance - TEER).

Here, we show that CEACAM1 deficiency causes subcellular eNOS redistribution in endothelial cells (i.e. by eNOS depalmitoylation) and alters endothelial glycocalyx that confers antiadhesive properties to the endothelium (i.e. by repression of glycocalyx-degrading enzymes). Accordingly, our analysis revealed an increased leukocyte–endothelial interaction in CEACAM1-deficient endothelium. In addition, CEACAM1 age-dependently modulated basal and TNF-ALPHA–mediated endothelial barrier (EB) leakiness. In younger mice, CEACAM1 was protective for EB, whereas in aged mice it promoted EB leakiness. EB function depends on interendothelial adherence junctions formed by BETA-catenin/vascular endothelial–cadherin complexes. We show here that CEACAM1 influenced basal and TNF-ALPHA–mediated phosphorylation of BETA-catenin and caveolin-1, which are essential players in EB modulation. Both increased adhesiveness to leukocytes and EB modulation due to CEACAM1 deficiency may facilitate inflammatory cell transmigration into the vascular wall and subsequent plaque formation.

Collectively, these results identify a crucial role for CEACAM1 in endothelial homeostasis of adult blood vessels.

Poster 9

Titel:
Vascular adventitia-derived macrophages are crucial regulators of new vessel formation by vascular wall-resident progenitors

Autoren/Adressen:
Florian Kleefeldt (Julius-Maximilians-Universität Würzburg), Georg Eckner (Julius-Maximilians-Universität Würzburg), Berin Upcin (Julius-Maximilians-Universität Würzburg), Jan Allmanritter (Julius-Maximilians-Universität Würzburg), Jochen Bauer (Julius-Maximilians-Universität Würzburg), Heike Bömmel (Julius-Maximilians-Universität Würzburg), Philipp Wörsdörfer (Julius-Maximilians-Universität Würzburg), Uwe Rückschloß (Julius-Maximilians-Universität Würzburg), Süleyman Ergün (Julius-Maximilians-Universität Würzburg); florian.kleefeldt@uni-wuerzburg.de

Abstract:
Pathological angiogenesis contributes to tumor growth, metastasis and atherosclerotic plaque rupture. Vascular wall-resident stem and progenitor cells (VW-SCs) were shown to deliver vascular cells contributing to new vessel formation, but also non-vascular cells like macrophages. Therefore, we investigated the potential contribution of VW-SC-derived macrophages to new vessel formation.

To this end, we analyzed sprouting activity in aortic ring assays (ARA) with or without pharmacological interventions. Different cell types within the aorta were identified by immuno-histochemical analysis of respective marker proteins.

In freshly isolated mouse aortae, we detected CD34(+), VEGFR-2(+) and Ly6c(+) cells within the sub-endothelial space and the adventitial “vasculogenic zone” while almost no F4/80(+) macrophages were found. In contrast, a high number of F4/80(+) cells were present within adventitia and collagen gel among the sprouting cells after ARA. This was accompanied by a significant reduction of adventitial CD34(+) cell numbers. Immunostaining identified F4/80(+) VW-SC-derived macrophages as the main source of VEGF in ARA. Consequently, macrophage depletion by clodronate treatment as well as inhibition of VEGF signaling by Lenvatinib reduced cellular sprouting and capillary-like formation in ARAs. Remarkably, after both interventions the adventitial stem cell niche was maintained as confirmed by CD34 immunostaining.

These results show that VW-SC-derived macrophages generate high levels of VEGF that acts on VEGFR-2(+) VW-SCs in a paracrine and autocrine manner. By this, VW-SC-derived macrophages function as locally generated essential promoters of angiogenesis that need to be taken into account in establishing new pro- and/ or anti-angiogenic therapeutic strategies.

Poster 10

Titel:
VHL deletion in renal proximal tubules of mice ameliorates hallmarks of diabetic nephropathy

Autoren/Adressen:
Martina Böttner (University of Kiel), Alexey Larionov (Department of Medicine), Eileen Dahlke (University of Kiel), Jean-Luc Magnin (University of Fribourg), Franziska Theilig (University of Kiel); m.boettner@anat.uni-kiel.de

Abstract:
Diabetic nephropathy is the leading cause of end-stage renal disease and frequently affects patients with type 2 diabetes mellitus. Glomerular hyperfiltration and phenotypic changes in proximal tubule epithelial cells (PTECs) are the initial signs of diabetic nephropathy. Systemic activation of hypoxia-inducible factors (HIFs) was shown to prevent diabetes-induced alterations in kidney oxygen metabolism.It was the aim of the study to assess markers of diabetic nephropathy in diabetes-induced SGLT2Cre/VHLflox mice.

To analyze in detail whether renal tubular HIF activation is sufficient to prevent functional and morphological alteration of diabetic nephropathy, SGLT2Cre/VHLflox mice were generated and diabetes mellitus was induced by streptozotocin application.

FITC-inulin clearance analysis revealed a strong increase in diabetic VHLflox control mice which was abrogated in diabetic SGLT2Cre/VHLflox mice showing similar values as the non-diabetic control mice. Blood glucose concentration and urinary volume excretion were significantly increased in diabetic mice without significant differences between genotypes. However, osmotically-induced hyponatremia was only observed in diabetic VHLflox mice whereas diabetic SGLT2Cre/VHLflox had values similar to non-diabetic controls. Urinary electrolyte, glucose and phosphate excretion was increased in diabetic mice and more pronounced in diabetic SGLT2Cre/VHLflox compared to diabetic VHLflox mice.

In summary, our study reveals that induction of proximal tubular HIF´s are able to prevent diabetes induced glomerular hyperfiltration. Additionally, osmotically-induced hyponatremia was also prevented upon HIF activation most probably through reduced proximal tubular glucose reabsorption and thereby leading to its increased urinary excretion.

Poster 11

Titel:
The actin-binding protein adducin regulates Dsg3 trafficking, Dsg3-dependent adhesive functions and cell migration

Autoren/Adressen:
Matthias Hiermaier (University of Basel), Marie-Therès Wanuske (University of Basel), Jens Waschke (Ludwig-Maximilians-University Munich), Volker Spindler (University of Basel); matthias.hiermaier@unibas.ch

Abstract:
Desmosomes are cell-cell junctions which provide strong intercellular adhesion and couple the intermediate filament cytoskeleton to sites of cell-cell contact. We have previously shown that the actin-binding protein adducin is required for desmosome function. The aim of this project was to elucidate the adducin-dependent mechanisms of desmosome regulation.

Experiments were conducted in human and murine keratinocytes with knockdown or knockout of adducin. Intracellular trafficking was evaluated by life cell imaging of the desmosomal adhesion molecule desmoglein(DSG)3. Quantum dot-labeled single chain variable fragments were used to analyze DSG3 movement on the cell surface. Intercellular cohesion and Ca2+ sensitivity was quantified by dispase-based dissociation assays. Keratinocyte migration assays in murine explants and in vitro were conducted to investigate cell mobility.

Dispase-based dissociation assays confirmed an overall reduced intercellular binding strength in cells lacking adducin although DSG3 and other desmosomal adhesion molecules were increased in the membrane. Life cell imaging during junction formation demonstrated an undisturbed speed of DSG3-containing vesicles to the cell membrane although their transport was less directed. In contrast, single molecule tracking of DSG3 in the membrane revealed increased velocities and profoundly impaired confined movement in keratinocytes without adducin. These changes correlated with reduced Ca2+-insensitivity, a feature of mature desmosomes, and reduced overall cell motility in wound healing assays in vitro and ex vivo.

Our data indicate that adducin promotes intercellular adhesion by constraining DSG3 mobility in the membrane and regulating desmosome maturation.

Poster 12

Titel:
Osteogenic differentiation of canine mesenchymal stem cells under addition of zinc

Autoren/Adressen:
Michele Christian Klymiuk (Justus-Liebig-University), Aya Rymbekova (Justus-Liebig-University), Kathrin Wolf-Hofmann (Justus-Liebig-University), Sabine Wenisch (Justus-Liebig-University), Stefan Arnhold (Justus-Liebig-University);
michele.klymiuk@vetmed.uni-giessen.de

Abstract:
The differentiation of canine mesenchymal stem cells (cMSC) in an osteogenic lineage is still challenging due to the spare knowledge of osteogenic differentiation in cMSC. An important factor for osteogenic differentiation is the activity of the alkaline phosphatase (AP), which provides phosphate for the formation of calcium phosphate, the basic anorganic bone substance. In previous in vitro experiments we could show that the AP activity is exceptionally low compared to other species. In order to support the AP activity, the addition of zinc, an important co-factor of the enzyme, to cMSC while osteogenic differentiation was investigated.

For cMSC isolation small pieces of fat tissue were collected while surgery in pet dogs and the desired cells were isolated and cryopreserved by standard lab procedure. Passage 3 cells of cMSC were cultured in standard medium (negative control) and osteogenic differentiation medium with addition of 5, 15 or 30 µM zinc. Medium was refreshed twice a week until samples for further analysis were taken. On day 14 and 21 Alizarin red staining, AP activity measurement and RT-qPCR were performed to evaluate the osteogenic differentiation.

Due to the high variability in the different cMSC donors, no statistical change within the investigated zinc concentrations was detectable. But in some individuals a remarkable change in the osteogenic differentiation could be shown.

An increased zinc concentration in osteogenic differentiation media could be a step forward to a successful osteogenic differentiation, but further confirming studies have to be carried out.

Poster 13

Titel:
Is it possible to generate neuronal precursor cells from canine adipose tissue derived mesenchymal stem cells (cAdMSCs) in liquor-enriched media?

Autoren/Adressen:
Laura Beate Heilen (Justus-Liebig-University), Michele Christian Klymiuk (Justus-Liebig-University), Veronika Lehner (University of Antwerp), Manuela Heimann (Justus-Liebig-University), Stefan Arnhold (Justus-Liebig-University);
laura.b.heilen@vetmed.uni-giessen.de

Abstract:
There are various media known to differentiate cAdMSCs to neuron-like cells. Many of them contain toxic substances like BETA-Mercaptoethanol. Other protocols increasingly are using growth factors such as NGF. We wanted to investigate, if the supplementation of cerebrospinal fluid (CSF) positively influences the differentiation of cAdMSCs.

Canine fat tissue was collected during abdominal surgery. CAdMSCs were isolated and cultured according to standard protocols. The cells were seeded in 24 well plates with inlaying silicon pads to avoid attachement. The pre-induction medium contained DMEM/F-12 supplement with 2 % B27 supplement, 1% N2 supplement, bFGF (10 ng/ml) and EGF (20 ng/ml). Spheres were harvested after 3 days of incubation (37 °C; 5 % CO2). For further differentiation cells were plated in gelatin-coated wells using a medium consisting of DMEM/F-12 supplement, 2 % B27 supplement, 1 % N2 supplement, BDNF (10 ng/ml) and NGF (100 ng/ml). Both media were prepared with and without 10 % canine CSF. The size and quantity of cells per sphere were investigated. Nestin, BETA III-Tubulin, and MAP 2 expression were detected as markers for neuronal differentiation.

After 24 hours the cells generated small spheres in both media. When analyzing the amount of cells and area per sphere it became apparent that they are linearly distributed. The immunofluorescence shows an expression of all tested markers within the spheres with no obvious difference between the media.

To verify this, neural features such as the expression of neuronal and glial markers have to be further investigated. This should include western blot and RT-qPCR. Additionally it is anticipated to test various concentrations of CSF and its effect on cAdMSCs.

Poster 14

Titel:
YAP/TAZ role in neural crest development and malignancy

Autoren/Adressen:
Alexandra-Larisa Condurat (Institute of Anatomy and Cell Biology), Jan Pruszak (Institute of Anatomy and Cell Biology); larisa.condurat@sgbm.uni-freiburg.de

Abstract:
Signaling pathways directing early stages of neural crest (NCC) induction and differentiation are known to be reactivated and/or dysregulated during neuroblastoma tumorigenesis. We have recently shown that the Hippo/YAP signaling cascade plays a significant role in promoting NCC fate and migration (Hindley et al., Scientific Reports 2016). Our current work focuses on elucidating the role of the Hippo downstream effectors YAP and TAZ in the context of different neuroblastoma (NB) subtypes and their responsiveness to retinoic acid (RA) treatment strategies.

For this purpose, we used human embryonic stem (hES) and human induced pluripotent stem (hIPS)-derived cell systems as well as several metastatic neuroblastoma cell lines.

Our data highlight that YAP/TAZ expression is particularly high in NB cells derived from aggressive MYCN-non-amplified NB tumors and strongly associates with NB cell unresponsiveness to RA-induced differentiation. Moreover, RA stimulation of the highly YAP/TAZ-positive GI-ME-N cells has no apparent blocking effect on cell cycle progression or migration, while it leads to an upregulation of pro-migratory factors (α4-/β1-/β3-integrin subunits). Conversely, we show that verteporfin (VPF), an inhibitor of YAP/TAZ-TEAD interaction, efficiently blocks migration and cell cycle progression and promotes cell death in both YAP/TAZ-positive and N-MYC-positive NB cells.

Altogether, we report a strong association between YAP/TAZ expression and NB unresponsiveness to RA-induced differentiation stimuli and highlight the potential of VPF as a therapeutic agent against both MYCN-amplified and MYCN-non-amplified NB cells.

Poster 15

Titel:
Daytime-dependent changes of cannabinoid receptor type 1 and type 2 expression in rat liver

Autoren/Adressen:
Ivonne Bazwinsky-Wutschke (Martin Luther University Halle-Wittenberg), Alexander Zipprich (Martin Luther University Halle-Wittenberg), Faramarz Dehghani (Martin Luther University Halle-Wittenberg); ivonne.bazwinsky@medizin.uni-halle.de

Abstract:
Endocannabinoids exert their effects via cannabinoid receptor type 1 (CB1) and type 2 (CB2). Recent studies indicate the particular importance of both receptors for liver function. The liver is involved in the regulation of glucose metabolism and known as a potential target of the metabolic actions of endocannabinoids. The present study was performed to analyze the diurnal expression pattern of CB1 and CB2 in rat liver tissue.

Liver tissue was obtained from control groups of normoglycemic and streptozotocin (STZ)-treated (type 1 diabetic) male Wistar rats, young rats (12 weeks) and middle-aged rats (51 weeks). Hepatic Cnr1 and Cnr2 receptor mRNA levels were measured by real-time RT-PCR and hepatic relative protein levels were estimated by Western Blot.

Cnr1 and Cnr2 mRNAs were highly expressed during the light period (ZT3, ZT6, and ZT9) while the expression was low in the dark period (ZT18 and ZT21). Diurnal transcript expression pattern was accompanied by comparable changes of protein level for CB1. In liver tissue of STZ-treated 12- and 51-week-old rats both receptors Cnr1 and Cnr2 mRNA showed alterations in their diurnal profile compared to normoglycemic Wistar rats.

The current results support the conclusion that expression patterns of cannabinoid receptors are influenced by light/dark cycle and therefore seem to be under the control of a diurnal rhythm. Diurnal receptor changes are also influenced by age. An impact of diabetic state on diurnal expression levels of cannabinoid receptors is suggested.

Poster 16

Titel:
Differential contributions of desmoplakin and plakoglobin to cell cohesion in keratinocytes

Autoren/Adressen:
Marie-Therès Wanuske (University of Basel), Matthias Hiermaier (University of Basel), Jens Waschke (Ludwig-Maximilians-University Munich), Volker Spindler (University of Basel); m.wanuske@unibas.ch

Abstract:
Desmosomes are complex molecular adhesive structures conferring resistance to tissues exposed to pronounced mechanical stress, such as the epidermis. They connect adjacent cells via the cadherin-type adhesion molecules desmogleins (DSG) and desmocollins (DSC), which are linked to the intermediate filament cytoskeleton by plakoglobin (PG), plakophilins (PKP) and desmoplakin (DP). We here compared the role of DP in controlling cell-cell adhesion and signaling pathways to that of PG.

We generated DP knockout cell lines in human keratinocytes using the CRISPR/Cas9 system and compared them to previously established PG knockout cells. Characterization was carried out using immunostainings and western blot analysis. Cellular cohesion and calcium insensitivity, a feature of mature desmosomes referred to as hyper-adhesion, were quantified with dispase-based dissociation assays.

Both loss of DP and PG severely impaired cell cohesion. PG knockout cells showed slightly reduced DSG2 and increased β-catenin levels, whereas these proteins remained unaltered in DP knockouts, which in turn showed decreased protein levels of PKP1 and DSC3. In PG depleted cells, the activities of p38MAPK and ERK, which are known to impair cell cohesion, were elevated in the cytosolic soluble pool of a triton fractionation. In contrast, p38MAPK was not activated upon loss of DP and ERK phosphorylation was reduced. Calcium insensitivity was severely compromised in DP knockout cell lines, indicating impaired desmosome maturation.

Loss of either DP or PG lead to reduced cell cohesion, suggesting that these molecules distinctly alter desmosome configuration and contribute to cell adhesion through defined mechanisms.

Poster 17

Titel:
Isolation and characterisation of exosomes derived from adipose tissue derived mesenchymal stem cell (AdMSCs) conditioned medium

Autoren/Adressen:
Natalie Balz (Justus-Liebig-University of Giessen), Michele Klymiuk (Justus-Liebig-University of Giessen), Sabine Wenisch (Justus-Liebig-University of Giessen), Stefan Arnhold (Justus-Liebig-University of Giessen); Natalie.Balz@vetmed.uni-giessen.de

Abstract:
Exosomes are small cellular vesicles with the size of 50-150 nm which are produced by endocytic pathways playing an important role in cell-cell communication by the transfer of miRNA and proteins. As the regenerative effect of stem cells is partially due to their paracrine effects, exosomes may have the potential to be used in veterinary regenerative medicine. We investigate isolation methods and storage conditions of equine and canine exosomes.

AdMSCs were obtained and cultured by standard protocols. Exosomes were collected from the supernatant of cell culture via centrifugation and sterile-filtration, ultracentrifugation or filtration through “Centricons”. Number and size of exosomes were measured by the Nano-particle-tracking analysis (NTA). The morphology was examined with TEM. Immunogold labelling was carried out to prove the presence of exosomal markers such as CD9 and CD81. A therapeutic benefit of the exosomes was examined using the wound & healing assay. Methods of isolation were compared concerning the practicability, amount and function of exosomes.

Exosomes are shedded by equine and canine AdMSCs and can be obtained via centrifugation and sterile-filtration. As detected by NTA exosomes are in the size range of 50 to 150 nm. Equine exosomes express tetraspanins CD9 and CD81 as surface markers. EDTA prevents aggregation of exosomes, stored over three days under different conditions including freezing.

Exosomes can be isolated from AdMSCs conditioned medium by all three methods applied in this study. To store exosomes, EDTA is necessary to prevent aggregation. A practicable way to obtain exosomes without loss of integrity and function would be necessary for future therapeutic applications.

Poster 18

Titel:
Ultrastructural analysis of biogenesis and release of endothelial extracellular vesicles

Autoren/Adressen:
Clara Elsner (Julius-Maximilans-University Würzburg), Süleyman Ergün (Julius-Maximilans-University Würzburg), Wagner Nicole (Julius-Maximilans-University Würzburg); nicole.wagner@uni-wuerzburg.de

Abstract:
Extracellular vesicle (EV)-mediated intercellular communication through exosomes, microvesicles and apoptotic bodies has been shown to be implicated in various physiological as well pathological processes including cardiovascular and neurodegenerative diseases. While the cellular machinery controlling EV formation and composition has been studied during the past decade, very little is known concerning the morphological processes underlying EV formation and extracellular release of EVs.

Our studies focused on a detailed investigation of the ultrastructural nature of EVs during such processes. Therefore, different steps of EV formation and release were analysed using cultured aortic endothelial cells under serum starvation and under inflammatory stimulation, applying different electron microscopic approaches such as transmission-, scanning-, immun-, and serial block face electron microscopy.

The initial step of microvesicle formation requires outward budding of the plasma membrane, leading to the formation of growing membrane blebs, which is associated with modifications in cytoskeleton protein organization and exclusion of intracellular components. In contrast to the formation of microvesicles, exosome biogenesis is initiated by vesicle internalization within early endosomes, resulting in the maturation of multivesicular bodies (MVBs). While it has been postulated that these intraluminal vesicles are released as exosomes into the extracellular environment upon fusion of MVBs with the plasma membrane, we discovered a potential second mechanism of exosome release, which we analyzed in detail.

Our data will contribute to a profound understanding of EV biogenesis and release, a fundamental prerequisite for the development of new methods to manipulate their formation, composition and secretion in the (patho) physiological context.

Poster 19

Titel:
Expression kinetics of human periodontal ligament fibroblasts in the early phases of orthodontic tooth movement

Autoren/Adressen:
Agnes Schröder (University Medical Hospital Regensburg), Kathrin Bauer (University Medical Hospital Regensburg), Gerrit Spanier (University Medical Hospital Regensburg), Peter Proff (University Medical Hospital Regensburg), Michael Wolf (University Hospital RWTH Aachen), Christian Kirschneck (University Medical Hospital Regensburg); christian.kirschneck@ukr.de

Abstract:
Human periodontal ligament (hPDL) fibroblasts hold an important mediating role in orthodontic tooth movement (OTM). We investigated the expression kinetics of genes and proteins associated with the early phase of OTM to gain insight into the timing and regulation of molecular signalling and transformation processes occurring in compressive areas of the periodontal ligament during OTM.

We stimulated hPDL fibroblasts with physiological orthodontic compressive forces of 2g/cm2 for 24h, 48h, 72h and 96h under cell culture conditions. At each time point, we quantified relative expression of genes involved in osteoblastogenesis (ALPL), inflammation (COX2, IL-6), extracellular matrix reorganization (COL1A2, P4HA1, FN1, MMP8) and angiogenesis (VEGF-A) by RT-qPCR as well as expression of proteins responsible for bone remodelling like osteoclastogenesis-inducing RANK-L by immunofluorescence staining and its decoy receptor OPG by ELISA relative to pressure-untreated controls incubated for corresponding time periods. In addition, we performed co-culture experiments with osteoclast precursor cells to evaluate hPDL-fibroblast-mediated osteoclastogenesis by TRAP staining.

We observed an induction of genes associated with angiogenesis, inflammation, osteoblastogenesis and the remodelling of the extracellular matrix as primary response to compressive forces within 24h. RANK-L expression was slightly inhibited after 24h and increased not until after 48h. OPG secretion was already inhibited after 24h of pressure application. Major hPDL-mediated osteoclastogenesis was observed after 72h with minor, non-RANK-L-dependent osteoclastogenesis occurring as early as after 24h of compressive force application.

hPDL fibroblasts play a major mediating role in the early phase of OTM with a differentiated, time-dependent regulation and expression pattern of cytokines and other mediators.

Poster 20

Titel:
Effects of ethanol on human periodontal ligament fibroblasts in a model of simulated orthodontic tooth movement

Autoren/Adressen:
Agnes Schröder (University Medical Hospital Regensburg), Erika Calvano Küchler (School of Dentistry of Ribeirão Preto, University of São Paulo), Marjorie Omori (School of Dentistry of Ribeirão Preto, University of São Paulo), Gerrit Spanier (University Medical Hospital Regensburg), Peter Proff (University Medical Hospital Regensburg), Christian Kirschneck (University Medical Hospital Regensburg); christian.kirschneck@ukr.de

Abstract:
Consumption of toxic substances like alcohol is widespread in the general population and thus also in patients receiving orthodontic treatment. Human periodontal ligament (hPDL) fibroblasts play an important mediating role in orthodontic tooth movement (OTM). In this study, we wanted to investigate, whether ethanol modulates the physiological activity and expression pattern of hPDL fibroblasts during simulated orthodontic force application.

hPDL fibroblasts were pre-incubated for 24h with different ethanol concentrations, corresponding to casual (0.41‰) and excessive (1.79‰) alcohol consumption. At each ethanol concentration, hPDL fibroblasts were incubated for another 48h with and without an additional physiological compressive force of 2g/cm2 occurring during OTM in compression areas of the periodontal ligament. Thereafter expression and secretion of genes and proteins involved in OTM regulation were analyzed by RT-qPCR and ELISA. Additionally, we performed co-culture experiments to investigate hPDL-fibroblast-mediated osteoclastogenesis.

We observed no effects of ethanol on apoptosis, necrosis, cytotoxicity or cell viability of hPDL fibroblasts in the concentrations applied. But ethanol showed an enhancing effect on angiogenesis, activity of alkaline phosphatase and collagen synthesis. Simultaneously, ethanol reduced the induction of IL-6 and increased prostaglandin E2 synthesis as well as hPDL-fibroblast-mediated osteoclastogenesis without affecting the RANK-L/OPG-system.

hPDL fibroblasts seem to be quite resistant to ethanol, as no cytotoxic effects or influence on apoptosis or necrosis were detected. High ethanol concentrations, however, seem to promote bone formation, angiogenesis and collagen synthesis. Ethanol at 1.79‰ also enhanced hPDL-induced osteoclastogenesis, indicating increased bone resorption and thus tooth movement velocity to be expected during orthodontic therapy.

Poster 21

Titel:
Bone-intrinsic dysregulation in Spinal Muscular Atrophy (SMA)

Autoren/Adressen:
Hermann Brickwedde (Hannover Medical School), Niko Hensel (Hannover Medical School), Lisa Marie Walter (Hannover Medical School), Claudia Neunaber (Hannover Medical School), Frank Tavassol (Hannover Medical School), Peter Claus (Hannover Medical School); Hermann.Brickwedde@stud.mh-hannover.de

Abstract:
Spinal Muscular Atrophy (SMA) is the most common fatal autosomal recessive neuromuscular disease in children. It is characterized by the degeneration of secondary motoneurons leading to progressive weakness of the limbs and trunk followed by muscle atrophy. The disease is caused by a lack of the SMN protein due to mutations in the SMN1 gene. SMN is ubiquitously expressed and – besides the neuromuscular system – peripheral tissues are also affected in SMA. In this project we aimed to analyze alterations in bone since some SMA patients show decreased bone mineral density and increased risk of bone fractures.

Here, we analyzed if the observed bone phenotype is a secondary effect due to muscle atrophy or if there is a bone intrinsic mechanism. To test this, we used a severe SMA mouse model at a presymptomatic postnatal stage. We examined the skeleton with X-ray analysis, µCT and non-decalcified histology. In addition, we established an osteoblast cell culture to determine mineralization capacity of osteoblasts. On a molecular level, we screened a panel of factors important for bone development in SMA and control mice.

We found a significant reduction in femur lengths in SMA mice compared to control animals. Analysis of the transcript patterns showed a significant reduction in the expression of two factors. Analysis of histology and osteoblast cell culture is still ongoing.

Our results indicate that bone intrinsic alterations in SMA are largely independent of the muscle phenotype.

Poster 22

Titel:
Expression of bitter taste receptors and components of the taste transduction cascade is restricted to chemosensory cells in the mouse gall bladder

Autoren/Adressen:
Anna-Lena Ruppert (Philipps University), Maryam Keshavarz (Justus-Liebig University), Sarah Winterberg (Philipps University), Mirjam Meiners (Justus-Liebig University), Petra Hartmann (Justus-Liebig University), Katharina Fleck (Justus-Liebig University), Alexander Perniss (Justus-Liebig University), Klaus Deckmann (Justus-Liebig University), Shuja Liu (Max Planck Institute for Lung Research), Stefan Offermanns (Max Planck Institute for Lung Research), Gabriela Krasteva-Christ (Saarland University), Wolfgang Kummer (Justus-Liebig University), Burkhard Schütz (Philipps University); schuetzb@staff.uni-marburg.de

Abstract:
Taste-sensing type 2 (bitter) receptors (Tas2R) are widely expressed in extra-oral tissues. In chemosensory (brush) cells of various epithelia they are linked to the canonical taste transduction cascade (CTTC), and serve to detect hazardous compounds at the mucosal surface. Airway and urinary bladder smooth muscle relax in response to bitter agonists through a still ill-defined pathway. Previously, we found that mouse gall bladder (GB) epithelium harbors many brush cells. We now determined the repertoire and cellular distribution of Tas2R and the CTTC in the mouse GB, and their impact on smooth muscle tone.

GB Tas2R expression patterns were assessed by RT-PCR and with in situ hybridization (ISH). ISH and immunohistochemistry were utilized to show the localization of the CTTC components. GB contraction was studied in organ bath recordings in wildtype, TRPM5-/-, and Tas2R triple-knockout mice (Tas2R143/135/126-/-). Intracellular calcium concentration ([Ca2+]i) was recorded in isolated cells.

mRNAs for several Tas2R, including members 108, 126, 135, 137, 138, and 143, were present in the GB, and localized to solitary cells in the epithelium. Also expression of CTTC members, including α-gustducin, PLCß2, and TRPM5, was restricted to brush cells. Bitter tastants (denatonium, quinine, noscapine) dose-dependently relaxed pre-contracted GB in wildtype, TRPM5-/- and Tas2R143/135/126-/- mice. Quinine and denatonium caused an increase in [Ca2+]i in isolated smooth muscle cells.

Bitter receptors and the taste transduction cascade are restricted to chemosensory cells in the mouse GB. The direct relaxant effect of various bitter agonists on GB smooth muscle operates through an independent pathway.

Poster 23

Titel:
Non-Neuronal Acetylcholine Released by Tracheal Solitary Chemosensory Cells Activates Mucocililary Clearance in the Murine Trachea

Autoren/Adressen:
Alexander Perniss (Justus Liebig University Gießen), Bernd Bufe (University of Applied Sciences Kaiserslautern, Zweibrücken), Keshavarz Maryam (Justus Liebig University Gießen), Amir Rafiq (Justus Liebig University Gießen), Anna-Lena Ruppert (Philips University), Aya Soultanova (Justus Liebig University Gießen), Gabriela Krasteva-Christ (Saarland University), Jochen Klein (Goethe-University Frankfurt), Ignaz Wessler (Johannes Gutenberg University Mainz), Frank Zufall (Saarland University), Burkhard Schütz (Philips University), Wolfgang Kummer (Justus-Liebig-University Giessen); Alexander.Perniss@anatomie.med.uni-giessen.de

Abstract:
Acetlycholine (ACh) is a powerful stimulator of mucociliary clearance (MC). Solitary chemosensory cells (SCC) express the ACh producing enzyme, choline acetyltransferase (ChAT) and components of the taste transduction cascade. We previously showed that bacterial signal peptides are detected by SCC. We here asked whether ACh is released by SCC upon stimulation.

Particle transport speed (PTS) was assessed on the mucosal surface of explanted tracheas. ACh release was quantified by HPLC in supernatants. Stimuli were either bacterial peptides in C57BL/6J-mice or LED stimulation (8Hz) in ChAT-ChR2(H134R)-YFP-mice. Channelrhodpsin-2-YFP (ChR2) expression in SCC was analysed by (immune)fluorescence microscopy.

The bacterial signal peptide FL185 (10 µM), produced by various pathogens, e.g. E. coli and Salmonella typhimurium, increased PTS from 44±2 to 75±3 µm/s (mean±SEM; $p<0.0001$; n=23). This effect was abolished by the muscarinic antagonists atropine (74±8 to 18±7%; p=0.0025; n=7) and 4-DAMP (66±16 to 11±4%; p=0.0037; n=6), but persisted after blocking nicotinic receptors (mecamylamine; 72±31 to 67±24%; p=0.7508; n=5) or voltage gated sodium channels (TTX; 124±43 to 103±24%; p=0.5207; n=5). ACh levels increased after stimulation with FL185 (70±17 to 158±36 nM; p=0.0203; n=6) in wildtype but not in TRPM5-KO mice (component of the taste transduction cascade); (112±70 to 60±20 nM; p=0.5000; n=3). LED stimulation of ChAT-ChR2-mice increased ACh from 18±5 to 40±9 nM, (p=0.0329; n=4). ChR2-YFP expression in the trachea was exclusively detected in SCC but not in nerve fibres or other cells.

Poster 24

Titel:
The scaffolding protein PATJ regulates ciliogenesis and epithelial morphogenesis

Autoren/Adressen:
Johanna H. Hinrichs (University Hospital of Münster), Pavel I. Nedvetsky (University Hospital of Münster), Michael P. Krahn (University Hospital of Münster);
j_hinr03@uni-muenster.de

Abstract:
Morphogenesis and formation of tissues and entire organs from single cells, requires a proper environment and external signal as well as their correct interpretation by the cells. Exposed to such an environment, cells polarize, differentiate and subsequently stop proliferation. In this study we investigate the role of the tight junction-associated polarity protein PATJ during formation of 3-dimensional epithelial structures (cysts) in Madin-Darby Canine Kidney (MDCK) epithelial cells.

We generated a stable PATJ-knockout MDCK cell line (dPATJ-MDCK) using CRISPR/Cas gene editing. To validate that the effects observed were due to deletion of PATJ, rescue experiments by reintroducing GFP-tagged PATJ were performed. All cell lines were analyzed in conventional and 3D cell culture.

When grown in 3D culture wild type MDCK cells formed well-polarized single lumen cysts. In contrast, dPATJ-MDCK formed much larger cysts with multiple lumens. These effects were rescued by reintroducing GFP-PATJ into dPATJ-MDCK cells. While proliferation of dPATJ-MDCK was drastically increased, multiple pro-proliferative signaling pathways (e.g. YAP-, Notch- and mTor-dependent signaling) were downregulated. Tight junction defects were rather modest in dPATJ-MDCK cells but their ability to form primary cilia was strongly perturbed.

We concluded that PATJ is essential for proper epithelial morphogenesis most likely due to its so-far unrecognized role in cilia formation suggesting a potential role of PATJ in the pathogenesis of ciliopathies.

Developmental Biology

Poster 25

Titel:
Birds and mammals share patterns of developmental apoptosis in the posterior placodal area

Autoren/Adressen:
Stefan Washausen (Westfälische Wilhelms-Universität Münster), Wolfgang Knabe (Westfälische Wilhelms-Universität Münster); w.knabe@uni-muenster.de

Abstract:
Placodes are focal thickenings which transiently exist in the head surface ectoderm of vertebrate embryos. They develop from anterior or posterior subsections of the panplacodal primordium and, together with neural crest cells, generate the peripheral nervous system. Recent experimental evidence shows that, in the posterior placodal area (PPA) of mammals, apoptosis assists in establishing physically separated otic and epibranchial placodes by eliminating anlagen of the mechanosensory lateral line system of anamniotes. Here, we aim to clarify whether conserved patterns of apoptosis exist in the PPA of mammals and birds.

To identify apoptotic patterns in Brown Leghorn embryos, histological, immunohistochemical and 3D reconstruction techniques were applied. Anti-Sox2 (Sex determining region y-box 2) was used to detect the paratympanic placode which, in chick embryos, additionally springs from the PPA.

Apoptosis starts along the ventral margin of the invaginating otic placode (HH11-HH13). From HH14 to HH18, apoptosis is found (1) adjacent to the otic porus/stalk, (2) between the otic placode and epibranchial placodes 1, 2 and/or 3, and (3) within dorsal parts of the epibranchial placodes 1 and 2. Thereafter, “interplacodal” apoptosis gradually disappears, whereas epibranchial placodes are apoptotically eliminated (HH19-HH26).

Our present (and previous) 3D reconstructions demonstrate that basically identical patterns of apoptosis pass through the PPA of chicks, C57BL6/N mice and primate-related Tupaia belangeri. We therefore expect that anlagen of the mechanosensory lateral line system also exist in birds. Pharmacological inhibition of apoptosis in chicks might help to fully explore the developmental potential of vestigial lateral line precursor cells in amniotes.

Poster 26

Titel:
Rhythmic patterns of neurulation-associated apoptosis in the spinal cord

Autoren/Adressen:
Stefan Washausen (Westfälische Wilhelms-Universität Münster), Thomas Scheffel (Medizinische Hochschule Brandenburg), Guido Brunnett (Technische Universität Chemnitz), Wolfgang Knabe (Westfälische Wilhelms-Universität Münster);
w.knabe@uni-muenster.de

Abstract:
Neural tube defects are found with an incidence of 0.5-2 per 1000 pregnancies. Hence, numerous studies have been carried out to clarify the mechanisms underlying proper neurulation. Whether and how spatiotemporally regulated patterns of apoptosis may contribute to neurulation is still subject to controversial debate. We therefore have investigated this issue by examining the developing spinal cord of the primate-related Tupaia belangeri.

To identify patterns of apoptosis in 13- to 17-day-old Tupaia embryos, histological, immunohistochemical and 3D reconstruction techniques were applied. Anti-Pax3 immunohistochemistry was used to detect premigratory neural crest cells.

Unexpectedly, it turned out that at least two craniocaudal waves of neurulation-associated apoptosis successively pass through the dorsal spinal cord. The first wave appears to be involved in neural fold fusion and/or postfusion remodeling, but probably also eliminates subpopulations of (displaced) premigratory neural crest cells. The second wave precedes and parallels formation of the roof plate. It thus may assist in establishing this signaling center which determines dorsal cell fates.

Craniocaudal waves of neurulation-associated apoptosis which pass at least twice through the dorsal spinal cord but, most probably, interact with different combinations of ectodermal cell types, are a fine example to highlight the difference between biological patterns and rhythms (Whitehead 1919). Our findings further help to reconcile seemingly inconsistent reports on chick and mouse embryos, and to create rules for computer simulations. Taking into account previous observations in T. belangeri, joint simulations of neurulation in the spinal cord and brain will have to simultaneously operate with different apoptotic rhythms.

Poster 27

Titel:
Spatiotemporal Pattern of Pax6 and Pax7 Expression in the Developing Neural Tube of Human Embryos

Autoren/Adressen:
Aimar Namm (1University of Tartu, 2Estonian University of Life Sciences), Andres Arend (University of Tartu), Marina Aunapuu (Estonian University of Life Sciences); aimar.namm@ut.ee

Abstract:
Regionalization of the neural tube is fundamental event in the development of the central nervous system (CNS). Early patterning of the CNS results in subdivision of the neural tube along the dorsal-ventral and cranial-caudal axes into the forebrain, midbrain, hindbrain and spinal cord. In human embryos Pax6 and Pax7 were identified as signalling molecules that are involved in the formation of the early spinal cord and brain.

The expression of Pax6 and Pax7 was examined in 29 human embryos by immunohistochemistry. The embryos were collected after legal abortions, fixed in 4% paraformaldehyde, embedded in paraffin and tissue blocks were serially cut in transversal direction. The embryos were classified according to Carnegie stages (CS). For immunohistochemistry the slices were incubated with primary antibodies of Pax6 and Pax7 and with a universal secondary antibody.

The expression of Pax6 and Pax7 had a tendency to increase in the later stages of the development both in the spinal cord and the brain. In the earlier stages of development these proteins were detected mostly in the dorsal part of the forming neural tube, confirming the role of Pax6 and Pax7 in the dorsal-ventral regionalization of the spinal cord and in the subdivision of the neural tube into vesicles. In CS 16-20 of the developing brain, Pax6 and Pax7 were expressed in the different regions of the forebrain midbrain and hindbrain

Thus, Pax6 and Pax7 are involved in the differentiation of neurons and establishment of cranial-caudal and dorsal-ventral boundaries of the developing human neural tube.

Poster 28

Titel:
Ossification of the Embryonal Human Spine

Autoren/Adressen:
Hannes Stofferin (Medical University of Innsbruck), Romed Hörmann (Medical University of Innsbruck), Michael Blumer (Medical University of Innsbruck), Helga Fritsch (Medical University of Innsbruck); hannes.stofferin@i-med.ac.at

Abstract:
Development of bones via a cartilaginous model involves the formation of ossification centers, comprises hypertrophy and mineralization of the cartilage, angiogenesis, resorption of the cartilage along with bone matrix deposition. Ossification of the human spine presumably requires similar processes, but convincing data is scarce. Therefore, we investigated in a series of human embryo-fetal specimens the formation of the vertebral ossification centers.

Eight embryo-fetal specimens from gestational week (GW) 8 to 12 as part of legal abortions according to Austrian law and parental consent were used. The whole spine was prepared from the samples, fixed in 4% paraformaldehyde buffered in PBS and embedded in paraffin. Frontal serial sections (7µm) were made through the entire spine and each twentieth section was stained with HE. Other sections were subjected to immunohistochemistry for localization of vascular endothelial growth factor (VEGF) and type I collagen (Col1). Furthermore, we investigated the occurrence of tartrate-resistant acid phosphatase (TRAP) cells.

At GW 9 chondrocytes were hypertrophic and small vessels penetrate into the cartilaginous anlagen of the vertebrae. The vessels were surrounded by mesenchyme and are similar to cartilage canals found in the epiphysis of long bones before the establishment of a secondary ossification center. Furthermore, at GW 12 a distinct layer of Col1 surrounded the canals and first signs of small ossification centers were present in several vertebrae.

Our results suggest that vertebrae ossify via the formation of cartilage canals that contribute to the establishment of the ossification centers.

Poster 29

Titel:
Effect of low oxygen tension on the multipotency and the regenerative potential of rat tendon-derived stem cells

Autoren/Adressen:
Mohamed I. Elashry (Justus Liebig University of Giessen), Asmaa Eldaey (Justus Liebig University of Giessen), Sabine Wenisch (Justus Liebig University of Giessen), Stefan Arnhold (Justus Liebig University of Giessen);
mohammed.elashry@vetmed.uni-giessen.de

Abstract:
Optimization of the stem cells niche would impact on the multipotent capacity. We examine the influence of low oxygen concentrations on the multipotency of rat tendon-derived stem cells (TSCs).

Achilles tendon of male Wister rats (n=4) were processed for TSCs extraction. TSCs was selected by using CD44 and CD90 labelled magnetic beads. The cells were induced for adipogenic, osteogenic and chondrogenic fate under normoxic (21% O2) and hypoxic (3% O2) atmosphere for up to 21 days. The expression of scleraxis and tenomoduline in TSCs were examined by using immunohistochemistry. TSCs viability was evaluated by using the MTT assay. TSCs adipogenesis, osteogenesis and chondrogenesis were examined using histological stainings for fat vacuoles, mineralization and mucopolysaccharides. The expression of adipogenic (FABP4 and PPARγ) and osteogenic (osteocalcin and osteopontin) gene expression were analyzed using RT-qPCR. Results were analyzed using a two-way ANOVA.

Immunofluorescence of TSCs showed cytoplasmic localization of scleraxis and tenomodulin. Hypoxia induced significant increase in cell viability after adipogenic and osteogenic inductions but not chondrogenic differentiation at days 7 and 14 compared to normoxia. Enhanced fat vacuoles and matrix mineralization were observed under hypoxia at days 7 and 21 post induction, respectively. Upregulation of the adipogenic and osteogenic relative markers were detected under hypoxia compared to normoxia. The expression of HIF1α revealed upregulation in all experimental groups under hypoxic culture conditions.

Hypoxia might enhance the multipotency of TSCs, the data point out that manipulation of oxygen tension should be considered during stem cells therapy.

Poster 30

Titel:
Electron microscopic analysis of ciliogenesis in multiciliary cells of the tracheal epithelium in the chicken embryo

Autoren/Adressen:
Alexander Reinke (University Medicine Rostock), Marcus Frank (University Medicine Rostock); marcus.frank@med.uni-rostock.de

Abstract:
Multiciliary cells with multiple motile cilia are present in various epithelia including the tracheal epithelium lining the upper airways. The proper generation and function of these cilia is critical for transport and efficient clearance of mucus. Ciliogenesis occurs during final differentiation of the multiciliary cells with the generation of hundreds of basal bodies that dock on the apical membrane and emerge nascent cilia. In the chicken embryo this differentiation starts following embryonic day 15 (E15). Recently, molecular mechanisms that govern basal body formation have been revealed, yet many signals and requirements for this developmental process remain largely unknown.

Tracheal epithelia of E14 to E18 chicken embryos were processed for scanning (SEM) and transmission electron microscopy (TEM). Anterior and distal (posterior) parts of the epithelium were examined for cilia formation. The ultrastructural studies were supplemented with immunofluorescence of ciliary markers, e.g. acetylated tubulin on paraffin sections.

SEM analysis shows that at E14 most epithelial cells presented with primary cilia only. preceeding a massive phase of ciliary sprouting in a rather short time interval between E15,5 and E17 in line with earlier TEM and histological observations. While we do not find an obvious anterior to posterior gradient in ciliogenesis along the tracheal epithelium, appearance of ciliated cells frequently occurs in distinct patches and stripe-like patterns. This suggests distinct signals from underlying cells or tissues that are no longer obvious in later developmental stages.

Comparative electron microscopy is a valuable tool to study structural-functional implications and related signaling during ciliogenesis in more detail.

Poster 31

Titel:
Core pluripotency factor Sox2 is not expressed in primordial germ cells of the rabbit“
Experimental Morphology

Autoren/Adressen:
Bernd Püschel (University Medical Center, Georg-August-Universität), Christoph Viebahn (University Medical Center, Georg-August-Universität); bpuesch@gwdg.de

Abstract:
Germ cells are exceptional in their competence to generate a totipotent zygote upon fertilization; they preserve and tightly control this extraordinary potential from specification of the germ cell lineage onwards, which – in mammals – occurs during gastrulation. Here, the early epiblast expresses the reciprocally acting transcriptional regulators Oct4, Sox2, and Nanog, of which the latter two are then downregulated upon primordial germ cell (PGC) specification. In mice, PGC expression of Sox2 and Nanog is soon regained while human and porcine PGCs reactivate Nanog only. Contrary to mouse Sox17 seemed to be a key regulator of human and porcine PGC specification taking effect upstream of Blimp1.

We speculated that the mouse may not be the odd one out in this respect and fixed day-6 to -8 rabbit embryos in 4% paraformaldehyde and subjected them either to in situ hybridization with riboprobes for Blimp1, Sox2, Nanog and Sox17 or to immunostaining using Oct4 and the PGC specific antibody PG2.

Similar to the mouse Nanog and Sox2 expression is reduced at the posterior pole of gastrulating rabbit embryos (stage 3-4). Later, Nanog expression is mostly gone at neurulation (stages 5-8) except for a strong upregulation at the posterior embryonic margin in single cells whose distribution is matched by Blimp1, PG2, or Oct4 labeled PGCs. Sox2 expression persists in the neuroectoderm only while PGCs harboring areas show no Sox2 labeling.

Whether posterior Sox17 expression in PGC harboring areas is taking effect upstream of Blimp1 in the rabbit as well, will be subject of future examinations.

Poster 32

Titel:
Human alveolar epithelial type I cells reconstructed in 3D – more than simple squamous cells

Autoren/Adressen:
Jan Philipp Schneider (Hannover Medical School), Christoph Wrede (Hannover Medical School), Jan Hegermann (Hannover Medical School), Matthias Ochs (Hannover Medical School), Christian Mühlfeld (Hannover Medical School); Jan Philipp Schneider (Hannover Medical School), Christoph Wrede (Hannover Medical School), Jan Hegermann (Hannover Medical School), Matthias Ochs (Hannover Medical School), Christian Mühlfeld (Hannover Medical School)

Abstract:
Alveoli of the lung are lined by alveolar epithelial type 1 (AE1) and alveolar epithelial type 2 cells. About 97% of the surface is covered by attenuated AE1 cell extensions to facilitate gas exchange. Previous studies suggested that AE1 cells can be branched and cross the interalveolar septa with their thin extensions. The current study explored the morphologic complexity of AE1 cells via serial block-face scanning electron microscopy (SBF-SEM) and three-dimensional (3D) reconstructions.

An archival sample from a human lung (Gehr et al. Respir Physiol. 32:121-140, 1978) was embedded according to Deerinck et al. (Microsc Microanal. 16:1138-1139, 2010), which includes ferrocyanide-reduced osmium tetroxide, thiocarbohydrazide, osmium tetroxide, uranyl acetate and lead aspartate. The tissue block was imaged by SBF-SEM to generate a stack of 2046 images (6144x6144 pixel², 18.5 nm/pixel, section thickness 80 nm). A substack of 901 images, comprising 110x105x72 µm³, was prepared for 3D reconstructions in IMOD (Kremer et al. J Struct Biol. 116,71-76, 1996).

Three entire AE1 cells were reconstructed. Reconstructions revealed the morphologic diversity of AE1 cells, a cell body spanning through the entire interalveolar septum, close proximity of excentric AE1 cell nuclei to AE2 cells, cell junctions between different domains of the same cell and a torus-like structure comparable with fenestrated endothelia or T-tubules in muscles. The latter properties enable closing the epithelial ring of an interalveolar pore by a single AE1 cell.

The results indicate an AE1 cell structure and plasticity unknown so far, which may be relevant during lung epithelial development, renewal and repair.

Poster 33

Titel:
Differential effects of high-fat or high-sucrose diet on murine alveolar epithelial type II

Autoren/Adressen:
Dagmar Jütte (Hannover Medical School), Christian Mühlfeld (Hannover Medical School), Julia Schipke (Hannover Medical School); juette.dagmar@mh-hannover.de

Abstract:
Obesity poses an increasing global health issue and is a risk factor for respiratory diseases like asthma or COPD. The objective of this study was to determine obesity-related effects induced by high-fat diet (HFD) and high-sucrose diet (HSD) on murine alveolar type II (AEII) cells.

Male C57BL/6N mice were fed control diet (CD), HFD and HSD for 30 weeks (n=5-7). Left lungs were instillation-fixed and processed according to design-based stereological standards. The samples were analyzed using light and electron microscopy.

Body-weight was significantly increased ($P<0.001$) in the HSD and HFD group compared to the CD group. Left lung volume was significantly higher in HFD, but not in HSD. No significant difference was found in the number-weighted mean volume or the apical surface of AEII cells. However, the total cell number was significantly higher in HFD ($P<0.001$) and HSD ($P<0.014$) compared to CD. The total volume, surface and volume-to-surface-ratio of lamellar bodies per AEII cell were not significantly altered by either diet. Only HSD-fed animals showed a significant increase in lipid droplet accumulation ($P<0.048$).

HFD induced more prominent increases in weight, lung volume and AEII cell number in comparison to HSD, however, only HSD led to lipid accumulation in AEII cells. Thus, AEII cell specific lipid accumulation seems to be influenced by other factors rather than lung volume or body mass.

Gross Anatomy/Clinical Anatomy

Poster 34

Titel:
Anthropological investigations at 4 skulls of the Meckel-Family

Autoren/Adressen:
Horst Claassen (Martin-Luther-University of Halle-Wittenberg), Rüdiger Schultka (Martin-Luther-University of Halle-Wittenberg); horst.claassen@medizin.uni-halle.de

Abstract:
We analyzed similarities between 4 skulls of the Meckel-Family: #1 Philipp Friedrich Theodor Meckel (1755-1803), #2 Heinrich Theodor Meckel (1785-1829), #3 Philipp Friedrich Meckel (1819-1847), #4 Bernhardt Albrecht Meckel (1823-1851).

All skulls were X-rayed, photographed and measured. The median sagittal outlines of the skulls were drawn with the help of X-ray photographs and grouped together.

The skulls are brachycran (#1-3) or ultrabrachycran (#4). The mediansagittal outlines of all 4 skulls lay cloth together, except for the glabella of #2. Concerning the forehead region, #3 and 4 have a persistent frontal suture while #1 and 2 lack it. The parietal bone region above the lambdoidal suture is flattened out in #1 and 4 only. If orbital height and breadth are compared, the orbital index of all is hypsiconch, and, if nasal height and breadth are compared, the nasal index of all is leptorhin. Furthermore, all skulls share a concave inverted nasal bone.

All 4 members of the Meckel-Family, representing grandfather (#1), son (#2) from the second marriage of #1 and two grandchilds (#3 and 4) belonging to another son of the second marriage of #1, named Albrecht August Meckel (1798-1829), show a lot of similarities. This is emphasized by the values for the orbital height: 40.5 mm in #1 and 4 or 35.5 mm in #2 and 3. Also nasal heigth of 55.0 and 53.0 mm in #1 and 2 and 48.0 and 50.0 mm in #3 and 4 reflects this relationship.

Poster 35

Titel:
Variations of digastric muscle and accessory bellies- a study of gross anatomic dissections and systematic review of literature

Autoren/Adressen:
Gürsel ORTUG (Bahcesehir University School of Medicine), Berna SIPAHI (Bahcesehir University School of Medicine), Alpen ORTUG (Istanbul Medipol University School of Medicine), Hasan Orkun IPSALALI (Bahcesehir University School of Medicine); alpenortug@gmail.com

Abstract:
The abnormality of anterior belly of digastric muscle (ABDM) is reported for long time and is not uncommon. However, there is still not a full consensus about the classifications. The aim of this study is to classify anterior belly of digastric muscle variations and compare with already existing data.

In the present study, the digastrics muscle has been analyzed bilaterally in 40 adult formalin fixed human cadavers. Dissections were performed at routine medical school laboratory classes

10 different types of variations in 15 dissections on anterior belly of digastric muscle and intermediate tendon were obtained out of 40. Previous results were utilized for classification of the variations. Anterior belly, intermediate tendon and posterior belly variations were evaluated according to unilateral, bilateral, central and unspesific. Thus, four different types of variations were unilateral and four different types of variations were bilateral at ABDM. Only one accessory belly was found obliquely residing at central and can be classified as “mentohyoid muscle” and the last case had intermediate tendon variation as piercing stylohyoid muscle. Remaining variation was crossing anterior belly of digastrics muscle fibers at central line. Posterior belly exhibited no variation.

Reporting the abnormalities and variations of digastric muscle is very important in surgical attempts and evaluation of the lymphadenopathy of submental area and in the floor of the mouth tumors, numerous clinically significant esthetic surgeries for head and neck surgeons, radiologists and plastic surgeons. More detailed information about ABDM can be obtained with ultrasonography, CT and MR.

Poster 36

Titel:
Gross anatomical variations among and within the incisive foramen

Autoren/Adressen:
Hasan Orkun İpsalali (School of Medicine, Bahcesehir University), Nil Kocanali (School of Medicine, Bahcesehir University), Alpen Ortug (School of Medicine, Medipol University), Gursel Ortug (School of Medicine, Bahcesehir University); hoipsalali@outlook.com

Abstract:
The aim of this study is to present the locational variations of the foramina of Scarpa and the incisive canals as well as the shape and the location of the incisive foramen itself

97 skull specimens were collected from different sources to create a pool of randomized skulls and were analyzed by Celestron brand digital microscope. The specimens were photographed. Additional analysis on the photographs were remade.

Out of 97 specimens (42 men, 55 women), 3 were excluded due to broken bone. In the analysis of the incisive foramen shape, 6 shapes were classified and are as follows; piriformis 34%, butterfly 7.4%, oval 26.6%, triangular 25.5%, rhomboid 5.3% and ellipsoid 1.1%. Majority (92%) of the foramen were aligned at the midline of the hard palate, where 4.3% was positioned to the right and 3.2% positioned to the left. The study presented a single incisive canal located on the midline in 40%, and two incisive canals located on the right and the left sides of the midline in 60% of the specimens. The presence of the foramen of Scarpa analysis revealed that a single foramen of Scarpa was present in 24.4% of the specimen and two foramina of Scarpa were present in 10.6 % of the specimen all located on the midline.

The gross anatomical analysis of the foramen and its contents are analyzed poorly in the literature; such variations and presences should be acknowledged and must be considered for the procedures involving this area

Poster 37

Titel:
Loss of muscle strength prior to knee replacement: a question of anatomical crosssectional area or specific-strength?

Autoren/Adressen:
Adam Culvenor (Paracelsus Medical University), Felix Hamler (Paracelsus Medical University), Jana Kemnitz (Paracelsus Medical University), Wolfgang Wirth (Paracelsus Medical University), Felix Eckstein (Paracelsus Medical University); adam.culvenor@pmu.ac.at

Abstract:
To elucidate whether loss in thigh muscle strength prior to knee replacement (KR) is caused by reductions of muscle anatomical cross sectional area (ACSA) or specific-strength.

All 100 Osteoarthritis Initiative participants who received a KR (cases) and had thigh isometric muscle strength and MRI recorded (58 women, age 65±8years, BMI 29±5kg/m2) were matched with a control (no KR) for age, sex, height, BMI, and radiographic severity. Thigh muscle ACSAs were determined from MRI at the visit before KR (T0), and two years before T0 (T-2). Specific-strength was calculated (strength÷ACSA) and the measures compared by conditional logistic regression.

KR cases displayed significantly smaller extensor ACSA than controls at T0 (women: pain-adjusted OR [ORadj] 1.89; 95%CI 1.05-3.90; men: ORadj 2.22; 95%CI 1.04-4.76), whereas no significant differences were found at T-2. Women with KR (but not men) displayed lower extensor specific-strength at T0 (OR 1.59 [1.02-2.50]), although this difference did not maintain significance after adjustment for pain (ORadj 1.22 [0.71-2.08]). Female cases lost significantly more extensor specific-strength between T-2 and T0 than controls (ORadj 3.76; 95%CI 1.04-13.60), whereas no significant differences were noted at T-2, or in men.

In women, a significant reduction in knee extensor strength prior to KR appears to occur through two mechanisms: one driven by pain (loss in specific-strength) and one independent of pain (loss in muscle ACSA). Men with KR displayed significantly lower extensor ACSA, but not significantly lower strength or specific-strength.

Poster 38

Titel:
Age-related Reductions in Thigh Muscle Strength in Men and Women are Due to Loss in Muscle Mass, but not Loss in Muscle Quality

Autoren/Adressen:
Rojina Shakya (Kathmandu University School of Medical Sciences), Jana Kemnitz (Paracelsus Medical University Salzburg and Nuremberg), Adam Culvenor (Paracelsus Medical University Salzburg and Nuremberg), Wolfgang Wirth (Paracelsus Medical University Salzburg and Nuremberg), Rajeev Shresta (Kathmandu University School of Medical Sciences), Felix Eckstein (Paracelsus Medical University Salzburg and Nuremberg); felix.eckstein@pmu.ac.at

Abstract:
Muscle strength and mass (anatomical cross-sectional areas; ACSA) are known to decline with age, but it is controversial whether this is accompanied by an age-dependent loss in muscle quality (“specific” strength, i.e. strength per muscle ACSA).

We studied 174 (of 4796) Osteoarthritis Initiative participants (71 men, 46-79y; 103 women, age 45-78y) who did not have radiographic knee osteoarthritis or knee/hip pain in the limb studied, and not more than infrequent knee/hip pain in the contralateral limb. Isometric knee extensor and flexor strength (N) were determined from the “Good Strength Chair”, and thigh muscle, adipose tissue and femoral bone ACSAs from axial thigh MRIs.

Older age was associated with less extensor strength (r^2=7% in men; p=0.03, and r^2=9%; p=0.002 in women), and with lower quadriceps ACSAs (r^2=25%; p<0.001; r^2=30%; p<0.001, respectively). However, extensor specific strength (men: p=0.71; women: p=0.94), intermuscular fat (men: p=0.31; women: p=0.81), and subcutaneous fat (women: p=0.62) were not significantly associated with age, except for a marginally significant relationship of less subcutaneous fat in older men (r^2=6% p=0.04). Findings for flexor strength, ACSAs, and specific strength were similar to those in the extensors.

Age-related associations in thigh muscle strength appear to be due to loss in muscle mass, but not to loss in muscle quality (specific strength) in both men and women. Maintaining muscle mass hence appears key to maintaining strength and function during aging.

Poster 39

Titel:
The usefulness of sigmoid colon mesentery in fetal ultrasonography in normal pregnancy. A pilot study

Autoren/Adressen:
Slawomir Wozniak (Department of Human Morphology and Embryology, Wroclaw Medical University,), Jerzy Florjanski (Wroclaw Medical University), Henryk Kordecki (Wroclaw University of Technology), Marzena Podhorska-Okolow (Department of Human Morphology and Embryology), Zygmunt Domagala (Department of Human Morphology and Embryology, Wroclaw Medical University);
slawomir.wozniak@umed.wroc.pl

Abstract:
The aim of this project is to measure a square area of sigmoid colon mesentery in fixed foetuses and disclose the mesocolon mesentery in routine fetal ultrasound examination in living subjects.

209 formalin fixed human fetuses (100 female and 109 male) aged from 4th to 7th gestational months (102-203 days) were examined. The square surface area of the mesocolon was measured. Correction for formalin induced shrinkage was applied. Pilot ultrasound fetal examinations were performed in living pregnant women.

The square surface areas of the sigmoid colon mesentery found for respective gestational months were as follows: month 4: 33.24-51.95mm2; month 5: 49.63-77.6mm2; month 6: 106.89-167.15mm2 and month 7: 145.69-272.53mm2. We conducted the pilot measurements in living subjects – the sigmoid colon mesentery was disclosed and measured.

The disclosing and measurement of the sigmoid colon mesentery can be used as a simple parameter applied in routine fetal ultrasonographic to evaluate the status of intrauterine status.

Poster 40

Titel:
Evaluation of soft tissue damage during minimal invasive hallux valgus surgery – an anatomical study

Autoren/Adressen:
Lukas F Reissig (Medical University of Vienna), Lukas Albrecht (Medical University of Vienna), Stefan Quadelbauer (AUVA Trauma Hospital Lorenz Böhler), Joachim Klikovics (Federal Hospital Güssing), Wolfgang J Weninger (Medical University of Vienna), Martin Kaipel (AUVA Trauma Hospital Lorenz Böhler);
lukas.reissig@meduniwien.ac.at

Abstract:
Surgical correction of a hallux valgus deformity according to BOESCH is a standard procedure. Yet, the risk of damaging the periarticular soft tissues during surgery is unknown and the impact of the surgeon's experience with this technique on the outcome of the operation was never examined.
This study aims at examining the risk of injuring surrounding soft tissues when performing a BOESCH operation and at evaluating the influence of the experience of the surgeon on the outcome of the operative procedure.

A BOESCH operation was performed on 40 fresh, non-embalmed feet with hallux valgus deformity stemming from body donors that donated their body to the Medical University of Vienna, Division of Anatomy. 20 specimens were assigned to group A and were operated by an experienced surgeon. Another 20 specimens were operated by untrained residents (group B). After surgery the specimens were dissected and the soft tissue damage was evaluated with special attention to the nerves and arteries of the hallux.

In 1 specimen assigned to group A the lateral dorsal digital nerve of the hallux was injured. In group B the nerve was injured in 6 specimens. Also other complications occurred significantly more often in group B.

The results show an increased risk of perioperative injury of the lateral dorsal digital nerve of the hallux and a significant influence of the surgeon's experience on the overall complication rate.

Poster 41

Titel:
Morphofunctional relationship between the stomatognathic and the locomotory system

Autoren/Adressen:
Jochen Fanghänel (University Medical Hospital Regensburg), Peter Proff (University Medical Hospital Regensburg), Christian Kirschneck (University Medical Hospital Regensburg); christian.kirschneck@ukr.de

Abstract:
Numerous authors (Barré 1926, Costen 1997 et al.) have already confirmed that there is a connection between the stomatognathic and the locomotory system. These interrelations are based on structural, pathological and syndromal commonalities; in addition, body and lower jaw posture are known to influence each other (Piekarz, Andreotti 2005).

The morphological and functional interdependencies are manifold due to corresponding anatomical structures. There are bony, muscular, fascial, ligamentous, vascular, lymphatic and neurological connections between the stomatognathic and the locomotory system. Pathological and syndromal relationships manifest themselves in joint involvement of both systems in diseases.

Scoliosis patients, for example, showed more midline shifts and posterior crossbites in our own investigations. The following diseases and syndromes are related to both the stomatognathic and the locomotory system: osteoporosis, osteophytes, fibrodysplasia ossificans progressiva, rheumatoid arthritis, ankylosing spondylitis, spinal muscular atrophy, muscular dystrophy (Duchenne type), vascular disease (e.g. atherosclerosis), neurological diseases (e.g. Parkinson's disease) and tumors (e.g. multiple myeloma).

From our investigations it must be concluded that the (dental) medical view in diagnosis and treatment must not be limited to a single organ system. Interdependencies and connections between the stomatognathic and the locomotory system should be taken into account, if a holistic therapy of the patient is to be achieved.

Poster 42

Titel:
The abnormality of lateral femoral cutaneous nerve of thigh positioning

Autoren/Adressen:
Nikityuk D.B.1, Klochkova S. V.2, Alexeeva N. T.3, Kvaratskheliya A.G.3
1Federal Research Centre of the Nutrition, the Biotechnology and the Food Safety
2Fist Sechenov State Medical University, Moscow, Russia
3Burdenko Voronezh State Medical University, Voronezh, Russia; alexeevant@list.ru

Abstract:
The abnormality of lateral femoral cutaneous nerve of thigh positioning was first mentioned in 19th century. Described its atypical position to the anterior superior iliac spine. In typical cases, the nerve emerges under the lateral edge of the psoas muscle, sometimes penetrates through it. Located under the iliac fascia, it directed along the anterior surface of the iliac muscle to the anterior upper iliac spine. It appears on the thigh medial to the last under the lateral part of the inguinal ligament in the muscle lacuna behind the deep artery, enveloping the Ilium. It then penetrates the fascia lata of the thigh, and then branches into the skin on its outer surface, passing to the knee joint.
During the dissection of cadaver fixed in 10% formalin the abnormality is discovered – dystopia of right and left lateral femoral cutaneous nerve of thigh. The right lateral femoral cutaneous nerve of thigh went down the inguinal ligament in muscular lacuna on the border between her middle and inner thirds more laterally than femoral nerve. The left lateral femoral cutaneous nerve of thigh went through the muscular lacuna in the same way as the right but at the level middle third of inguinal ligament. 25% of the population has variant lateral nerve of thigh positioning. The above-described case of bilateral abnormality of external cutaneous nerve of thigh needs to be considered in traumas in anterior surface upper third of the thigh, herniectomy and operations in area of iliac fossa.

Immune Biology

Poster 43

Titel:
Significant impact of leptin on the morhology and intra-cellular cofilin of Natural Killer cells

Autoren/Adressen:
Jana Oswald (Martin Luther University Halle-Wittenberg), Maximilian Büttner (Martin Luther University Halle-Wittenberg), Simon Jasinski-Bergner (Martin Luther University Halle-Wittenberg), Philip Rosenstock (Martin Luther University Halle-Wittenberg), Roland Jacobs (Hannover Medical School), Heike Kielstein (Martin Luther University Halle-Wittenberg); oswaldjana@web.de

Abstract:
Obesity is a medical condition characterized by excessive body fat and is a serious and escalating public health problems affecting all age and socioeconomic groups. Obesity is associated with hyperleptinemia and as a consequence with immune dysfunction and carcinogenesis. Natural Killer (NK) cells, are mediators of anti-tumor immunity and the most actively migrating cells among leukocytes. Actin rearrangement, promoted by cofilin plays a central role in cellular migration.

We used human NK-92 cells to explore the in vitro effects of leptin on phosphorylation of cofilin, co-localization of cofilin and F-actin and on morphological changes in NK cells. NK-92 cells were incubated with different leptin concentrations (10 and 100 ng /ml). After 30min and 24h immunohistochemistry was performed and proteins were isolated. Western blot analysis was conducted to evaluate the influence of leptin on phosphorylation of cofilin. Utilizing confocal microscopy, the co-localization of cofilin and F-actin and the cellular morphology was analyzed.

Results show that the co-localization of cofilin and F-actin as well as changes in the length of filopodia were affected by leptin. No effects of leptin on the phosphorylation of cofilin in NK-92 cells could be observed.

In summary, the present study demonstrates an impact of a leptin stimulation on the filopodia length, and a time-dependent effect on the co-localization of cofilin and F-actin in NK- 92 cells. An altered migration competence may be causal for a decreased tumor cell killing of NK cells in obese individuals.

Poster 44

Titel:
Macrophage polarization and crown-like structures in the epicardial and subcutaneous fat of heart surgery patients

Autoren/Adressen:
Tomáš Kučera (First Faculty of Medicine, Charles University), Aneta Pierzynová (First Faculty of Medicine, Charles University), Jana Kloučková (First Faculty of Medicine, Charles University), Jaroslav Lindner (Charles University and General University Hospital), Michal Lipš (First Faculty of Medicine, Charles University and General University Hospital), Martin Haluzík (Institute for Clinical and Experimental Medicine); tkucer@lf1.cuni.cz

Abstract:
In both animal as well as human adipose tissue in obesity, crown-like structures (CLS) consisting of adipocytes surrounded by macrophages can be observed. The macrophages in adipose tissue represent two different populations, situation described as macrophage polarization. In our work, we focused on the detection and quantification of CLS in epicardial fat (EF) and subcutaneous fat (SF) of patients with obesity (OP) and ischemic heart disease (IHD).

We used adipose tissue samples obtained from heart surgeries. A total of 44 EF samples, and 45 SF samples were divided into groups according to whether they were in OP group, IHD group or not. Immunohistochemistry was used to visualize CD68-PGM1+ macrophages, CD11c+ M1 cells and active-caspase3-positive apoptotic cells in CLS.

In all samples from both SF as well as EF tissue we found numerous scattered CD68-PGM1+ cells. At least one CLS was observed in 4 EF samples and 6 in SF. In SF of OP, 4 out of 19 samples contained CLS, while 2 out of 26 samples from non-obese group was CLS+. All CLS+ samples from EF tissue were from IHD patients. All CLS were also positive for M1 cell marker CD11c. Apoptotic cells were not detected in CLS.

CLS structures are occasionally found in both subcutaneous as well as epicardial tissue of patients undergoing open heart surgery. The relationship between CD11c+ CLS in EF tissue and IHD suggests the role of EF macrophage polarization in IHD pathogenesis.
Supported by Project AZV 15-26854A and PROGRES Q25.

Poster 45

Titel:
The number of mast cells in epicardial adipose tissue is increased in cardiac surgery patients with coronary artery disease

Autoren/Adressen:
Karolína Rozsívalová (First Faculty of Medicine, Charles University), Aneta Pierzynová (First Faculty of Medicine, Charles University), Jana Kloučková (First Faculty of Medicine, Charles University), Jaroslav Lindner (First Faculty of Medicine, Charles University), Michal Lipš (First Faculty of Medicine, Charles University and General University Hospital), Martin Haluzík (First Faculty of Medicine, Charles University and General University Hospital), Tomáš Kučera (First Faculty of Medicine, Charles University and General University Hospital); tkucer@lf1.cuni.cz

Abstract:
Inflammation of adipose tissue can affect its metabolic activity associated with the pathogenesis of coronary artery disease (CAD). Mast cells represent an important component of the innate defense system. In our work, we quantified mast cell counts in epicardial adipose tissue (ET), subcutaneous adipose tissue (ST), and atrial myocardium (AM) in patients undergoing open heart surgery with various metabolic diseases.

Bioptic samples of ET (n = 44), ST (n = 42) and AM (n = 17) were fixed by 4% paraformaldehyde and embedded into paraffin. An anti-mast cell tryptase antibody was used for immunohistochemical detection and quantification of mast cells. The expression of CD117 and chymase was also demonstrated immunohistochemically.

In ET of patients with CAD, a higher number of mast cells was found compared to patients without CAD (3.7 ± 2.6 vs. 2.1 ± 1.2 cells / mm2). Higher number of mast cells in ET of patients with obesity and type 2 diabetes mellitus (DM2T) was not statistically significant. In ST and AM there was no difference in the number of mast cells in patients with obesity and without obesity, neither in patients with CAD or without CAD nor in patients with DM2T and without DM2T. Mast cells in ST, ET and AM expressed CD117 and chymase.

An increased number of mast cells in ET patients with CAD may indicate the specific role of these inflammatory cells in relation to epicardial adipose tissue and coronary arteries affected by atherosclerosis.
Supported by Project AZV 15-26854A and PROGRES Q25.

Methods/Teaching

Poster 46

Titel:
Evolution of the human organ systems

Autoren/Adressen:
Bath M., Polzer C., Horn F. Innsbruck (Austria); post@florian-horn.de

Abstract:
Ausgehend von der alten Idee einer vergleichenden Anatomie, soll der Frage nachgegangen werden, ob es für die Lehre hilfreich und die Forschung spannend ist, sich gründlicher mit der Evolution der menschlichen Organsysteme zu beschäftigen.

In die Anatomie fügen sich diese Überlegungen nicht nur als Grundlage für die Embryologie, sondern auch, weil sich Evolution maßgeblich auf histologischer Ebene abspielt.

Als Hintergrund seien auch Fortschritte in der ‚Evolutionären Medizin' (mit einem Institut in Zürich) genannt, wodurch sich die grundsätzliche Frage nach einem Beitrag der Anatomie stellt.

Innerhalb von knapp zwei Jahren sind in der Anatomie in Innsbruck Seminare zur ‚Evolution und Embryologie der Organsysteme' entwickelt und gehalten worden; zudem wurden zentrale Themen auch direkt in den Sezier-/ Präparierkurs integriert.

Die Ausgangsfrage, warum Organe so (und nicht anders) aufgebaut sind und funktionieren, beschäftigt Studenten erfahrungsgemäß in besonderer Weise. Sie wird in zahlreichen Einzelpublikationen seit einigen Jahren verstärkt wissenschaftlich bearbeitet, die Inhalte sollten aber zusammengeführt (und gelehrt) werden.

Da es sich um eine Neubelebung alter Forschungsüberlegungen mit neuen Möglichkeiten handelt, stehen die Grundlagen für viele Forschungsfragen offen. Als Beispiel seien Überlegungen zur Evolution des Stoffwechsels genannt: Insulin in Einzellern, Evolution des Pankreas, Embryologie … Metabolisches Syndrom.

Betrachtungen des Gesamtorganismus' gehören mit ihrer Entstehungsgeschichte trotz (oder wegen) ihrer Interdisziplinarität in die Anatomie. Das Eindringen evolutionärer Überlegungen in die Medizin wird fortschreiten, und die Anatomie sollte dabei eine inhaltliche Führungsrolle übernehmen.

Poster 47

Titel:
Storing of primary antibodies for weeks in a pre-diluted formulation has no impact on staining quality

Autoren/Adressen:
Henrike Link (Ludwig-Maximilians-University of Munich), Markus Kipp (Ludwig-Maximilians-University of Munich); henrike.link@live.de

Abstract:
Immunohistochemistry (IHC) is used for disease diagnosis or biological research. The use of primary antibodies makes IHC a relatively expensive method. The storage of pre-diluted antibodies for a later immunohistochemical application would be a cost effective strategy. Therefore, the objective of this project was to test whether primary antibodies can be stored before using them in IHC experiments.

Stability of pre-diluted primary antibodies was tested by diluting them in normal goat serum, and storage at 4°C for one month. The following antibodies were included: Anti-IBA1 to detect microglia, anti-OLIG2 to detect oligodendrocytes, anti-GFAP to detect astrocytes, and anti-PLP to detect myelin. Sequential mouse brain slides were stained with either stored antibodies, or antibodies prepared freshly prior to IHC experiments. The staining with each primary antibody was done in parallel under the same staining conditions. Staining intensity was finally quantified by either measuring the optical density or counting particle densities in a blinded fashion.

No obvious difference with respect to cellular morphology and staining intensity was observed in slides stained with either fresh or stored primary antibodies. In particular, densities of microglia, astrocytes and oligodendrocytes were equal. Comparably, anti-PLP staining intensities in the white matter tract corpus callosum were similar.

Storage of pre-diluted antibodies in the refrigerator is an efficient, cost-effective and economical strategy for IHC. This is especially relevant for primary antibodies requiring high dilutions. Further experiments have to show whether this applies for other primary antibodies as well, and how long such pre-diluted antibodies are stable.

Poster 48

Titel:
Anatomical Museums For All Ages Anatomy Education

Autoren/Adressen:
Nil Kocanali (School of Medicine, Bahcesehir University), Ahmet Midi (School of Medicine, Bahcesehir University), Hasan Orkun İpsalali (School of Medicine, Bahcesehir University), Gürsel Ortuğ (School of Medicine, Bahcesehir University); nilkocanali@gmail.com

Abstract:
The history of the "museum" movement is quite old in Western culture. From the beginning, the aim and the content have been improved by the purpose. Universities had an important role within the "museum" activity and in the creation of special "Science Museums". In every discipline, besides the collection and classification of its own preparations and their presentation, the use of these scientific-based materials in research and teaching is also very effective way of education.

After the beginning of establishing a small boutique anatomy museum in Bahçeşehir University School of Medicine, has been organizing lecture conferences and seminars for different age groups for the past four years presenting history of human anatomy and human body. This study has been approved and supported by TUBITAK (the project number : 118B441).

Department receives many applications from different schools (primary school to high school) for being a part of the project.

Eventhough improved technnology allows people to search online and visualize human body easily, modern anatomy museums would give comparatively real specimens with normal and pathological anatomy. Another point of view is these museums are an open educational field for not only medical or health sciences students but also from primary school kids to high school students. In addition to this, besides the activities such as conferences, workshops, material preparation carried out with regards to various age groups, instructional movies and information to be given about the diseases, under the roof of “Anatomy Museum”, will play an important role on creating a healthy society.

Poster 49

Titel:
The advantages of the interactive mixed reality ‘Magic Mirror‘ system as an additional tool for integrated radiology and gross anatomy education

Autoren/Adressen:
Felix Bork (Technical University Munich), Leonhard Stratmann (Ludwig-Maximilians-University Munich), Stefan Enßle (Ludwig-Maximilians-University Munich), Nassir Navab (Technical University Munich), Jens Waschke (Ludwig-Maximilians-University Munich), Daniela Kugelmann (Ludwig-Maximilians-University Munich); Daniela.Kugelmann@med.uni-muenchen.de

Abstract:
Interpretation of radiological images is highly relevant for clinical purpose. Integrating radiology into the anatomical course has been recognized as an effective way to achieve early exposure to medical images while simultaneously increasing students’ motivation and their understanding of both radiology and gross anatomy.

749 medical students worked on additional tutorial exercises during the dissection course with the ‘Magic Mirror’ system and the Anatomage virtual dissection table. To compare the potential of these systems with respect to their additional teaching value, we surveyed all participants during the course and evaluated their feedback. To quantitatively verify these findings and to measure the learning effect of both systems, we established a follow-up elective course with 76 students with equal anatomical knowledge and compared the performance of students during a series of tests for three different groups: Anatomage, ‘Magic Mirror’, and atlas-based theory.

Our results demonstrate that the ‘Magic Mirror’ can be used effectively as a low-cost AR teaching device for interactive, student-centered learning during an integrated radiology and gross anatomy course. We observed a comparable learning effect in all groups, with slight advantages for the ‘Magic Mirror’ system. In the theory group, students with high mental rotation test (MRT) scores improved significantly more that students with low MRT scores. Interestingly, this was not the case for the ‘Magic Mirror’ and Anatomage groups.

Exploring radiological data with both systems improves the students' spatial understanding of anatomical structures independent of their comprehension in this area and thus may serve to enhance learning outcome.

Poster 50

Titel:
Voronoi tesselation for quantification of the microvascular pattern in the histological section of the myocardium

Autoren/Adressen:
Jaromír Šrámek (First Faculty of Medicine, Charles University), Aneta Pierzynová (First Faculty of Medicine, Charles University), Tomáš Kučera (First Faculty of Medicine, Charles University); jsram@lf1.cuni.cz

Abstract:
Pathogenesis of the cardiovascular diseases involves usually disturbances in several processes including remodelation of the microvascular network. Common technique used to the description of the microvascular network is assessment of the microvascular density in the histological section. Unfortunately, this technique neglects disturbances in the regularity of the microvascular pattern. Main goal of our work is to improve description of the microvascular pattern in the histological section.

We used several samples of the myocardium from both left and right chambers obtained during the cardiac surgery. The samples were processed for routine immunohistochemical staining, the CD31 immunostain was used in order to highlight the cross-sections through the capillaries perpendicular to the plane of section. To analysis of the point-pattern, we performed the Voronoi tesselation with the centerpoints in manually marked vessels. Each vessel was represented using the area of given Voronoi cell

We tried to use the Pareto's, log-normal, and gamma distribution for fitting of the areas of Voronoi cells. We performed non-linear fitting in the R language (ver. 3.2.3) using packages fitdistrplus, stats, and rmutil. For each sample, the best value of the Akaike information criterion was obtained for the log-normal distribution. Therefore, parameters of this model, i.e. the mean and the standard distribution of logarithms of areas of Voronoi cells, describe the point-pattern by the best way.

Voronoi tesselation-based approach can improve quantitative description of the microvascular pattern on the histological section. Especially, parameters of the model contain more information than the microvascular density alone.

Poster 51

Titel:
Evaluation of students' exam preparation strategies and the satisfaction of students'- and teachers'- with current anatomical testing strategies at the Charité-Universitaetsmedizin Berlin

Autoren/Adressen:
Anna Neugebauer (Charité- Universitaetsmedizin), Susanne Werner (Charité-Universitaetsmedizin), Maren März (Charité- Universitaetsmedizin), Irene Brunk (Charité- Universitaetsmedizin); anna.neugebauer@charite.de

Abstract:
We evaluated the exam preparation strategies of medical students in anatomy and intended to reflect the level of satisfaction among students and teachers to stabilize or even improve the quality of anatomical education.

We conducted an anonymous survey to evaluate possible varieties in students' exam preparation for three different anatomical testing strategies (3D-MC-examination using specimen, practical oral examination using specimen, written MC-examination). Furthermore, we investigated the level of satisfaction among teachers and students regarding the different anatomical testing strategies.

91.52% of the conducted students stated to use anatomical specimen during the exam preparation for the 3D-MC-examination (n= 224). To prepare for the practical oral examination, 80.58% of the students declared to use anatomical specimen (n= 103). Whereas only 35.94% of the students used anatomical specimen to prepare for the MC-examination (n= 207). 54.39% of the students (n= 228) and 94.12% of the teachers (n= 17) preferred the oral practical examination in anatomy to written exams. At the same time, 80.80% of the students chose the 3D-MC-examination to be the most effective anatomical exam strategy. Whereas 88.24% (n= 17) of the teachers preferred the oral practical examination. At the same time, 86.41% (n=103) of the students graded the oral practical examination with the highest level of difficulty.

An important aspect of learning anatomy is to understand the topography between organs. Testing anatomy using the written MC-examination neglects these three-dimensional aspects. Our survey shows that students and teachers prefer the oral and practical exam strategies. Nevertheless, the current development of the anatomical testing does not reflect this position.

Poster 52

Titel:
Volumetric reconstruction of digitized histological serial sections from a human fetal tissue sample

Autoren/Adressen:
Michael Scholz (FAU Erlangen-Nürnberg), Simone Gaffling (FAU Erlangen-Nürnberg), Friedrich Paulsen (FAU Erlangen-Nürnberg), Bernhard N. Tillmann (CAU Kiel); michael.scholz@fau.de

Abstract:
For a better understanding of the specific tissue situation of anatomical structures, volumetric 3D data out of histological serial sections can be analyzed. The reconstruction of the original tissue from a digitized histological image sequence can immensely facilitate the perception of the morphology and the spatial tissue structure.

An important requirement is, however, that the final reconstruction should restore the anatomy in a way that ideally matches to the original in vivo tissue situation before it was histologically processed.

We present the volumetric reconstruction of histological serial sections on the example of the head of an early human fetus (3 fm), which was digitally scanned and virtually reconstructed with the HiD (HistoDigital) application.

The goal of our presentation is to demonstrate and highlight the state of the art progress in virtual tissue reconstruction.

Poster 53

Titel:
Realization of the new Maternity Protection Law in the anatomical education of students of human medicine

Autoren/Adressen:
Christoph Kulisch (Charité - Universitätsmedizin Berlin), Evelyn Heuckendorf (Charité - Universitätsmedizin Berlin), Jana Langheinrich (Charité - Universitätsmedizin Berlin), Imre Vida (Charité - Universitätsmedizin Berlin), Irene Brunk (Charité - Universitätsmedizin Berlin); christoph.kulisch@charite.de

Abstract:
The new hazard classification of formaldehyde from 01.04.2015 prohibits the participation of pregnant and breast-feeding students in anatomical dissection courses. A recent change of the Maternity Protection Law on 01.01.2018 included an expansion on students. Both necessitated alternatives for the regular courses and exams.

The inventory of plastinates and models within the department of anatomy had to be reviewed and expanded to cover the anatomical curriculum of the third and fourth semesters. New concepts had to be developed for these weekly practical courses. In order to fully convey the content, teaching strategies partially different from the regular practical course had to be chosen (i.e. mini-reviews of students, more descriptions by the lecturer, broader discussions). The exam at the end of each semester had to be adapted to the changed situation as well.

Writing new concepts for both semesters with concomitant handbooks for students required half a year of intensive planning and structuring. The alternative practical courses were carried out in the summer semester of 2017 for the first time, and have continued since then. In the summer and winter semester of 2017, 5 to 12 students attended the two courses. First exams indicate that these students are not disadvantaged in learning anatomy.

These alternative courses perfectly realize the new Maternity Protection Law in anatomical teaching.
Because students do not dissect a human body by themselves, we recommend this form of anatomical teaching only as an exceptional solution for pregnant and breast-feeding students.

Poster 54

Titel:
Double electroporation to target gene expression in two adjacent tissues in chicken embryos

Autoren/Adressen:
Margarethe Draga (Universität zu Köln), Valentina Safronjuk (Universität zu Köln), Martin Scaal (Universität zu Köln); margarethe.draga@uk-koeln.de

Abstract:
In ovo-electroporation is a well-established method to introduce transgenes into a number of tissues in chicken embryos, e.g. neural tissue, limb mesenchyme and somites. This method has been widely used to investigate cell lineage, cell morphology and molecular pathways by localized expression of fluorescent reporter constructs and of genetic constructs for gain- and loss-of-function experiments.

We have developed a new technique to double electroporate two adjacent tissues with two different plasmids using an electroporation chamber.

As proof of principle, we electroporated the dorsal surface ectoderm with a reporter construct expressing mCherry, and the subjacent somites with a reporter construct expressing EGFP.

This technique allows to separately target different genes of interest in neighboring tissues and will be useful to investigate the cellular and molecular interaction between neighboring tissues during embryonic development.

Poster 55

Titel:
PEMP - the exact morphology quantification procedure of podocyte foot processes by super-resolution microscopy

Autoren/Adressen:
Florian Siegerist (University Medicine Greifswald), Nadine Artelt (University Medicine Greifswald), Alina Ritter (University Medicine Greifswald), Florian Tesch (University Medicine Greifswald), Olaf Grisk (University Medicine Greifswald, Karlsburg), Rabea Schlüter (University Medicine Greifswald), Karlhans Endlich (University Medicine Greifswald), Nicole Endlich (University Medicine Greifswald); nicole.endlich@uni-greifswald.de

Abstract:
Objective
Proper morphology of podocyte foot processes is the prerequisite for renal function. In the past, podocyte foot process morphology could only be visualized by electron microscopy, a sophisticated and time-consuming technique. Our goal was to establish an automatized method for podocyte foot process measurement by super-resolution microscopy.

Methods
Three dimensional structured illumination microscopy was used as a technique of superresolution microscopy. Kidney biopsies were stained with an antibody against nephrin and/or synaptopodin.

Results
By PEMP (***p****odocyte* ***e****xact morphology* ***m****easurement* ***p****rocedure),* we measured a mean foot process width of 0.249±0.068 µm in healthy kidneys and a significantly increased mean foot process width of 0.675±0.256 µm in minimal change disease patients (MCD), indicating effacement of foot processes. We then hypothesized that the filtration slit length per glomerular capillary surface area (filtration slit density - FSD) could be used as an equivalent for the diagnosis of effacement. Using custom-made software, we measured a mean value of 3.10±0.27 µm-1 in healthy subjects and 1.83±0.49 µm-1 in the patients suffering from MCD. As foot process width was highly correlated with FSD (R2=0.91), we concluded that our approach is a valid method for the diagnosis of foot process effacement. Furthermore, we used PEMP to measure the FSD of double-stained kidney sections of rats as well as mice, demonstrating that FSD increases from humans to rats and mice.

Conclusion
We developed a new technique to quantify podocyte damage, which combines superresolution microscopy with automatized image processing. Due to its diverse advantages, we propose PEMP to be included into routine diagnostics of glomerular histopathology as well as into research on animal models.

Poster 56

Titel:
Zebrafish - a model for focal segmental glomerulosclerosis

Autoren/Adressen:
Kerrin Hansen (Universität Greifswald), Florian Siegerist (Universität Greifswald), Karlhans Endlich (Universität Greifswald), Nicole Endlich (Universität Greifswald); nicole.endlich@uni-greifswald.de

Abstract:
Objective
Loss of podocytes, highly differentiated cells of the renal glomerular filtration barrier, leads to proteinuria, activation of parietal epithelial cells and glomerular scarring resulting in progressive loss of kidney function. Increasing prevalence of glomerular diseases makes the development of curative drugs necessary. Due to its suitability for high-throughput experiments and a high morphological and molecular homology to mammals, zebrafish larvae is an ideal model for this purpose. The aim of the present study was to investigate whether focal segmental glomerulosclerosis (FSGS) can be induced in zebrafish larvae.

Methods
We used the modified nitroreductase/metronidazole model of targeted podocyte ablation (Siegerist et al. 2017). Metronidazole was used at low dose to deplete a subset of podocytes. We titrated the concentration to a level at which larvae survived until three days after washout. Analysis of the glomerular morphology was done by histology, immunofluorescence and electron microscopy.
Survival and phenotypes were counted as well as glomerular filtration barrier was assessed by injection of fluorescently labelled high molecular-weight dextran.

Results
Larvae developed severe edema, a hallmark of kidney failure which was confirmed by analysis of the filtration barrier after injection of fluorescently labelled high molecular-weight dextran. Morphological and ultrastructural examination of the glomeruli revealed podocyte effacement, the development of sub-podocyte pseudocysts as well as parietal cell activation, all signs of FSGS.

Conclusions
Our specific zebrafish larvae injury model shows morphological and functional features of FSGS making this model ideal for therapeutic drug screening.

Poster 57

Titel:
Ultra high-field MRI determination of diffusion rates in human lenses of different ages

Autoren/Adressen:

Stahnke T. (Rostock University Medical Center), Guthoff R.F. (Rostock University Medical Center), Stachs O. (Rostock University Medical Center), Wree A. (Rostock University Medical Center), Jünemann A. (Rostock University Medical Center), Grabow N. (Rostock University Medical Center), Lindner T. (Rostock University Medical Center), Polei S. (Rostock University Medical Center);

Abstract:
Purpose:
Accommodation loss affects clinically every human individual older than 45. This effect is predominantly caused by increased lens stiffness. The aim of this investigation was to analyze effects in presbyopia in human lenses of different ages ex vivo using ultra high-field magnetic resonance imaging (UHF-MRI).
Methods:
After enucleation intracapsular lens extractions were performed. Optically clear lenses (N=23) were photographed, weighed, and embedded in cooled 0.5% agarose solved in culture medium. UHF-MRI (7 Tesla, BioSpec 70/30, Bruker, Germany) was conducted to analyze anatomical characteristics, equatorial lens diameters and thicknesses in the central layer of each lens using T2-weighted Turbo-RARE sequences (Resolution: (75x75x800) µm3). Spin-echo based diffusion sequences (6 directions, 4 b-values between (100-1000) s/mm²) were used to obtain Apparent Diffusion Coefficient (ADC) values. A simple (double-) Gaussian fit routine was applied in order to examine two individual modes.
Results:
There was an age-dependent increase in lens weight, which was from 0.18 g for younger lenses (31-40 years) to 0.28 g for older lenses (81-90 years). In T2-weighted images the measured increase in lens diameter from 8.57 to 9.74 mm and in lens thickness (3.71 to 5.65 mm) was also age-related. Additionally, T2-weighted images revealed a hyperintense area of the lens cortex, which decreased in signal intensity stepwise towards the nucleus. Histogram analysis of the ADC values of individual lenses showed occurrence of bimodal distributions. Peak positions of the first mode (lower ADC values) in the ADC histogram appear to remain constant (~0.5*10-3 mm²/s), whereas for the second mode peak positions tend towards lower ADC values with increasing age.
Conclusions:
Ex vivo UHF-MRI allowed detailed examinations of anatomical characteristics and diffusion coefficients of human lenses. In conjunction with the local distribution of ADC values within the lens the results indicate that the first mode represents mainly the lens nucleus where there was no change, whereas there is an age dependent decrease in the cortex region of the lens, represented by the second mode. Age dependent space-resolved ADC values showed unexpected alterations only in the cortical areas, the clinically obvious age dependent hardening of the nucleus is not correlated with changes in the Apparent Diffusion Coefficient.

Poster 58

Titel:
Persistent hyper-innervation in the murine vagina in response to complete Freund's adjuvant

Autoren/Adressen:
Barry C. (Flinders University Adelaide), Huigol K. (Flinders University Adelaide), Vilimas P. (Flinders University Adelaide), Haberberger R.V. (Flinders University Adelaide);

Abstract:
Proliferation of vaginal nerve fibres is the major pathological feature of vulvodynia, a prevalent chronic pain disorder of unknown cause. In most patients with vulvodynia, hyperinnervation is present without overt evidence of inflammation. We recently showed hyperinnervation is present in the murine vagina 7 & 14 d following exposure to the pro-inflammatory agent complete Freund's adjuvant (CFA). The present study aimed to determine if nerve fibre proliferation and inflammation are present 4 weeks following exposure to CFA.
Mild chronic inflammation was induced using microinjection of CFA (5 µl) in the distal vagina of C57Bl/6 mice (n = 4). Control mice received saline (n = 2) naïve mice (n = 4) no treatment. Inflammation (swelling) was assessed by measuring lamina propria cross-sectional area in H & E sections. Nerve fibres were identified using the pan-neuronal marker PGP9.5 and the T-cell marker CD3. Micrographs were acquired from 18–30 regions of interest per mouse, analysed blinded to the treatment group. Data were analysed using ANOVA followed by Bonferroni post-tests.
Hyperinnervation was present in vagina at 28 days following CFA ($F_{1, 6} = 8.7$, $p = 0.03$) in absence of edema but with present infiltration by CD3-immunoreactive cells. Innervation density was increased in proximal and distal regions of the vagina. Edema was absent suggesting acute inflammation has resolved. The inter-animal differences within each treatment group in the present study were small, indicating this model is useful to study mechanisms underlying inflammation-related vaginal nerve fibre proliferation and the impact of potential interventions.

Poster 59

Titel:
Ultra high-field MR-Imaging of a biodegradable, subconjunctival drug delivery system - in vitro, ex vivo and in vivo examinations

Autoren/Adressen:
Kopp F. (Universität Rostock), Polei S. (Universität Rostock), Falke K. (Universität Rostock), Eickner T. (Universität Rostock), Witt M. (Universität Rostock), Stachs O. (Universität Rostock), Guthoff R.F. (Universität Rostock), Lindner T. (Universität Rostock);

Abstract:
Purpose:
Drug delivery systems have gained increasing importance in managing chronic ocular diseases such as glaucoma. During the development of biodegradable drug depots, information about dimension and the degradation process is of high importance. In this study, we assess the suitability of ultra high-field MR-imaging (UHF-MRI) for the characterization of depot localization and degradation.
Methods:
An in situ polymerizing drug delivery system, consisting of hyaluronic acid and the cross-linking agent ELA-NCO, an isocyanate-functionalized 1,2-ethylene glycol bisdilactic acid derivative, was investigated using UHF-MRI at 7 Tesla (Biospec 70/30, Bruker, Germany). *In vitro* measurements of the components before and after polymerization were conducted to establish optimal scan parameters. *Ex vivo* images after depot injection into the subconjunctival space of an enucleated pig eye were compared with hematoxylin and eosin (H&E) stained sections of the same eye. *In vivo* imaging and volumetric quantification of the drug depot were performed 3 and 12 weeks after injection into the subconjunctival space of a New Zealand White rabbit.
Results:
The individual components as well as the polymer mixture could be analyzed *in vitro* with an optimal signal-to-noise ratio, using proton-density-weighted sequences with TE/TR = 7/2000 ms. Distinctly different polymer morphologies were observed based on the progress of the polymerization reaction. *Ex vivo* measurements of the polymer depot in a pig eye with TE/TR = 42/4550 ms achieved an in-plane resolution of 100 x 100 µm. Correlation of the MRI data with H&E stained sections confirmed the localization of the depot between sclera and conjunctiva. *In vivo* imaging of the drug depot using T2-weighted Turbo RARE sequences (TE/TR = 70/5180 ms) yielded an in-plane resolution of 120 x 120 µm. Volumetric analysis revealed a 36 % reduction between 3 and 12 weeks after depot injection.
Conclusions:
UHF-MRI can be used as a noninvasive imaging tool during the development of subconjunctival drug delivery systems. T2-weighted Turbo RARE sequences allow high resolution depiction of the polymeric drug depot and its distinction from surrounding ocular tissues. Biodegradation of the drug depot can be monitored and quantified using volumetric analysis, which provides valuable information for the study and adaptation of drug release kinetics.

Poster 60

Titel:
Examination of the photoreceptor - RPE interaction using the microphysiological Retina-ona-chip

Autoren/Adressen:
Jasmin Haderspeck (Universität Tübingen), Kevin Achberger (Universität Tübingen), Christopher Probst (Fraunhofer Institute for Interfacial Engineering and Biotechnology IGB, Stuttgart), Julia Rogal (Fraunhofer Institute for Interfacial Engineering and Biotechnology IGB, Stuttgart), Johanna Chuchuy (Fraunhofer Institute for Interfacial Engineering and Biotechnology IGB, Stuttgart), Wadood Haq (University of Tuebingen), Peter Loskill (Fraunhofer Institute for Interfacial Engineering and Biotechnology IGB, Stuttgart), Stefan Liebau (Universität Tübingen); stefan.liebau@uni-tuebingen.de

Abstract:
ABSTRACT BODY:
Retinal disorders such as age-related macular degeneration or retinitis pigmentosa are a leading cause for blindness and curative treatment options are currently not available. Basic research on disease mechanisms involved in the pathology as well as the development of new pharmacological treatment options require reproducible and accessible model systems that accurately represent the physiological situation in humans. Existing retinal model systems such as retinal explants or animal models are either hardly accessible and not suitable for long-term culture or differ substantially from the human physiology. Both, therefore, do not represent an accurate model system. Retinal organoids are 3-dimensional organ-like structures that can be derived from human pluripotent stem cells and feature all major retinal cell types as well as a retinal layering that closely resembles the human in vivo situation. Nevertheless, retinal organoids are still restricted in their applicability as a model system, due to limitations in retinal connectivity and maturation as well as reproducibility issues due to variability of culture conditions or insufficient media supply. To overcome these limitations, we have combined retinal organoids with microfluidic organ-on-a-chip technology to develop a 3D Retina-on-a-chip (3D RoC). The 3D RoC enables the co-culture of retinal organoids with retinal pigment epithelium (RPE) and other cell types to create a defined physiological microenvironment providing optimized and reproducible culture conditions. We have successfully integrated retinal organoids and RPE derived from human induced pluripotent stem cells into the chips and were able to show viability and physiological interactions, essential for nutrition, survival and normal metabolism of the retina.

Poster 61

Titel:
Noninvasive monitoring of embryonic chick eye development in ove using 7 Tesla MRI

Autoren/Adressen:
Oliver Stachs (University Medicine Rostock), Ronja Klose (University Medicine Rostock), Tobias Lindner (University Medicine Rostock), Felix Streckenbach (University Medicine Rostock), Thomas Stahnke (University Medicine Rostock), Stefan Hadlich (University Medicine Greifswald), Jens-Peter Kühn (University Medicine Greifswald), Rudolf F. Guthoff (University Medicine Rostock), Andreas Wree (University Medicine Rostock), Anne-Marie Neumann (University Medicine Rostock), Marcus Frank (University Medicine Rostock), Sönke Langner (University Medicine Greifswald);

Abstract:
Purpose: The avian embryo serves as an excellent model for monitoring embryonic development. Ultrahigh field magnetic resonance imaging (UHF-MRI) is an invaluable tool for noninvasive and high resolution tissue imaging. The purpose of this study was to characterize the embryonic eye development during incubation in ovo and to analyze the putative influence of repetitive UHF-MRI measurement procedure on ocular developments.

Methods: A total population of 38 fertilized chicken eggs has been divided into two sub-groups: 36 eggs were examined pairwise only on one day, starting at embryonic day 3 (E3) to day 20 (E20) and have been sacrificed immediately after MR imaging (Group A). For comparison, the second group of two eggs (Group B) was examined repeatedly on daily manner during the developmental time course E3 to E20 to evaluate the influence of daily MRI-scanning. Moderate cooling of the eggs was performed before and during UHF-MRI at 7.1 Tesla for about 50-70 minutes to reduce possible artifacts due to natural embryo movements. Ganglion cell counting was performed using HE-staining at E20 in both groups.

Results: Using fast T2 weighted MR-sequences, we could provide a biometry of the eye with an in-plane resolution of 74 µm starting from E5. Data show a rapid growth of the chicken eye with a steep increase of intraocular distances and of bulbus volume during initial development until E10, followed by a phase of reduced growth rate in later developmental stages. The length of the pecten, a nutritive structure specific to the bird eye, could be evaluated from E12. No differences in ocular development could be determined comparing the two sub-groups A and B.

Conclusions: We conclude that UHF-MRI provides a powerful imaging technique for noninvasive and longitudinal studies of avian eye development. The technique allows an investigation of the maturation of the chicken eye in ovo from E5 onwards. Daily MR scanning in combination with moderate cooling of chicken eggs during MR imaging does not alter ocular development. The MR based imaging technique could become a routine approach for longitudinal embryonic studies.

Poster 62

Titel:
Use and validation of an easy to integrate e-learning concept in medical education

Autoren/Adressen:
Mona Eulitz (University Witten/Herdecke), Hannah Plachta (University Witten/Herdecke), Berit Geis (University Witten/Herdecke), Jan Ehlers (University Witten/Herdecke); mona.eulitz@uni-wh.de

Abstract:
Use of the Internet has become part of everyday life for students. At the Witten/Herdecke University, however, the combination of internet-based e-learning with the well established method of problem based (oriented) learning (POL) and teaching has so far been realized only marginally. This study examines the potential benefits of integrating simple and easily portable e-learning tools (Moodle combined with an audience response system (ARS)) for teaching human anatomy.

Medical students of the first four semesters in the field of anatomy were divided into two randomized groups as part of a randomly selected POL case. Participants in the study group had access to specific questions in Moodle and also the opportunity to anonymously answer questions with their smartphones (ARS) during classroom teaching. All students were then asked to complete a questionnaire and an anonymous test with case-related questions.

Participants of the study group scored significantly higher than the students of the control group. It could also be shown that students are more willing to take part in ARS exercises during classroom teaching than learning with Moodle exercises alone at home. They were mainly motivated by ARS exercises.

It is possible to improve medical education at the Witten/Herdecke University with relatively low effort. Various simple e-learning offers such as Moodle and ARS can be combined or integrated individually into the already existing lesson.This contemporary teaching meets a high level of acceptance and should be taken into account more frequently in the future.

Poster 63

Titel:
Test anxiety among anatomy students

Autoren/Adressen:
Christian Hammer (Friedrich-Alexander-Universität Erlangen-Nürnberg), Michael Scholz (Friedrich-Alexander-Universität Erlangen-Nürnberg), Friedrich Paulsen (Friedrich-Alexander-Universität Erlangen-Nürnberg), Pascal Burger (Psychiatrische Universitätsklinik Zürich); christian.hammer@fau.de

Abstract:
To characterize test anxiety among first year medical students and to test medical hypnosis as a therapeutic option.

A validated psychological test anxiety questionnaire was used to probe the development of test anxiety among participants of the first year course “Functional Anatomy of the Locomotor System” at the Department of Anatomy, Erlangen, Germany. The course included two mandatory oral tests. At the commencement of winter term 2017/18, the questionnaire was used for the first survey. Two days before the first and the second oral test, the second and third surveys were conducted. In each survey, prevalence and severity of test anxiety were determined. Moreover, the four different dimensions of test anxiety “emotionality”, “worry”, “interference” and “lack of confidence” were investigated. The severest 46 cases (out of 196) were selected after the first survey and organized into an intervention group receiving medical hypnosis prior to the first exam and a matched control group. The effect of hypnotherapy on test anxiety was investigated by comparison of the group-specific survey outcomes.

More than half of the study participants showed pronounced test anxiety in at least one dimension during the semester. “Lack of confidence” was reduced significantly ($p<0.05$) and effectively ($d=0.80$) by hypnotherapy. The same was true for the total, dimension-independent test anxiety score ($p<0.05$; $d=0.85$). “Emotionality” was reduced significantly in the course of the semester in both groups.

Test anxiety is very prevalent among medical students. Hypnotherapy should be considered as a treatment option, especially for students exhibiting pronounced “lack of confidence”.

Poster 64

Titel:
Pearls and Pitfalls of post mortem computer tomography in teaching human gross anatomy

Autoren/Adressen:
Alexander Schneller (University Heidelberg), Kerstin Klopries (University Heidelberg), Sara Doll (University Heidelberg), Frederik L. Giesel (University Hospital Heidelberg), Ralph Nawrotzki (University Heidelberg), Thomas Kuner (University Heidelberg), Daniel Paech (German Cancer Research Center / University Heidelberg);
a.schneller@stud.uni-heidelberg.de

Abstract:
Radiologic imaging modalities, such as cadaver-specific post mortem computer tomography (CSPMCT), are increasingly used in gross anatomy teaching. The purpose of this study was to further specify the advantages and weaknesses of (CSPMCT) in preclinical anatomy teaching and to identify particular strengths and possible “pitfalls” of this method.

All anatomical lecturers and tutors (n=28) were invited to answer a 32-item questionnaire with a five-point Likert scale in order to identify specific advantages of this teaching method compared to conventional manual dissection (1 =lowest score, 5=highest score). Thereby, we focused on the determination of anatomical structures that particularly profit from the CSPMCT method. Furthermore we analyzed all CSPMCT scans in order to identify typical artefacts (e.g. metal of artificial hips or contrast extravasation) yielding a quantitative assessment of typical CSPMCT “pitfalls”.

Based on the questionnaire evaluation we identified the Willis Circle (median CSPMCT=5; overall agreement (OA)=90,91% vs. median dissection =3; OA=47,83%), the crania foramina (median CSPMCT =4; OA=73,33% vs. median dissection =3; OA=26,09%), and small intracranial arteries (e.g A. ophthalmica) (median CSPMCT =5; OA=80,00% vs. median dissection =3; OA=43,48%) as specific “pearls” of the CSPMCT teaching approach. Most common pitfalls were ‘contrast-enhancement in the mucosa of the gastrointestinal tract’ in 14/15 CSPMCT data sets and ‘extravasation of contrast agent into the gastric lumen’ in 12/15 CSPMCT cases.

Identification of specific advantages and disadvantages, the “pearls” and “pitfalls” of cadaver-specific post mortem computer tomography, is one of the big cornerstones for a successful implantation of this teaching method into the macroscopic dissection course.

Neuroanatomy/Neurobiology

Poster 65

Titel:
GPR17 expression in oligodendrocyte progenitor cells: Relevance for remyelination

Autoren/Adressen:
Stella Nyamoya (Faculty of Medicine, RWTH Aachen University), Patrizia Leopold (Ludwig-Maximilians-University of Munich), Birte Becker (Faculty of Medicine, RWTH Aachen University), Cordian Beyer (Faculty of Medicine, RWTH Aachen University), Fabian Hustadt (UCB BioPharma), Christoph Schmitz (Ludwig-Maximilians-University of Munich), Anne Michel (UCB BioPharma), Markus Kipp (Ludwig-Maximilians-University of Munich); snyamoya@ukaachen.de

Abstract:
During the formation of Multiple sclerosis (MS) lesions, oligodendrocytes die, leading to focal demyelination and axonal degeneration. Remyelination is one example of tissue repair in the human adult central nervous system. While remyelination is widespread in some MS patients, it is sparse in others. The underlying mechanism(s) for remyelination failure is not fully understood. Since G protein-coupled receptors have been found to be regulators of oligodendrocyte development and maturation, we investigated the expression of GPR17 (G protein-coupled receptor 17) during de- and remyelination. Here we investigate GPR17 expression levels in lesions with robust and impaired endogenous remyelination capacity.

Different in vivo models were used to characterize the expression of GPR17 during acute and chronic demyelination and early remyelination. The expression of GPR17 was analyzed by immunohistochemistry, in situ hybridization and real-time PCR.

Lesions with robust endogenous remyelination (i.e. acute cuprizone and lysophosphatidylcholine lesions) showed robust expression of GPR17, while lesions with low endogenous remyelination (i.e. chronic cuprizone and EAE lesions) showed moderate expression of GPR17. Immuno-fluorescence double labelling studies revealed expression of GPR17 in oligodendrocytes but not other glia cells or neurons. Finally, GPR17+ cells displayed a multipolar, highly ramified morphology both, in mice and human tissues.

Our findings demonstrate that GPR17 expression induction correlates with acute demyelination and sufficient endogenous remyelination. This strengthens the view that manipulation of this receptor might be a therapeutic opportunity to support endogenous remyelination.

Poster 66

Titel:
DOT1L and H3K79 methylation establish transmittable layer identity in progenitors of the cerebral cortex

Autoren/Adressen:
Henriette Franz (Albert-Ludwigs-Universität Freiburg), Alejandro Villarreal (Albert-Ludwigs-Universität Freiburg), Tanja Vogel (Albert-Ludwigs-Universität Freiburg);

Abstract:
Cortical development is orchestrated by different temporo-spatial transcriptional programs initiated by molecules such as transcription factors. Yet, it is still under debate how cell fate and regionalization in the different cortical layers are transmitted during progenitor cell division.

Deletion of Dot1l, methyltransferase of H3K79 methylation, in the murine telencephalon leads to cortical layering defects. Genome-wide analysis of gene expression profiles and H3K79me2 patterning show that H3K79 methylation control not only the cell cycle and division mode of cortical progenitors but also transmit layering information from early to late progenitors.

For that, DOT1L establishes with H3K79me2 stable transcriptional programs characteristic for upper layer neurons (Satb2, Pou3f3, Cux1, Cux2) already at an early developmental stage, and thereby restricts cell fate of late cortical progenitors.

In line with these results overexpression of DOT1L via in-utero electroporation induces an upper layer cell fate in cortical progenitors, proofing its role in progenitor cell specification.

Poster 67

Titel:
Connectomics of the rat thalamus

Autoren/Adressen:
Oliver Schmitt (uni-rostock), Sebastian Schwanke (uni-rostock), Peter Eipert (uni-rostock), Felix Lessmann (uni-rostock), Frauke Russ (uni-rostock), Adrian Karnitzki (uni-rostock), Paulinne Morawska (uni-rostock);schmitt@med.uni-rostock.de

Abstract:
So far, the intrinsic connectome of the rat thalamus has not been analysed. Therefore, all neuronal connections which are documented in 7800 tract-tracing studies have been collated manually to obtain a complete database of all known thalamic connections. The aim of the study was to determine most important regions of the thalamus which build the connectional backbone of the intrinsic thalamic network architecture.

Peer reviewed articles documenting neuronal tract-tracing results in rats were evaluated. The neuronal connections in normal adult rats were collated and imported into a connectome analysis software (neuroVIISAS). 70 thalamic regions were selected to obtain a fully connected network. The network was analysed with regard to global, local parameters and motif analysis. A functional characterization was computed by applying diffusion models and a coupled FitzHugh-Nagumo neuron network.

The 70 regions are interlinked by 347 neuronal connections. The average degree per region is 9. 84 reciprocal connections were identified. The average pathlength is 2.7 and the average clustercoefficient is 0.37. The Klemm-Eguilez null-model is most similar with the empirical network. The connectome has a large small-worldness of 5.3. Modularity analysis shows 5 significant modules.The largest ranks among 50 local network parameters have the reuniens, ventral lateral geniculate and parafascicular nuclei

The intrinsic thalamic connectome could approximated by one surrogate network, only. It has a distinct small-worldness property which correlates with the 5 connectional modules.
Most important regions are the reuniens, ventral lateral geniculate and parafascicular nuclei.

Poster 68

Titel:
The human vomero nasal organ is an estrogen target

Autoren/Adressen:
Enrico Wollbaum (University Hospital), Maxim Maximov (University Hospital), Veronika Gebhart (University Hospital), Andrea Rodewald (University Hospital), Gustav Jirikowski (University Hospital); gustav.jirikowski@med.uni-jena.de

Abstract:
A vomeronasal organ (VNO) is present in humans during development. Its persistence in postnatal live is discussed. A vomeronasal duct (VND) in the lower nasal cavity has been described only in up to 20% of adults.

Here we studied postmortem tissue samples of nasal mucosa and tissue samples obtained after surgical correction of nasal septum. We examined histological sections with antibodies to olfactory marker protein (OMP), to sex hormone binding globulin (SHBG) and to estrogen receptor alpha (ER□). mRNA was extracted from tissue homogenates and subjected to RT- PCR in order to amplify the respective transcripts.

In all samples we found epithelial cells within the mucosa of the lower part of the nasal septum which exhibited the morphological features of sensory neurons and which showed immunostaining for OMP. These cells were interposed by ciliated cells, goblet cells and small capillaries. Only occasionally we found such cells within a defined epithelial duct. In most cases we found OMP- positive cells either in epithelial cavities or just embedded in the respiratory epithelium. Double immunostaining for either SHBG or for ER□ indicated that some of the OMP positive cells were estrogen targets. RT-PCR revealed the existence of OMP-, SHBG- and ER□ encoding transcripts, suggesting intrinsic expression of these proteins.

We conclude that a functional VNO is regularly conserved in humans although a VND is only present in few individuals. Similar to the VNO of macrosmatics the human VNO may be involved in recognition of aerosolic steroids, known to have pheromone properties.

Poster 69

Titel:
Spine density of dentate granule cells is independent of expression levels of actinmodulating protein Synaptopodin

Autoren/Adressen:
Michael Rietsche (Goethe-University Frankfurt), Dinko Smilović (University of Zagreb), Meike Fellenz (Goethe-University Frankfurt), Tassilo Jungenitz (Goethe-University Frankfurt), Stephan W. Schwarzacher (Goethe-University Frankfurt), Mandy Paul (Goethe-University Frankfurt), Andreas Vlachos (University of Freiburg), Mario Vukšić (University of Zagreb), Domenico Del Turco (Goethe-University Frankfurt), Thomas Deller (Goethe-University Frankfurt); rietsche@med.uni-frankfurt.de

Abstract:
Dendritic spines are sites of synaptic plasticity and play a key role in memory and learning. The actin-binding protein Synaptopodin (SP) is an essential component of the spine apparatus (SA), an organelle regularly found in mature dendritic spines. SP/SA have been linked to different forms of synaptic plasticity, including long-term potentiation (LTP), long-term depression (LTD) and homeostatic synaptic plasticity. Mechanistically SP/SA are involved in the regulation of intracellular calcium stores, actin-modulation and the accumulation of GluA1-receptor subunits in dendritic spine heads. Here we tested the hypothesis that SP/SA could also affect the number of dendritic spines by using loss-of-function (Synaptopodin-deficient mice) and gain-of-function (Synaptopodin-transgenic mice) approaches. While SP-deficient mice are devoid of SA, SP-overexpressing mice show an enlarged organelle.

Adult male SP-deficient mice, wildtype mice and SP-transgenic mice were transcardially perfused and paraformaldehyde-fixed. Using intracellular injection technique of fluorescent dyes in fixed tissue, identified granule cells located in the suprapyramidal blade of the dentate gyrus were labeled. Confocal z-stacks of dendritic segments in the outer molecular layer (OML) were acquired and spine densities were determined using standardized counting methods with the investigator blind to genotypes.

Dendritic spine density of SP-deficient mice showed no significant differences to control mice (C57BL/6J-background), consistent with earlier results using Golgi-staining in pyramidal cells of CA1. SP-overexpressing animals likewise revealed a normal density of spines and no difference to wildtype controls.

We conclude that the density of dendritic spines is independent of the expression-level of the actin-modulating protein SP. (supported by DFG, DAAD, Dr. Senckenbergische Stiftung).

Poster 70

Titel:
The role of lipocalin 2 in brain diseases

Autoren/Adressen:
Fatemeh Ranjbar Taklimie (Faculty of Medicine, RWTH Aachen University), Natalie Gasterich (Faculty of Medicine, RWTH Aachen University), Markus Beissel (Faculty of Medicine, RWTH Aachen University), Miriam Scheld (Faculty of Medicine, RWTH Aachen University), Tim Clarner (Faculty of Medicine, RWTH Aachen University), Adib Zendedel (Faculty of Medicine, RWTH Aachen University), Cordian Beyer (Faculty of Medicine, RWTH Aachen University); fatemeh.ranjbar.md@gmail.com

Abstract:
Lipocalin 2 (LCN2) plays a crucial role in the pathogenesis of brain diseases like stroke and multiple sclerosis (MS). LCN2 acts as a promoter of glia cells-migration towards injured tissue through the induction of chemokines. In this study, we examined the time course of LCN2 expression animal models related to MS and ischemic stroke.

In a rat model of transient middle cerebral artery occlusion, LCN2 was analyzed by RT–qPCR and immunohistochemistry during the post-ischemic course in the peri-infarct zone.For MS studies, we used cuprizone (Cup) treatment, the experimental autoimmune encephalomyelitis (EAE) model, and a combination of both. In addition, we applied an in vitro hypoxia model by using the microglia cell line BV-2 and neonatal astrocytes

In the stroke model, LCN2 expression (protein and mRNA) and in the serum was increased 72 h after ischemia onset. Immunofluorescence double staining showed that microglia and astrocytes are the main sources of LCN2. In vitro, hypoxia induced LCN2 in BV-2 and astrocytes. In the MS animal models, LCN2 expressing cells (astrocytes and natural killer cells) were only seen in the brain of EAE and Cup/EAE animals where we also found an increase of the chemokines CXCL9, CXCL10 and CXCL11.

Our data showed that LCN2 is present in a chronic and an acute brain disorder at the affected brain site. Further, LCN2 seems to be secreted through exosomes (under investigation) into the blood serum. In both models, astroglia and microglia are LCN2-producing cells.

Poster 71

Titel:
Combined effects of Schwann cells and 17β-estradiol in a spinal cord injury model

Autoren/Adressen:
Adib Zendedel (Uniklinikum), Tim Clarner (Uniklinikum), Fatemeh Ranjbar-Taklimie (Uniklinikum), Cordian Beyer (Uniklinikum); azendedel@ukaachen.de

Abstract:
Spinal cord injury (SCI) is a devastating traumatic event which burdens the affected individuals and the health system. Schwann cell (SC) transplantation is a promising repair strategy after SCI. However, SCs do not easily survive following transplantation. Previous studies demonstrated that 17β-estradiol (E2) protects different cell types and reduces tissue damage in experimental SCI animal models. In the current study, we evaluated the protective potential of E2 on SCs in vitro and investigated whether the combinatory thereapeutic strategy with E2 and SC improves the outcome after SCI.

Primary isolated SCs were incubated with E2 for 72 h. In a subsequent experiment, thoracic contusion SCI was induced in male rats followed by sustained administration of E2 or vehicle. Eight days after SCI, DiI-labeled SCs were transplanted into the epicenter of vehicle and E2-treated animals.

The combinatory regimen decreased behavioral deficits and protected neurons and oligodendrocytes in comparison to vehicle rats. Moreover, E2 and SC significantly attenuated the SCI-induced astrogliosis and microgliosis post SCI. Transmission electron microscopy demonstrated increased mitochondrial numbers and size in neurons after SCI which was antagonized by the combinatory treatment. Similarly, we found that E2/SCs modulated gene expression of mitochondrial fusion and fission and restoring mitochondrial respiratory enzyme activity in the injured spinal cord.

These data demonstrate that E2 protects SCs against hypoxia-induced SCI and improves the survival of transplanted SCs.

Poster 72

Titel:
Lipocalin 2 as a regulator of astrocyte reactivity and neuroinflammation

Autoren/Adressen:
Natalie Gasterich (Faculty of Medicine and JARA-BRAIN, RWTH Aachen University), Fatemeh Ranjbar Taklimie (Faculty of Medicine and JARA-BRAIN, RWTH Aachen University), Markus Beissel (Faculty of Medicine and JARA-BRAIN, RWTH Aachen University), Sophie Wetz (Faculty of Medicine and JARA-BRAIN, RWTH Aachen University), Miriam Scheld (Faculty of Medicine and JARA-BRAIN, RWTH Aachen University), Adib Zendedel (Faculty of Medicine and JARA-BRAIN, RWTH Aachen University), Ralf Weiskirchen (Faculty of Medicine, RWTH Aachen University), Cordian Beyer (Faculty of Medicine and JARA-BRAIN, RWTH Aachen University), Tim Clarner (Faculty of Medicine and JARA-BRAIN, RWTH Aachen University); ngasterich@ukaachen.de

Abstract:
Reactive astrocytes are central players in brain inflammation and neurodegeneration. After activation, they undergo severe morphological and functional changes to differentiate either into a detrimental (A1=proinflammatory) or protective (A2=antiinflammatory) phenotype. Pathfinding experiments using mouse models for multiple sclerosis (MS) showed strong expression of lipocalin 2 (LCN2) in an astrocyte subpopulation in the vicinity of inflammatory infiltrates. LCN2 is thought to regulate astrocyte activation in an autocrine and paracrine manner. What stimuli trigger LCN2 expression in astrocytes is not established and the functional impact of LCN2 on astrocytes also remains elusive.

Primary astrocyte cultures from WT and LCN2-deficient mice were stimulated with LCN2, LPS and IFNγ. Gene and protein expression of LCN2, typical A1/A2 markers and inflammasome components were measured by means of RT-qPCR, ELISA and/or immunoblotting. IL1b ELISA and caspase 1 assays were additionally performed to confirm functional inflammasome activation. Using scratch wound assays, LCN2 effects on astrocyte migration, proliferation and morphological changes were investigated in dependency of LCN2.

LPS caused LCN2-expression, inflammasome activation, induction of A1 marker expression, morphological changes and faster wound closure. These LPS effects were partly dependent of LCN2 signaling. LCN2 treatment induced responses comparable to LPS treatment in WT cells. Faster scratch wound closure in LCN2-treated cells was independent of proliferation and migration but mainly the result of morphological changes.

Our results clearly demonstrate the importance of LCN2 for the activation of astrocytes. Further studies using astrocyte-specific LCN2-deficent mice will elucidate the exact role of astrocytic LCN2 in neuroinflammation.

Poster 73

Titel:
Unilateral Botulinum neurotoxin-A injection into the caudate-putamen of C57BL/6 mice leads to a different motor behavior compared to rats

Autoren/Adressen:
Veronica Antipova (Medical University of Graz), Andreas Wree (Rostock University Medical Center), Carsten Holzmann (Rostock University Medical Center), Teresa Mann (Rostock University Medical Center), Nicola Palomero-Gallagher (Research Center Jülich), Karl Zilles (Research Center Jülich), Oliver Schmitt (Rostock University Medical Center), Alexander Hawlitschka (Rostock University Medical Center); veronica.antipova@medunigraz.at

Abstract:
In hemiparkinsonian rats injection of botulinum neurotoxin-A (BoNT-A) into the ipsilataral caudate-putamen (CPu) annuls apomorphine-induced rotations up to 6 months. The beneficial effect of BoNT-A is possibly based on the reduction of striatal hypercholinism of Parkinson´s disease or changes in receptor densities. As shown in rats, BoNT-A injection into the mouse CPu does not alter the number of choline acetyltransferase-immunoreactive (ChAT-ir) interneurons. Additionally, ChAT-ir BoNT-A-induced varicosities (BiVs) are found both in the mouse rat CPu. In contrast to rats, tyrosine hydroxylase-immunoreactive (TH-ir) BiVs are never present in BoNT-A-injected mice.

In order to investigate the behavioral consequences of the differing morphological outcome of intrastriatal BoNT-A-injections in naïve rats and mice, we studied various motor behaviors in mice up to 9 months after BoNT-A. Apomorphine- and amphetamine-induced rotational behavior, spontaneous motor behavior as well as lateralized sensorimotor integration and hindlimb clasping were studied in mice after injection of single dosages of BoNT-A into the right CPu, the results compared those obtained in rats.

Unilateral intrastriatal injection of BoNT-A in mice induced significantly increased contralateral apomorphine-induced rotations 1 to 3 months as well as significantly increased contralateral amphetamine-induced rotations 1 to 9 months after injection. Lateralized sensorimotor integration, forelimb preference and forelimb stepping were significantly impaired on the left side.

The differences in motor behaviors between rats and mice after unilateral intrastriatal BoNT-A may be caused by different BoNT-A effects on ChAT-ir and TH-ir fibers in rat and mouse striata, interspecies differences in striatal receptor densities, and different connectomes of the basal ganglia.

Poster 74

Titel:
Endocannabinoid actions at hypothalamic AgRP/NPY neurons

Autoren/Adressen:
Henrike Horn (University of Leipzig), Beatrice Böhme (University of Leipzig), Marco Koch (University of Leipzig); henrike.horn@medizin.uni-leipzig.de

Abstract:
Endocannabinoids (eCBs) and their related lipid messengers have been hypothesized to be critically involved in the control of feeding behavior, in the regulation of energy metabolism and consecutively in the maintenance of body weight. With the world-wide increase of obesity and due to its detrimental health effects, a better understanding of the circuits underlying hunger and food intake are needed.

The arcuate nucleus (ARC) of the hypothalamus is a pivotal brain region controlling energy homeostasis. One resident neuronal population, Agouti-related peptide (AgRP)/neuropeptide Y (NPY) co-expressing neurons are active during states of caloric need and represent essential drivers of hunger. Whether eCBs directly affect the neuronal activity AgRP/NPY hunger neurons is not clear. Therefore, we generated a mouse line in which AgRP/NPY neurons are deficient for the eCB-synthesizing enzyme NAPE-PLD (AGRP-NAPE-/-). Since AGRP-NAPE-/- mice will likely generate reduced amounts of eCBs, we aim at examination of the resulting metabolic phenotype under standard diet and diet-induced obesity on a molecular, metabolic and behavioral level.

Our first results revealed that AGRP-NAPE-/- mice are hyperphagic after fasting-induced re-feeding, while basal food intake remained unchanged upon standard diet. Effects on glucose metabolism and body fat composition are still under investigation.

Overall, the observed hyperphagic phenotype let us suggest that local eCBs in the ARC inhibit AgRP/NPY hunger neurons. Lack of/ or reduced amounts of eCBs in AGRP-NAPE-/- mice might therefore lead to dis-inhibition of AgRP/NPY hunger neurons.

Poster 75

Titel:
Innate fear response in wild and laboratory rats - behavioral and neuroanatomical differences

Autoren/Adressen:
Katharina Gottswinter (Otto-von-Guericke Universität Magdeburg), Silke Storsberg (Otto-von-Guericke Universität Magdeburg), Rafal Stryjek (Polish Academy of Sciences), Andrea Kröber (Otto-von-Guericke Universität Magdeburg), Klaudia Modlińska (Polish Academy of Sciences), Markus Fendt (Otto-von-Guericke Universität Magdeburg), Thomas Roskoden (Otto-von-Guericke Universität Magdeburg); katharina.gottswinter@st.ovgu.de

Abstract:
Rats are among the most frequently used species in laboratory research. They have been bread for generations in laboratories which led to alterations in physiology and anatomy compared to wild strains.

In this project rats of the wild strain Warsaw Wild Captive Pisula Stryiek (WWCPS) and rats of the laboratory strain Lister Hooded (LH) were exposed to a component of predator odor, 2,3,5-trimethyl-3-thiazoline (TMT), present in fox feces.
The present study analyzes neuronal activation in specific brain regions connected to aversive olfactory stimuli and fear responses by using immunohistological detection of the proto-oncogene c-fos.

Previous studies in our research group have shown behavioral differences between the two rat strains in this setup which triggers innate fear responses.

Here further significant (neuro)anatomical and new behavioral variations are presented.

Poster 76

Titel:
Noradrenergic innervation of mouse urinary bladder

Autoren/Adressen:
Uwe Pfeil (Institute for Anatomy and Cell Biology), Petra Mermer (Institute for Anatomy and Cell Biology), Wolfgang Kummer (Institute for Anatomy and Cell Biology); uwe.pfeil@anatomie.med.uni-giessen.de

Abstract:
To investigate distribution, density, and potential cotransmitters of noradrenergic nerve fibers innervating mouse urinary bladder.

Tyrosine hydroxylase (TH) was used as a marker for noradrenergic nerve fibers. Distribution of TH-positive fibers was investigated by immunohistochemistry in cleared urinary bladders prepared by the PACT (passive CLARITY technique) method in Bl6, ChAT (choline acetyltransferase)-GFP, and nAChR-alpha3-GFP mice. Colocalization with calcitonin gene-related peptide (CGRP), substance P, nitric oxide synthase I (NOS I), neuropeptide Y (NPY), vasoactive intestinal peptide (VIP), and ChAT was investigated in tissue sections and cleared urinary bladders.

TH-positive nerve fibers were found in nerve bundles, around blood vessels and as single nerve terminals. While nerve terminals were found frequently within the detrusor muscle and in the subepithelial layer, fiber bundles and TH-positive perivascular fibers were located more to the outer surface. Density of TH-positive fibers was highest at the bladder base, but innervation was still clearly obvious at the bladder body and dome. In nerve fibers, TH was colocalized with NPY but not with NOS I, VIP, substance P, and ChAT. Some very rare TH-positive fibers were also positive for CGRP. In intrinsic ganglion cells, colocalization of TH with NPY and ChAT was found.

The findings of this study indicate that the mouse urinary bladder receives extensive noradrenergic, probably sympathetic innervation. If this also applies to humans, it may have impact on treatment of overactive bladder and related diseases.

Poster 77

Titel:
Evaluation of non-motor symptoms of BoNT-A treatment in hemiparkinsonian rats

Autoren/Adressen:
Veronica Antipova (Medical University of Graz), Andreras Wree (Rostock University Medical Center), Alexander Hawlitschka (Rostock University Medical Center), Carsten Holzmann (Rostock University Medical Center);
carsten.holzmann@med.uni-rostock.de

Abstract:
While Parkinson's disease is mainly regarded as a movement disorder, patients suffer not only from motor symptoms, but also from non-motor symptoms which can significantly debilitate patient's activities as well as the quality of life and may appear before the motor symptoms. The loss of dopaminergic neurons in the substantia nigra leads to a disinhibition of cholinergic interneurons in the striatum. Recently we showed that injection of botulinum neurotoxin-A (BoNT-A) into the striatum of hemiparkinsonian (hemi-PD) rats reduced apomorphine-induced rotation behavior significantly for at least 3 months. The beneficial effect of BoNT-A possibly is based on the reduction of striatal hypercholinism in hemi-PD or changes in receptor densities. We previously showed that bilateral intrastriatal BoNT-A injections in Wistar rats does not cause cognitive impairments but reduce anxiety.

We here report further behavioral tests to evaluate possible side effects of intrastriatal BoNT-A treatment in hemi-PD rats. Forelimb akinesia was assessed using a modified version of a stepping test. Spontaneous horizontal locomotor activity and anxiety were estimated via the open field and elevated plus maze tests. Depressive behavior was testes using the forced swim test.

Concerning corridor task and stepping test BoNT-A injection did not change hemi-PD rats´ behavior significantly. In contrast to our study using wildtype rats we observed no changes in anxiety related behavior in hemi-PD rats. However, we detected significantly less depressive behavior in BoNT-A-treated hemi-PD rats compared to sham-treated and untreated hemi-PD rats.

Intrastriatal BoNT-A has a positive effect on motor and non-motor symptoms in hemi-PD rats.

Poster 78

Titel:
Quantitative morphology of mesencephalic dopaminergic progenitor cells in fibroblast growth factor-2 (FGF-2) isoform-specific knockout mouse embryos

Autoren/Adressen:
Ina Leiter (Hannover Medical School), Friederike von Hövel (Hannover Medical School), Claudia Grothe (Hannover Medical School); leiter.ina@mh-hannover.de

Abstract:
An increased number of dopaminergic neurons as well as an enlarged volume of the substantia nigra pars compacta (SNpc) has been described in adult mice genetically lacking all FGF-2 isoforms (Fgf2tm1Zllr). Additionally, FGF-2 knockout mice have shown an increased number of Lmx1a positive dopaminergic progenitor cells at embryonic day E14.5 and a reduced apoptotic rate at P0 in the SNpc. This study will identify the specific role of the respective isoforms during early and late events of development of DA neurons.

A timed mating was performed with four animal groups (n = 6 – 8): knockout mice (Fgf2tm2Doe/J) lacking the low molecular weight isoform of FGF-2 (LMW-ko), wildtype mice of the same strain (LMW-wt), knockout mice (Fgf2tm3Doe/J) lacking the high molecular weight isoforms of FGF-2 (HMW-ko) and the respective wildtype mice (HMW-wt). At embryonic day E13.5, bromodeoxyuridine (BrdU) is injected intraperitoneally into the pregnant females to mark proliferating cells. At E14.5, embryonic brains are prepared. The cryostat sections (40 µm) are double-stained for Lmx1a and BrdU. Double-stained cells in the subventricular zone of the ventral mesencephalon are quantified via stereology by a blinded investigator.

The absence of the LMW isoform could have a major impact on the amount of dopaminergic progenitor cells in the ventral mesencephalon as the HMW isoforms are barely detectable at this time point. Alternatively, the determining event concerning the higher dopaminergic neuron number in adult mice could occur later in the ontogenic development with possible involvement of the HMW isoforms.

This is under current investigation.

Poster 79

Titel:
PSEN1 / gamma-secretase plays a key role in the initial migration and (dendritic) differentiation of Cajal-Retzius cells

Autoren/Adressen:
Laura Hagemann (Universität Bonn), Dieter Hartmann (Universität Bonn), Angelika Zoons (Universität Bonn), Birgit Rau (Universität Bonn); s4lahage@uni-bonn.de

Abstract:
Investigation of the mechanism(s) governing the tangential migration of Cajal-Retzius cells and the elaboration of their dendritic architecture in the early marginal zone.

Analysis of the cortical anlage of wild-type and PSEN1-deficient mice between embryonic days (E) 13 and E 17 by immunohistochemistry and electron microscopy supplemented by cell culture assays investigating the effect of □-secretase inhibition on neurite development.

Absence of PSEN1 in vivo leads to a loss and maldistribution of Cajal-Retzius cells in the early marginal zone throughout prenatal development. Most notably, their in wild-type mice elaborate dendritic tree featuring multiple secondary branches running perpendicular up to the brain surface is reduced and stunted in KO mice. Pharmacological inhibition of □-secretase in vitro causes a retraction of neurites characterized by retraction bulbs immunoreactive for (among others) APP, APLP2 and mitochondrial markers in combination with alteration of the actin/ tubulin cytoskeleton which could be seen as an equivalent of dendritic vulnerability in vivo, where it is paralleled by a fragmentation of reelin deposition in the marginal zone.

Our results indicate that next to the established chemotactic control of CR cell positioning by CXC-L12 and CXC-R4 and –R7, signaling mechanisms based upon regulated intramembrane proteolysis play a crucial role especially for cell orientation and dendritic development.

Poster 80

Titel:
Functional cell groups and transmitter inputs in the abducens nucleus in human

Autoren/Adressen:
Anja Horn (Ludwig-Maximilians Universität), Annie Horng (RZN-Radiologisches Zentrum), Adam Dombi (Aurelious Augenzentrum), Norbert Buresch (Ludwig-Maximilians Universität), Ahmed Messoudi (Ludwig-Maximilians Universität), Wolfgang Härtig (Universität Leipzig); Anja.Bochtler@med.uni-muenchen.de

Abstract:
The abducens nucleus (nVI) contains several functional cell groups: motoneurons of singly-innervated twitch fibers (SIF-MNs), motoneurons of multiply-innervated tonic fibers (MIF-MNs) of the lateral rectus muscle, internuclear neurons (INTs) and paramedian tract-neurons (PMT) projecting to the flocculus. In monkey these cells can be delineated by combined tract-tracing and their chemical signature. Here we identified the homologous neurons and the inhibitory transmitter inputs in the human nVI compared to monkey.

Monkey and human brainstem sections were immunostained for choline-acetyltransferase (ChAT), chondroitin-sulphate proteoglycan (CSPG), glutamate decarboxylase (GAD) and glycine transporter 2 (GLYT2), as well as for acetylcholine esterase (AChE) activity. In two monkey cases the INTs were prelabelled by retrograde tract-tracing after Choleratoxin subunit B injection into the oculomotor nucleus (nIII).

We found: ChAT- and CSPG-positive SIF-MNs, ChAT-positive, but CSPG-negative MIF-MNs and ChAT-negative, but CSPG-positive INTs. ChAT-negative neurons with strong AChE-activity were identified as PMT neurons by correlating them with the location of anterograde tracer labelling from nIII. In accordance to monkey the homologous cell groups were identified in the human nVI by their chemical signature. Unlike in monkey a strong GABAergic input was found in the human nVI, in addition to a glycinergic input present in both species.

In conclusion, the nVI neuron populations were identified in man by their specific histochemical properties established in monkey, which allows correlative clinic-pathological studies on a cellular level. The study further demonstrated that “glycine as the main inhibitory transmitter of the horizontal oculomotor system” does not hold true for human.
Supported: DFG Ho 1639/4-4

Poster 81

Titel:
Olfactory performance as an indicator for protective treatment effects in an animal model of neurodegeneration

Autoren/Adressen:
Anne Gläser (Carl von Ossietzky University Oldenburg), Anja Meyer (Rostock University Medical Center), Anja U. Bräuer (Carl von Ossietzky University Oldenburg), Andreas Wree (Rostock University Medical Center), Jörg Strotmann (University of Hohenheim), Arndt Rolfs (Rostock University Medical Center), Martin Witt (Rostock University Medical Center); anne.glaeser@uni-oldenburg.de

Abstract:
Here we use a rare neurovisceral lipid storage disorder, Niemann-Pick disease C1 (NPC1), to illustrate disease-specific dynamics of olfactory dysfunction and its reaction upon therapy. Previous findings in a transgenic mouse model (NPC1-/-) show severe morphological and electrophysiological alterations of the olfactory epithelium (OE) and the olfactory bulb (OB) that ameliorate under therapy with combined 2-hydroxypropyl-ß-cyclodextrin (HPßCD)/allopregnanolone/miglustat or HPßCD alone.

Olfactory performance was tested using buried pellet test. Changes of olfactory key markers and several olfactory receptors were analysed via qRT-PCR. To investigate cell dynamics of the OB, we determined proliferative (BrdU) and apoptotic activities (Cas-3). Immunohistochemistry and western blotting for microglia (Iba1), astroglia (GFAP) and tyrosine hydroxylase was also performed.

The buried pellet test revealed a significant olfactory deterioration in NPC1-/- mice, which reverted to normal levels after treatment. In the OE, olfactory receptors localized in a small patch in the center OE are reduced in untreated NPC1-/- mice. In the OB, BrdU and Cas-3 data show increased proliferation and apoptotic activity of untreated NPC1-/- mice. At the protein level, Iba1 and GFAP indicate increased microgliosis and astrogliosis, which were prevented by treatment.

Olfactory deficits are likely due to central deficits, e.g., at the level of the OB. Due to the unique plasticity especially of peripheral olfactory components, the results show a successful treatment in NPC1 condition with respect to normalization of olfaction. Our results demonstrate that olfactory testing may be used as a suitable biomarker to evaluate the course of neurodegenerative signs and symptoms of NPC1.

Poster 82

Titel:
Enhanced expression of components of the neurotrophin signaling system in the hypertensive carotid body

Autoren/Adressen:
Dimitrinka Atanasova-Dimitrova (1) Institute of Neurobiology, Bulgarian Academy of Sciences; 2) Faculty of Medicine, Trakia University), Nikolay Dimitrov (Faculty of Medicine, Trakia University), Nikolai Lazarov (Medical University of Sofia); didiatan@bio.bas.bg

Abstract:
The carotid body (CB) is a small, neural crest-derived paired structure located at the carotid bifurcation. It senses the oxygen level in blood and monitors ventilation. The spontaneously hypertensive rat (SHR) is considered a good animal model for primary hypertension.

The present study is designed to determine the survival requirements of the CB cell populations in response to hypertensive stress. We examined the immunohistochemical localization of some neurotrophic factors and their corresponding receptors in the CB of SHRs, and then specifically compared their expression patterns with that of age-matched normotensive rats.

The CB was composed of two cell types, neuron-like glomus cells and glial-like sustentacular cells, which were aggregated in highly vascularized cell clusters, densely innervated by the sympathetic nerve system. Our immunohistochemical experiments revealed the presence of the nuclear Ki-67 protein in the sustentacular cells, and nerve growth factor, brain-derived neurotrophic factor, neurotrophin-3 and their corresponding receptors p75NTR, TrkA, TrkB and TrkC in the majority of glomus cells and in a subset of sustentacular cells. In addition, virtually all glomus cells expressed glial cell line-derived neurotrophic factor and its specific GDNF family receptor alpha1. The obtained quantitative image analysis data demonstrate that in glomus cells of the hypertensive CB there is more enhanced expression of components of the neurotrophin signaling system than in the in age-matched normotensive CB.

Our results suggest that hypertension can alter the neurotrophic factor profiles in the CB which results in parenchymal growth.

Poster 83

Titel:
Gradual development of dendritic and synaptic plasticity in adult newborn granule cells

Autoren/Adressen:
Tassilo Jungenitz (Goethe University), Marcel Beining (Ernst-Strüngmann Institute (ESI)), Tijana Radic (Goethe University), Lucas Alberto Mongiat (Universidad Nacional del Comahue-CONICET), Hermann Cuntz (Ernst-Strüngmann Institute (ESI)), Thomas Deller (Goethe University), Peter Jedlicka (Justus Liebig University Giessen), Stephan W. Schwarzacher (Goethe University); tassilo.j@gmx.de

Abstract:
Adult neurogenesis has been implicated in hippocampal forms of learning and memory. Here we study structural maturation, synaptic integration and plasticity of adult newborn GCs (abGCs) in young adult rats in vivo.

We virally labeled abGCs and mature GCs (mGCs). Structural analysis and reconstructions were combined with in vivo high-frequency stimulation (HFS) of the medial perforant path (MPP) known to induce homosynaptic LTP (hom LTP) at tetanized synapses and simultaneous heterosynaptic LTD (het-LTD) at non-tetanized synapses of the lateral perforant path.

2h HFS of the MPP induced hom-LTP in the middle molecular layer (MML) and conversely het-LTD in the outer molecular layer (OML). Analysis of mushroom spines revealed a homosynaptic spine head enlargement in the stimulated MML and heterosynaptic spine head shrinkage in the adjacent OML. Spine enlargement and shrinkage occurred in parallel on dendritic segments of the same neuron and appeared gradually in abGCs between 28 dpi and 35 dpi. Application of the non-competitive NMDA receptor antagonist MK-801 abolished hom-LTP and het-LTD and completely blocked concurrent structural spine changes. We further correlated GC morphologies with HFS responsiveness using the immediate-early gene Arc as a marker of synaptic activation. Only abGCs at 28 and 35 dpi but neither old abGCs nor mGCs responded to stimulation with a remodeling of their dendritic arbor.

AbGCs stay distinct from mGCs, exhibit NMDA receptor dependent structural hom-LTP and het-LTD of dendritic spines gradually from 28-35 dpi on and their dendritic arbor can be shaped by afferent activity during a narrow critical time window of 28-35 dpi.

Neurodegeneration/Neuroregeneration/Neuroimmunology

Poster 84

Titel:
Expression profile of pattern recognition receptors in skeletal muscle of ALS mice and sporadic ALS patients

Autoren/Adressen:
Sonja Johann (Medical Faculty Heinrich-Heine University Düsseldorf), Sonja Lehmann (Medical Faculty RWTH Aachen), Eliza Esch (Medical Faculty RWTH Aachen), Philipp Hartmann (Medical Faculty RWTH Aachen), Goswami Anand (Medical Faculty der RWTH Aachen), Nikolin Stefan (Medical Faculty der RWTH Aachen), Weis Joachim (Medical Faculty der RWTH Aachen), Beyer Cordian (Medical Faculty der RWTH Aachen); sonja.johann@med.uni-duesseldorf.de

Abstract:
Amyotrophic lateral sclerosis (ALS) affects upper and lower motoneurons in the brain and spinal cord, which leads to progressive muscle wasting and paralysis. It is well-accepted that inflammatory processes significantly contribute to disease progression. Inflammasomes are cytosolic multiprotein complexes and important players in innate immunity. They consist of a pattern recognition receptor (PRR), apoptosis-associated speck-like protein (ASC) and caspase 1 and are essential for interleukin (IL) processing. Recently, we described inflammasome activation in the spinal cord of ALS patients and in SOD1(G93A) ALS mice. Since pathological changes in the skeletal muscle are early events in ALS, we hypothesized that PRRs might be abnormally expressed in muscle fibre degeneration.

Muscle biopsies from SOD1(G93A) mice and sporadic ALS patients were analysed by Western Blot, immunohistochemistry and real-time PCR. Expression sub-cellular localization of PRRs, including NOD-like receptors (NLRs) NLRP1, NLRP3, NLRC4 and the DNA sensor, AIM2 (absent in melanoma 2) was investigated. Additionally, expression of ASC, caspase 1, IL1 beta and IL18 was evaluated.

Expression of PRRs and ASC was detected in murine and human tissue. Notably, NLRC4, caspase 1 and IL1 beta were significantly elevated in denervated muscle of SOD1(G93A) mice and sALS patients. Furthermore, levels of caspase 1 and IL1 beta were already increased in presymptomatic animals.

Our findings suggest that inflammasome activation may be involved in skeletal muscle pathology in ALS. Furthermore, elevated levels of NLRC4, caspase 1 and IL1 beta reflect early changes in the skeletal muscle and may contribute to the denervation process.

Poster 85

Titel:
Inhibition of formyl peptide receptors activity reduced Aβ1-42 induced neurodegeneration in Alzheimer's disease

Autoren/Adressen:
Nicole Schröder (Uniklinik Aachen), Lea Weiler-Tersch (RWTH Aachen), Anja Schaffrath (RWTH Aachen), Josua Welter (RWTH Aachen), Angelika Griep (University of Bonn), Michael Heneka (University of Bonn), Thomas Pufe (Uniklinik Aachen), Lars-Ove Brandenburg (Uniklink Aachen); nischroeder@ukaachen.de

Abstract:
The chemotactic G-protein coupled formyl peptide receptor (FPR), a receptor linked with signal transduction and uptake of Aβ1-42 in glial cells, seems to be linked with the massive increase of gliosis in the brain due to the chronic inflammation of Alzheimer's disease (AD). Our work is focused on mice FPRs, mFPR1 and mFPR2, which are orthologous to human FPR1 and FPRL1. We assume that their activity is critically involved in neuroinflammation and neurodegeneration in the Aβ1-42 affected brain.

We used an AD mouse model to inject the pro-inflammatory FPR agonist fMLF, FPR1/2 antagonist Boc2 or anti-inflammatory FPR2 agonist Ac2-26 i.p. twice a week about 20 weeks. After a Morris water maze (MWM) glial cell density, Aβ1-42 plaque density and the impact on neuronal structures were analyzed through immunohistochemistry. Besides we investigated the mRNA level of neurotrophic factors and Aβ-degrading enzymes.

Interestingly, our results suggest that Boc2 treatment of AD mice leads to an improved MWM performance, a reduced glial cell density in the hippocampus and a reduced neurodegeneration in the dentate gyrus and layer V of the cortex. Additionally, the mRNA level of Aβ-degrading enzymes and neurotrophic factors where significantly increased. Furthermore we determined a reduction of small sized plaques in the hippocampus. Ac2-26 and fMLF treatment revealed a wide variety in their effects.

Altogether, our results suggest that FPRs have an impact on Aβ1-42 induced neurodegeneration and glial cell activation in the course of AD. Especially inhibition with Boc2 seems to be a promising approach.

Poster 86

Titel:
Are peroxisomes involved in Alzheimer´s disease pathogenesis?

Autoren/Adressen:
Eugen Semikasev (Justus-Liebig-University Giessen), Barbara Ahlemeyer (Justus-Liebig-University Giessen), Anne Schänzer (Justus-Liebig-University Giessen), Elke Rodenberg-Frank (Justus-Liebig-University Giessen), Till Acker (Justus-Liebig-University Giessen), Eveline Baumgart-Vogt (Justus-Liebig-University Giessen); Barbara.Ahlemeyer@anatomie.med.uni-giessen.de

Abstract:
Gene mutations causing the familial form of Alzheimer´s disease (AD) are well characterized, but the pathogenesis of sporadic forms of this disease providing more than 95% of the patients is still not clarified. The apopliprotein E EPSILON 4 allele is the best established risk factor pointing to a disturbance in lipid metabolism. Peroxisomes are required for the synthesis and degradation of important lipids. Indeed, increased levels of very long-chained fatty acids and decreased levels of plasmalogens have been found in the brain of AD patients (Kou et al. 2011, Acta Neuropathol. 122: 271). However, the authors analyzed the peroxisome density only in the frontal cortex and with an antibody against a metabolically altered peroxisomal lipid transporter, therefore probably not detecting all peroxisomes.

We used paraffin sections from human autopsy material of 10 different brain regions of 5 gender-matched groups based to the ABC-Score for clinicopathological staging (control, low, middle, high probabilities for AD and non-AD related tauopathy). Double-immunofluorescence staining was performed to quantify peroxisomes with an anti-PEX14 antibody, the best marker for this organelle independent of cell-specific metabolisms, in neurons and in the surrounding neuropil in areas with and without amyloid plaques (anti-Amyloid BETA antibody, 4G8) and neurofibrillary tangles (anti-PHF tau antibody, AT8). Image J software was used for quantitative analysis.

The severity of AD pathology in some areas (entorhinal cortex, temporal cortex) correlated well with a reduced number of peroxisomes.

In future experiments, a higher number of patients will be analyzed to verify our preliminary finding of a role of peroxisomes in AD.

Poster 87

Titel:
Of old mice and men - what do their brains have in common?

Autoren/Adressen:
Barbara Ahlemeyer (Justus-Liebig-University Giessen), Sascha Halupczok (Justus-Liebig-University Giessen), Elke Rodenberg-Frank (Justus-Liebig-University Giessen), Klaus-Peter Valerius (Justus-Liebig-University Giessen), Eveline Baumgart-Vogt (Justus-Liebig-University Giessen);
Barbara.Ahlemeyer@anatomie.med.uni-giessen.de

Abstract:
Amyloid BETA peptide (A BETA), paired helical filament-tau (PHF-tau) and ALPHA-synuclein are in the focus of neuroscience research because they aggregate in brains of patients with Alzheimer's and Parkinson´s disease. Transgenic mouse models were used containing the human genes for A BETA precusor protein/presenilin/tau or ALPHA-synuclein with the most frequent mutations to mimick these diseases. However, they are not ideal since most patients develop sporadic forms of the diseases with no causative single gene defect. Furthermore, the aggregation of human proteins in man is not necessarily the same in rodents. We hypothesized that for such cases the aged mouse could be an alternative model.

Endogenous A BETA, PHF-tau and ALPHA-synuclein were analyzed at mRNA and protein levels (immunofluorescence staining) in formaldehyde-fixed paraffin-embedded brain sections of newborn up to 15-months-old mice. A BETA levels were additionally quantified using the A BETA40/42 ELISA Kits from Wako Chemicals.

A BETA was below detectable levels at birth, but present at high levels in the 15 month-old mouse. Intracellular A BETA was found in neurons of the temporal cortex, cingulate area, pons and cerebellum; extracellular A BETA in the periventricular zone. Mouse brain was devoid of PHF-tau-positive neurofibrillary tangles. ALPHA-Synuclein was visible at high level in the marginal zone of the lateral cortex and average levels in the hippocampus, pons and cerebellum with a 3-fold increased over time. ALPHA-Synuclein resided in the neuropil and did not aggregate into Lewy-body like structures even in the aged mouse.

We suggest the aged mouse as an alternative model to study A BETA plaque formation.

Poster 88

Titel:
Laquinimod is protective in a novel multiple sclerosis animal model

Autoren/Adressen:
Philipp Riedler (LMU Munich), Christin Reinbach (LMU Munich), Julia Nedelcu (LMU Munich), Markus Kipp (LMU Munich); philipp.riedler@gmx.at

Abstract:
Myelin pathology was recently identified as a potent trigger for peripheral immune cell recruitment into the forebrain of multiple sclerosis patients. Laquinimod has well-documented anti-inflammatory effects, but little is known about its direct activity within the brain. Here we tested the hypothesis that Laquinimod protects oligodendrocytes and myelin during non-inflammatory demyelination, and that this myelin protection secondarily ameliorates auto-immune triggered inflammation.

We used cuprizone as a model for noninflammatory demyelination and cuprizone combined with the classic active experimental autoimmune encephalomyelitis (i.e. Cup/EAE model), to trigger inflammatory forebrain lesions. Histological and immunohistological analyses were performed to analyse myelin, oligodendrocyte and microglia densities. TSPO PET Imaging (Positron Emission Tomography) was performed to study pan-microglia activation. Antibodies directed against various glia-marker proteins were used to study the signature of astrocyte activation.

On the histopathological level, Laquinimod ameliorated cuprizone-induced demyelination, microgliosis, and acute axonal injury. These results were confirmed by TSPO PET Imaging, demonstrating severe TSPO-ligand binding in vehicle, but not Laquinimod-treated groups. Subsequent immunization with myelin oligodendrocyte glycoprotein 35-55 peptide, which induces myelin autoreactive T cells in the periphery, resulted in massive immune cell recruitment into the affected forebrain of vehicle, but not Laquinimod-treated mice. Furthermore, we observed a fine-tuned amelioration of astrocyte activation by Laquinimod.

This study demonstrates that a drug which modulates brain-intrinsic inflammatory cascades secondarily can dampen the influx of peripheral immune cells into the brain, and by this mode-of-action can ameliorate autoimmune inflammation.

Poster 89

Titel:
Vesicular glutamate transporter 1 is a reliable marker for axonal damage

Autoren/Adressen:
Sebastian Rühling (Ludwig-Maximilians-University), Markus Kipp (Ludwig-Maximilians-University), Tanja Hochstrasser (Ludwig-Maximilians-University);

Abstract:
Axonal damage is the main factor contributing to permanent disability in Multiple sclerosis (MS). Treatment options to prevent disease progression in chronic stages are still limited. A better understanding of the underlying mechanisms of axonal injury is therefore urgently needed. While the focal accumulation of amyloid precursor protein (APP) at sites of axonal injury is well documented, the accumulation of other proteins transported by the anterograde axonal transport machinery is less well investigated. Here, we assume that synaptic vesicles accumulate at sites of acute and chronic demyelination.

We treated C57BL/6J mice with 0.25% cuprizone and studied accumulation of anterograde transport cargos in the corpus callosum by immunohistochemistry and immunofluorescence microscopy.

We showed that not only APP, but also synaptic (vesicular glutamate transporter 1) proteins accumulate in demyelinated areas. Increased numbers of spheroids were identified after acute demyelination. After chronic demyelination, spheroids were still observable, albeit to a lower extent. Co-localization studies revealed that synaptic proteins co-localized with APP after acute and chronic demyelination. In MS patient tissue samples, synaptic spheroids were also found. However, we did not detect accumulation of inhibitory synaptic proteins (vesicular GABA transporter).

In conclusion, our data suggest vesicular glutamate transporter 1 as a reliable marker of axonal damage in the CNS in inflammatory/demyelinating conditions. Further studies are needed to understand which factors orchestrate breakdown of the axonal transport machinery.

Poster 90

Titel:
CPI17 regulates oligodendrocyte degeneration

Autoren/Adressen:
Daniel Ostertag (Ludwig-Maximilians-University of Munich), Moritz Rennet (Ludwig-Maximilians-University of Munich), Newshan Behrangi (Ludwig-Maximilians-University of Munich), Markus Kipp (Ludwig-Maximilians-University of Munich);
daniel-ostertag@t-online.de

Abstract:
Multiple sclerosis (MS) is an autoimmune disease of the central nervous system characterized by chronic inflammation, demyelination, gliosis, and neuronal loss. CPI-17, encoded by the gene ppp1r14a, is a 17 kDa cytosolic protein that functions as a potent inhibitor of the smooth muscle myosin phosphatase. In the brain, CPI-17 is, besides by smooth muscle cells, specifically expressed by mature oligodendrocytes. The role of CPI-17 for oligodendrocyte physiology and pathology is not known. The aim of the current study is, therefore, to evaluate the function of CPI-17 during toxin-induced oligodendrocyte apoptosis.

Two groups of mice (C57BL/6) were used: (1) CPI-17-/- and (2) wild type. Both groups were intoxicated with 0.25% cuprizone for 1 week. Densities of apoptotic oligodendrocytes (H&E-Staining), microglia cells (anti-IBA1), oligodendrocytes (anti-OLIG2) and cellular stress (anti-ATF3) were evaluated by immunohistochemistry.

Immunohistochemical studies showed that CPI-17 is exclusively expressed in mature oligodendrocytes. CPI-17 expressing cells are lost under various demyelinating conditions including the cuprizone model. The loss of mature oligodendrocytes was more severe in CPI-17-/- compared to wildtype mice. This was paralleled by more severe microgliosis and higher levels of cellular stress responses in CPI-17-/- mice.

Our data show that CPI-17 is a novel regulator of oligodendrocyte pathology during early demyelination. Future studies are now needed to evaluate the relevance of CPI-17 for regenerative processes.

Poster 91

Titel:
Study on the treatment effect of agomelatine in kainate-induced status epilepticus in a model of temporal lobe epilepsy

Autoren/Adressen:
Dimitrinka Atanasova-Dimitrova (1) Institute of Neurobiology, Bulgarian Academy of Sciences; 2) Faculty of Medicine, Trakia University - Stara Zagora), Jana Tchekalarova (Institute of Neurobiology, Bulgarian Academy of Sciences), Lidia Kortenska (Institute of Neurobiology, Bulgarian Academy of Sciences), Michaella Shishmanova-Doseva (Medical University of Plovdiv), Pencho Marinov (Institute of Information and Communication Technologies), Angel Dandov (Medical University of Sofia), Todor Kirov (Medical University of Sofia), Nikolai Lazarov (Medical University of Sofia); didiatan@bio.bas.bg

Abstract:
Recently much data appeared in literature on the anticonvulsant effects of the antidepressant agomelatine (Ago) in acute seizure tests on intact animals. The aim of this study was to evaluate the effect of Ago, when applied in kainic acid (KA)-induced status epilepticus (SE), on the epileptiform activity during the acute and chronic stage of epilepsy.

To perform the goals of the study we employed these methods: electrode implantation for EEG recording, KA-induced SE, pharmacological approach, 24-hour video-surveillance and EEG recording, morphological and immunohistochemical methods.

In Experiment no.1, Ago (40 mg/kg i.p.), applied at certain intervals during SE (15 min., 6, 24 and 48 hrs), did not seem to be effective for the number of the EEG registered paroxysms or their duration when compared with lacosamide (50 mg/kg i.p.). In Experiment no.2, Ago and lacosamide were injected once during the chronic stage of epilepsy to study their anticonvulsant effect from a single dose. The EEG-recorded paroxysms and their duration were not altered by Ago, whilst lacosamide decreased them when compared with the group injected only with the solvent. The multiple administration of Ago has a powerful neuroprotective and anti-inflammatory effect, as it suppresses the KA-induced microglosis and astrogliosis in CA1 and CA3c fields of the dorsal hippocampus.

In conclusion, Ago does not affect the epileptiform activity in KA-induced post-SE model of temporal epilepsy, though it provides neuoroprotection and selectively suppresses activated glia and astrocytes in CA1 and CA3c fields of the dorsal hippocampus.

Poster 92

Titel:
Demyelination, but not inflammation triggers oligodendrocyte progenitor activation

Autoren/Adressen:
Patrizia Leopold (Ludwig-Maximilians-University of Munich), Stella Nyamoya (RWTH Aachen University), Markus Kipp (Ludwig-Maximilians-University of Munich); patrizia-leopold@t-online.de

Abstract:
Remyelination is the natural repair mechanism of demyelination and can be a highly efficient process in multiple sclerosis (MS). However, in the majority of lesions, this regenerative approach is incomplete or even fails completely. An impaired oligodendrocyte progenitor cell (OPCs) activation has been suggested as one underlying mechanism of remyelination failure in MS. To what extend demyelination or inflammation trigger OPC activation in MS is, however, unknown. Here we investigate which mechanisms trigger OPC activation in the forebrain of MS-relevant animal models.

Demyelination of forebrain white matter bundles was induced by cuprizone intoxication. Inflammation with moderate demyelination was induced by MOG-peptide immunization (experimental autoimmune encephalomyelitis; EAE), or a combination of cuprizone and EAE (Cup/EAE). Densities of GPR17 (G protein-coupled receptor 17)-expressing OPCs was analyzed by immunohistochemistry. The cellular signature of GPR17-expressing cells was tested by immunofluorescence double labelling experiments.

During toxin-induced demyelination, there was a time-dependent increase of GPR17+ cell densities in the forebrain. No increase of GPR17+ cell densities was observed in inflammatory spinal cord lesions. In Cup/EAE mice, the activation of OPC was absent as well. In all models, most of the GPR17+ cells co-expressed the oligodendrocyte marker protein OLIG2.

This study nicely demonstrates that demyelination, and not inflammation, is the pivotal trigger for OPC activation in the central nervous system.

Poster 93

Titel:
Characterisation of the mitochondria in the cervical spinal cord of the wobbler mouse

Autoren/Adressen:
Jan Stein (Ruhr-University Bochum), Carsten Theiss (Ruhr-University Bochum), Veronika Matschke (Ruhr-University Bochum); Jan.Stein-d6t@ruhr-uni-bochum.de

Abstract:
Amyotrophic lateral sclerosis (ALS) is a chronic degenerative disease of the nervous system. The cardinal symptom is the atrophy of skeletal muscle, which leads to lethal outcomes.
Until now, many questions about the pathogenesis of ALS have not been finally resolved. Latest studies could detect a malfunction of the respiratory chain complexes I, III and IV in wobbler mice, an animal model of sporadic ALS, which lead to selective motor neuron vulnerability. On this basis mitochondria within the cervical spinal cord of wobbler mice were analyzed concerning their ultrastructure.

The Wobbler animal model (WR) plays an important role in ALS research, since the homozygous mice show typical hallmarks similar to human ALS. In order to study the distribution, size and morphology of mitochondria in more detail, spinal cord of WR and wildtype mice were investigated by electron microscopy. Here, certain parameters were analyzed using ImageJ. Additionally the network of mitochondria is checked using immunohistochical techniques. In the focus are proteins that regulate fusion and fission processes and thus influence the morphology and function of mitochondria. For this reason we analysed mRNA- and protein-expression of several candidates by qPCR and Western Blot.

We observed various parameters of mitochondria that differ in wobbler mice compared to wildtype ranging from disorganization of the cristae to swelling of the inner membrane.

Changes in the morphology and functionality of mitochondria play a major role in the wobbler mouse and should once again move into the focus of ALS research.

Poster 94

Titel:
ROS-Scavenging-Defects in motor neuron of wobbler mice as a possible explanation for oxidative stress in sALS

Autoren/Adressen:
Maya Junghans (Ruhr-University Bochum), Johann Matschke (University Hospital Essen), Carsten Theiss (Ruhr-University Bochum), Veronika Matschke (Ruhr-University Bochum); maya.junghans@rub.de

Abstract:
Amyotrophic lateral sclerosis is a chronic disease of the central and peripheral nervous system with degeneration of the 1st and 2nd motor neurons. In contemporary research the Wobbler mouse plays an important role as an animal model for sALS.
Recent studies have shown that levels of reactive oxygen species (ROS) in motor neurons of wobbler mice are elevated compared to those in wild-type motor neurons. In many neurodegenerative diseases (e.g. Alzheimer) Glutathione and its metabolism is impaired. As this is an important antioxidant the reducing conditions can no longer be maintained.

To uncover the processes leading to increased ROS levels, primary dissociated cultures from the spinal cord of wobbler mice and wild-type mice were used. These cultures are treated with different ROS scavengers, to distinguish between the most important ROS producers in wobbler motor neurons. As double strand breaks are a putative consequence of elevated ROS-levels, immunocytochemical staining of yH2AX, a double strand break marker, was performed.

With the aid of different ROS-scavengers we were able to show that the glutathione metabolism in motor neurons of Wobbler mice differs from wild-type motor neurons.

The increased ROS level in motor neurons of wobbler mice could be an important factor in the pathogenesis of ALS. As the glutathione homeostasis is altered in motor neurons of Wobbler mice, it can be assumed that glutathione as an antioxidant, plays a role in the development of ALS.

Poster 95

Titel:
Pulmonary homeostasis is affected in Niemann-Pick Disease Type C1

Autoren/Adressen:
Katarina Gerdinand (University of Rostock Medical Center), Andreas Wree (University of Rostock Medical Center), Michaeal Kasper (University of Technology Dresden), Marcus Frank (University of Rostock Medical Center), Martin Witt (University of Rostock Medical Center); katarina.gerdinand@med.uni-rostock.de

Abstract:
Niemann-Pick disease type C1 (NPC1) is a rare neurovisceral, autosomal recessive disorder caused by a mutation of the NPC1 gene. This gene encodes a transmembrane protein, which is relevant for the intracellular transport of unesterified cholesterol from late endosomes / lysosomes to the endoplasmic reticulum. Dysfunctions lead to interruption of this transport and intracellular accumulation of cholesterol and several glycolipids resulting in severe neuropathology, visceromegaly and pulmonary failure.
Until now there is no causal therapy of NPC1. It has been previously demonstrated that the use of either 2-hydroxypropyl-ß-cyclodextrin (“HPßCD treatment”) or a combination of miglustat, allopregnanolone and HPßCD (“combi treatment”) revealed positive effects with regard to neurologic and visceral symptoms.

We used an NPC1 mutant mouse model (BALB/c Nctr-Npc1m1N/J) to investigate ultrastructure, occurrence and distribution of alveolar epithelial cells and macrophages using immunohistochemistry and electron microscopy. We compared 6 groups: sham-treated NPC1-/-, HPßCD-treated NPC1-/-, combination-treated NPC1-/- mice and the correspondent wildtypes (NPC1+/+).

Preliminary results show that NPC1-/- animals show severe hemorrhage, alterations of the air/blood barrier, e.g., capillary endothelial and alveolar epithelial injury and increased numbers of foam cells when compared to NPC1+/+ animals.

In contrast to the situation in the CNS and liver, treatment effects in the lung of NPC1-/- mice are inconsistent and need further investigation.

Poster 96

Titel:
Induction of dopaminergic degeneration in organotypic slice cultures

Autoren/Adressen:
Sarah Joost (Rostock University Medical Center), Michael Tasler (Rostock University Medical Center), Andreas Wree (Rostock University Medical Center), Stefan Jean-Pierre Haas (Rostock University Medical Center); sarah.joost@med.uni-rostock.de

Abstract:
Experimental models which exhibit dopaminergic cell loss are pivotal in research on Parkinson's disease. The most common techniques are treatments with the neurotoxins 6-OHDA or MPTP in rodents or non-human primates. Both specifically lesion dopaminergic neurons and lead to a Parkinson-like degeneration of the substantia nigra pars compacta. In order to establish alternatives for these severe animal experiments, we are aiming to transfer these model systems to organotypic nigrostriatal slice cultures.

Whole-brain murine sagittal organotypic slices containing all components of the nigrostriatal pathway were cultured on semiporous membranes for two weeks. During culture, slices were treated with 6-OHDA or MPP+, the active metabolite of MPTP, to induce dopaminergic degeneration. Different neuronal and glial cell populations were analysed by immunofluorescent stainings. Dopaminergic neurons were detected by tyrosine hydroxylase immunohistochemistry and quantified.

Treatment of organotypic slice cultures with 6-OHDA at various time-points and for different durations of culture only led to minor dopaminergic degeneration. This is in contrast to the robust cell loss of about 80 % we documented after stereotactic injection of 6-OHDA in vivo. Results for treatment with MPP+ are still pending by the time of abstract submission.

Organotypic nigrostriatal slice cultures are a promising ex vivo model for Parkinson's research, still the inducibility of dopaminergic degeneration by treatment with neurotoxins has to be evaluated. Future studies will strive for the application of organotypic slice cultures in assessment of neuroprotective or neuroregenerative agents in Parkinson's disease treatment.

Poster 97

Titel:
Altered phosphorylation of profilin2 is a key factor of changed actin dynamics in Spinal Muscular Atrophy

Autoren/Adressen:
Lisa Marie Walter (Hannover Medical School), Sebastian Rademacher (Hannover Medical School), Robert Lindner (Hannover Medical School), Niko Hensel (Hannover Medical School), Peter Claus (Hannover Medical School); Walter.Lisa@mh-hannover.de

Abstract:
Spinal Muscular Atrophy (SMA) is a fatal autosomal recessive disorder in children characterized by degeneration of lower motoneurons leading to progressive muscle atrophy. The disease is caused by a lack of the survival of motoneuron (SMN) protein. However, the mechanisms of motoneuron susceptibility still remain elusive. The direct interaction between SMN and the actin-binding proteins Profilin1/2 (PFN) links SMA pathology to dysregulation of the actin cytoskeleton. Moreover, the RhoA/ROCK pathway is hyperactive in SMA leading to aberrant phosphorylation of PFN2 and cofilin. Additionally, we have shown the presence of cofilin-actin rods in several cellular and mouse SMA models. Here, we analyzed the contribution of specific PFN phosphorylation-sites on altered actin dynamics in SMA.

To test this, we mutated putative phospho-sites of PFN2 to obtain phospho-mutants. To further investigate the impact of phosphorylation on basic properties of PFN2, we performed a variety of binding assays with purified recombinant PFN2 mutants. Moreover, we examined the role of PFN2 on actin rod formation by transfection of different PFN2 constructs in motoneuron-like NSC34 cells.

We identified several putative phospho-sites of PFN2 altering its binding properties. Moreover, PFN2 knockdown significantly reduced the number of cells with actin rods. Phosphorylation of specific sites has an effect on actin rod formation in the cell.

Together, our results indicate the importance of the role of PFN2 in dysregulated actin dynamics observed in SMA. A correction of the phosphorylation pattern of PFN2 and other factors may contribute to the amelioration of the SMA phenotype.

Poster 98

Titel:
Cleavage of the guidance receptor PlexinD1 in Spinal Muscular Atrophy

Autoren/Adressen:
Cornelia Greb (Hannover Medical School), Sebastian Rademacher (Hannover Medical School), Niko Hensel (Hannover Medical School), Peter Claus (Hannover Medical School); Cornelia.Greb@stud.mh-hannover.de

Abstract:
Spinal Muscular Atrophy (SMA) is a neuromuscular disease characterized by degeneration of alpha-motoneurons in the spinal cord. This disorder is caused by mutations of the SMN1 gene resulting in muscle weakness and atrophy. Dysregulation of the actin cytoskeleton plays a critical role in SMA pathogenesis. Cytoskeletal rearrangement is mediated by either attractive or repulsive guidance cues binding to guidance receptors. PlexinD1 is a membrane-spanning cell surface guidance receptor activated by the Semaphorin SEMA3E. Previously, we showed that PlexinD1 is cleaved by metalloproteases in different models of SMA, causing a signal switch from attractive to repulsive. Here, we hypothesized that a specific metalloprotease is up-regulated inducing PlexinD1 cleavage and that the cleavage product is detrimental for motoneuron survival and maintenance in SMA.

To identify potential candidates, we screened for expressional changes of a large number of metalloproteases and tissue inhibitors of metalloproteases (TIMPs) by RT qPCR. The screening was performed in SMA-mice spinal cords and motoneuron-like NSC34 cells.

We identified altered expression of seven metalloproteases as well as two TIMPs in SMA mice. Based on these data, putative metalloproteases were further examined by Western Blot and investigations of actin rod formation under knockdown conditions. We have identified candidate metalloproteases responsible for cleaving PlexinD1 in SMA models.

Analyses and identification of metalloproteases and their inhibitors are an important milestone for modulating increased PlexinD1 cleavage in SMA and a putative base for novel therapeutic strategies.

Poster 99

Titel:
Exploring patho-mechanistic targets of Alzheimer's disease in its initial stages

Autoren/Adressen:
Fatemah Sakr (School of Medicine, Rostock University), Michel Grothe (German Center for Neurodegenerative diseases), Anja Bräuer (School of Medicine and Health Sciences, Carl von Ossietzky University Oldenburg), Stefan Teipel (School of Medicine, Rostock University); fatemah.sakr@med.uni-rostock.de

Abstract:
- Test the replicability of a recently described approach for in-vivo staging of regional cerebral amyloid-β burden enhancing the detection of its initial phases of deposition.
- Exploring lipid profile alterations in Alzheimer's disease patients in a trial to promote new dynamic biomarker candidates that could be of particular sensitivity to predict disease progression.

We projected the pre-processed amyloid PET data of the participants (n= 318) of the INSIGHT-preAD cohort, i.e. people with subjective memory complaints into the proposed in-vivo hierarchical staging model of amyloid-β progression. Individual adherence to this model was examined across all cases. For the second objective, exploratory comprehensive lipid profile analysis was carried out on a limited number of plasma samples as a first step. The identified lipid alterations would be further explored in a larger sample.

We provided evidence for the validity and replicability of the proposed in-vivo staging model of amyloid-β progression. Individual staging allowed for identifying 38.7 % of the individuals in this preclinical cohort as having evidence of regional amyloid deposition, opposed to the 20 % identified as amyloid-positive using the conventional method. Preliminary results from the lipid profile analysis identified alterations in several lipid members that should be further characterized using Mass spectrometry.

The in-vivo amyloid staging model provides a reproducible approach for a clinically relevant stratification of the initial phase of AD. Lipid profile analysis could represent a promising dynamic biomarker of Alzheimer's disease patho-mechanisms progression

Poster 100

Titel:
Cell biology of post-translational modifications of the survival of motoneuron (SMN) protein

Autoren/Adressen:
Tobias Schüning (Hannover Medical School), Nora Tula Detering (Hannover Medical School), Niko Hensel (Hannover Medical School), Peter Claus (Hannover Medical School); Tobias.Schuening@stud.mh-hannover.de

Abstract:
Spinal muscular atrophy (SMA) is an autosomal recessive neurodegenerative disease characterized by proximal muscular atrophy due to the loss of alpha motoneurons. SMA is caused by a homozygous deletion or mutation of the survival of motoneuron gene 1 (SMN1) leading to low levels of functional survival of motoneuron (SMN) protein. SMN is involved in several molecular processes dependent on its localization: this includes regulation of the actin cytoskeleton in the cytoplasm, snRNP biogenesis in the nucleus and cytoplasm, and pre-mRNA splicing within the nucleus. Previous studies revealed that the SMN protein level has an impact on neurite outgrowth as well as neuronal survival in vitro. Localization and stability of SMN are regulated by post-translational modifications such as phosphorylation. Thus, we hypothesized that altered phosphorylation state of SMN has a putative effect on cell survival and neurite outgrowth.

Therefore, we investigated selected SMN phosphorylation sites by generating non-phosphoryable SMN mutants and SMN phospho-mimics. Moreover, siRNA mediated knock-down of endogenous SMN was performed mimicking SMA conditions.

Since siRNA mediated knock-down of SMN alters cell survival, cell viability was rescued with selected SMN phospho-mutants. Hence, the phosphorylation state of SMN revealed an impact on neurite outgrowth in neuron-like cells.

The results indicate the potential role of altered SMN phosphorylation state for cell survival and neurite outgrowth under SMA conditions.

Poster 101

Titel:
[18F]FDG-PET/CT imaging in an animal model of Parkinson's disease

Autoren/Adressen:
Teresa Mann (Rostock University Medical Center), Bernd Joachim Krause (Rostock University Medical Center), Alexander Hawlitschka (Rostock University Medical Center), Jan Stenzel (Rostock University Medical Center), Tobias Lindner (Rostock University Medical Center), Stefan Polei (Rostock University Medical Center), Alexander Hohn (Rostock University Medical Center), Andreas Wree (Rostock University Medical Center), Jens Kurth (Rostock University Medical Center); teresa.mann@med.uni-rostock.de

Abstract:
In Parkinson's disease (PD) the progressive striatal dopaminergic cell loss is followed by hypercholinism in the striatum which additionally worsens motor impairments. Hemiparkinsonian (hemi-PD) rats display PD's pathophysiology and are frequently used in the preclinical testing of new therapeutical options. The dopaminergic cell loss is induced by unilateral injection of 6-hydroxydopamine (6-OHDA) into the medial forebrain. In former studies, we demonstrated intrastriatal application of the anticholinergic drug botulinum neurotoxin-A (BoNT-A) to improve motor deficits in hemi-PD rats. The underlying mechanisms leading to an improvement of motor functions after treatment with BoNT-A have not been fully disclosed. As literature provides indication that alterations in glucose utilization are associated with neurodegenerative diseases like PD, we used [18F]FDG-PET/CT studies to analyze regional glucose metabolism in 6-OHDA rats with and without BoNT-A treatment.

32 animals were measured in 3 groups: controls, BoNT-A-injected hemi-PD rats and sham-injected hemi-PD rats. Online blood-sampling via an arteriovenous shunt was performed during PET data acquisition for subsequent kinetic modeling. An individual MRI of each rat was used as a co-registration template and anatomical reference.

Calculation of Standard uptake values (SUV) in static images could not identify significant interhemispheric differences in hemi-PD and BoNT-A-treated hemi-PD rats. Interestingly, the new approach to fully quantify regional changes in dynamic images using kinetic modeling with arterial input curves derived from continuous blood activity data revealed relevant effects in our different experimental groups.

In summary, [18F]FDG-PET/CT imaging with online blood-sampling is a feasible tool to analyze disease processes and therapeutical benefits in hemi-PD rats.

Poster 102

Titel:
Survival of Motoneuron (SMN) does not localize to stress granules but regulates their formation

Autoren/Adressen:
Sabrina Kubinski (Hannover Medical School), Luisa Klemke (Hannover Medical School), Carina Müller (Hannover Medical School), Niko Hensel (Hannover Medical School), Peter Claus (Hannover Medical School); kubinski.sabrina@mh-hannover.de

Abstract:
Stress granules (SGs) are cytoplasmic complexes comprising mRNA molecules and different proteins. They are formed as a reaction to cellular stress. RNA molecules, not essential for cellular survival, are stored in SGs thereby preventing their translation. This is an energy-saving process and important for regeneration and survival after removal of the stressor. Thus, SGs are dynamic structures that assemble under stress and dissolve after stress elimination. However, SGs often become permanent aggregates in neurodegenerative diseases. Spinal Muscular Atrophy (SMA) is a fetal, autosomal recessive neurodegenerative disease affecting children. In SMA, the Survival of Motoneuron 1 (SMN1) gene is mutated or lost. Previous studies in fibroblasts suggested that SMN localizes to SGs.

All experiments were performed in motoneuron-like NSC34 cells. For co-localization studies we combined confocal microscopy with in-depth image analyses. Moreover, we used a high-throughput technology for SG quantification in siRNA-mediated SMN knock-down cells.

Here, we show that SMN does not localize to stress granules in motoneuron-like NSC34 cells. Interestingly SMN-interacting proteins Profilin 1 and Profilin 2 co-localize with SGs. siRNA-mediated knock-down of SMN results in a significant increase of SG numbers in stressed NSC34 cells.

These findings suggest that SG dynamics is indirectly altered in SMA. Furthermore, SMN reduction changes steady state levels of Profilins eventually resulting in a functional loss of SGs. In conclusion, SMN regulates SG dynamics in motoneuron-like cells.

Poster 103

Titel:
Role of fibroblast growth factor 2 on the development of dopaminergic neuronal subpopulations

Autoren/Adressen:
Alina Langenhagen (mh-hannover), Dietmar Schreiner (mh-hannover), Claudia Grothe (mh-hannover);langenhagen.alina@mh-hannover.de

Abstract:
Neurotrophic factors fulfill a pivotal role in the shaping and proper function of the CNS. Among others Fibroblast growth factor 2 (FGF2) is well known to play an important role during neuronal development and in the adulthood. Our previous studies revealed that genetic ablation of FGF2 caused hyperplasia of dopaminergic neurons in substantia nigra pars compacta (SNc) in mice, suggesting an important role of FGF2 for midbrain dopaminergic neurons (mDA). To get deeper insights into the functional role of FGF2 for dopaminergic neurons we aimed to analyze the impact of the FGF2 gene ablation on mRNA translation in the mDA neurons in adult animals.

For the isolation of actively transcribed mRNA from mDA neurons we applied the RiboTag approach. The isolated mRNA was sequenced on the Illumina platform and analyzed using bioinformatics approaches.

Several genes were identified to be differentially expressed in the FGF2-KO vs. wild-type littermates. Among others Otx2 transcriptional factor was identified to be strongly reduced in the adult mDA neurons of KO mice. Otx2 plays a central role during the development of mDA neurons. In the adulthood Otx2 is known to be expressed in a subset of mDA neurons which are located in the Ventral-Tegmental-Area. Additionally, analysis of the projection area of this neuronal population in the Nucleus accumbens showed reduced downstream dopamine signaling.

Our observations suggest that FGF2 might be involved in the differentiation and/or maintenance of Otx2 positive mDA neurons.

Poster 104

Titel:
Presenilin-associated rhomboid-like protease PARL is crucial for the postnatal maintenance of oligodendrocytes and neurons

Autoren/Adressen:
Sonja Magdalena Hilse (University of Bonn), Angelika Zoons (University of Bonn), Birgit Rau (University of Bonn), Dieter Hartmann (University of Bonn); s4sohils@uni-bonn.de

Abstract:
Analysis of the CNS phenotype of mice deficient for the rhomboid protease PARL, an enzyme involved both in apoptosis control and mitochondrial dynamics and turnover.

Analysis of brain morphology of PARL deficient mice by ultrastructural and immunohistochemical approaches as well as monitoring PARL and known PARL substrates (OPA-1, pink1/parkin, Htra2/Omi and PGAM5) by Western Blotting and quantitative RT-PCR.

We observed a major loss by apoptosis of oligodendrocytes and neurons most prominent in diencephalon, brain stem and deep cerebellar nuclei, with only moderate lesions of the striatum, while only minor defects occured in the (retrosplenial) cortex. Neural tissue destruction is characterized by a an apparent sequence of hydropic swelling of axonal mitochondria, detachment oft he inner myelin lamella and subsequent axonal collapse, remnants of which temporarily remain within a condensed myelin sheath.

Until now, deregulation of apoptosis by PARL deficiency has mainly been associated with lymphatic tissues. Here we show that loss of this protease has a related effect in the postpubertal murine brain. Interestingly, the initial steps seem to be restricted to myelinated axons, arguing for a specific and novel role oft he PARL/STOML2 complex in the interplay between axons and ther myelin sheaths.

Poster 105

Titel:
Deficiency for the mitochondrial rhomboid protease PARL causes an adult-onset photoreceptor degeneration in mice

Autoren/Adressen:
Barbara Deimling (University of Bonn), Angelika Zoons (University of Bonn), Birgit Rau (University of Bonn), Dieter Hartmann (University of Bonn);
dhartman@uni-bonn.de

Abstract:
Investigation of the retinal function of PARL, a protease of the inner mitochondrial membrane, and its association with the processing of OPA-1 and thus the pathogenesis of dominant optic atrophy

Analysis of retina and optic nerve in wild-type and PARL-deficient mice between postnatal weeks 4 and 12 by immunohistochemistry and electron microscopy. PARL and known PARL substrates (OPA-1, pink1/parkin, Htra2/Omi and PGAM5) were additionally monitored by Western Blotting and quantitative RT-PCR.

Despite the frequently cited role of PARL as a protease crucial for the activating cleavage of OPA-1 we did neither encounter a destruction of ganglion cells nor other degenerative lesions of the optic nerve chracteristic of DOA in PARL – deficient mice. Instead, we observed a progressive degeneration of rod photoreceptors primarily affecting their outer segments, but then progressing to the remainder of the cell. Outer segment foreshortening and destruction were preceded by a multifocal changes within photoreceptors of key components of the Krebs cycle as well as structural components of the cilia (rootlets).

Our results support recent data that in most tissues PARL is not crucial for OPA-1 dependent apoptosis control, which seem to rely more on mAAA proteases OMA and YME1L1. The late-onset degeneration of photoreceptor outer segments observed instead seems to be linked to both defective energy production and abnormalities of the ciliar rootlet. A striking novel link between these seemingly unrelated pathogenetic events could reside in functional defects of proteins controlling both mitochondrial turnover and cilia formation like HDAC6 and VDAC3.

Poster 106

Titel:
Expression of immunologically relevant genes in multiple sclerosis animal models

Autoren/Adressen:
Katherina Vicky Gantenbein (Ludwig-Maximilians-Universität München), Sophie Alina Mitter (Ludwig-Maximilians-Universität München), Markus Kipp (Ludwig-Maximilians-Universität München); k.gantenbein@campus.lmu.de

Abstract:
Multiple sclerosis (MS) is a common autoimmune disease that targets myelin in the central nervous system (CNS). Recent genome-wide association studies have uncovered more than 200 loci that independently contribute to disease pathogenesis. Many of these genes are accepted regulators of the acquired immune system. Since a growing body of evidence suggests that components of the innate immune system, particularly microglia and astrocytes, trigger the development of inflammatory MS lesions, we here investigated the expression of various MS-associated proteins in glia cells.

Reproducible demyelination accompanied by microglia and astrocyte activation was induced by cuprizone intoxication. In a separate cohort of mice, development of anti-myelin autoimmunity was triggered by immunization with the myelin oligodendrocyte glycoprotein 35-55 peptide (MOG_{35-55}) after cuprizone intoxication. Focal demyelination was induced by stereotactic injection of lysophosphatidylcholine. Gene and protein expression levels were investigated by gene array analyses, PCR and immunohistochemistry, respectively.

The expression of various immunoregulatory genes was observed to be induced in MS animal models, among the transcription factors STAT3 and c-MYC as well as VCAM1 and TNFRSF1A. While STAT3 was expressed by activated astrocytes, c-MYC was expressed by microglia cells. Furthermore, peripheral immune cells expressed such transcription factors.

This study clearly demonstrates the importance of innate immune cells for MS lesion development. A better understanding of factors regulating early inflammatory lesion development will pave the way for novel therapeutic treatment strategies.

Poster 107

Titel:
The immunregulatory gene CD44 is expressed by glia cells in a model of toxic demyelination

Autoren/Adressen:
Maria-Sophia Stadler (Anatomische Anstalt der Ludwig-Maximilians Universität München), Christin Reinbach (Anatomische Anstalt der Ludwig-Maximilians Universität München), Markus Kipp (Anatomische Anstalt der Ludwig-Maximilians Universität München), Tanja Hochstrasser (Anatomische Anstalt der Ludwig-Maximilians Universität München); mariasophia.stadler@med.uni-muenchen.de

Abstract:
Numerous studies report that the pathogenesis of multiple sclerosis (MS) is mediated by autoimmune inflammation, others have proposed that brain intrinsic degeneration is the initial factor driving lesion formation. We have shown that the immunologically relevant gene CD44 is expressed in the brain in a mouse model of intrinsic demyelination. The objective of this study was to determine the precise localization and induction of CD44.

Intoxicating C57BL/6 with cuprizone induced intrinsic degeneration only, to realise the autoimmunity liaison cuprizone was combined with active EAE (i.e. Cup/EAE). To determine the localization of brain intrinsic CD44, we used three distinct methods: immunogold labeling, immunofluorescence staining in hGFAP-eGFP transgenic mice and in-situ-hybridization. To test whether CD44 is expressed on peripheral immune cells outside the brain, monocytes, Th1-, Th17-, and regulatory T cells were examined by flow cytometry.

The observed staining pattern suggested expression of CD44 in GFAP-positive astrocytes in the corpus callosum of cuprizone animals. To test the interaction between intrinsic degeneration and autoimmunity on peripheral CD44 expression, both models were combined. First results show increased expression of CD44 on Th1, Th17, regulatory T cells and monocytes in Cup/EAE animals compared to controls.

This study demonstrates that CD44 is expressed by brain astrocytes and immune cells in MS relevant models. Expression of the immunologically relevant gene CD44 could play a major role for the progression of immune cells into the brain.

Poster 108

Titel:
Motor deficits in experimental autoimmune encephalomyelitis are ameliorated by copper chelation

Autoren/Adressen:
Vladislav Yakimov (LMU Munich), Felix Schweiger (LMU Munich), Markus Kipp (LMU Munich); vladislavvd15@gmail.com

Abstract:
Previous studies suggest that the copper-chelator cuprizone (Bis(cyclohexanone) oxaldihydrazone) might directly suppress T-cell reactivity. The immune system requires copper to perform several functions, of which little is known about the direct mechanism of action. Here we investigated whether copper-chelation via cuprizone feeding is protective in experimental autoimmune encephalomyelitis (EAE), one of the most commonly used multiple sclerosis (MS) animal models.

Female C57BL/6 mice were immunized with myelin oligodendrocyte glycoprotein 35-55 peptide, which induces the formation of myelin autoreactive T cells in the periphery. In parallel, mice were treated with cuprizone. Animals were scored daily for clinical deficits and sacrificed at the peak of the disease. Brains were (immune-) histochemically evaluated for the presence of perivascular inflammatory infiltrates, demyelination, glia reactivity and neurodegeneration.

Disease frequency was lower in cuprizone versus vehicle groups. Maximum disease score and cumulative disease score was lower in cuprizone versus vehicle groups. The amelioration of clinical deficits by copper chelation was paralleled by less severe histopathological alterations.

The chelation of copper by cuprizone robustly suppresses development of anti-myelin immune response. This strategy might be an attractive opportunity to halt development of MS and other autoimmune diseases.

Poster 109

Titel:
The influence of purine metabolism on excitotoxic neuronal injury

Autoren/Adressen:
Joshua Kleine (Martin Luther University Halle-Wittenberg), Fahim Ebrahimi (Martin Luther University Halle-Wittenberg), Tim Hohmann (Martin Luther University Halle-Wittenberg), Chalid Ghadban (Martin Luther University Halle-Wittenberg), Urszula Grabiec (Martin Luther University Halle-Wittenberg), Faramarz Dehghani (Martin Luther University Halle-Wittenberg);

Abstract:
Stroke is one of the leading causes for disability and mortality in adults. Current therapies harbors many limitations and therefore are not suitable for all patients. The immunosuppressant mycophenolate mofetil (MMF) has the capacity to inhibit microglial and astrocytic activation and to reduce the extent of cell death after neuronal injury.
This study was designed to analyze the therapeutic windows of MMF and the temporal dynamics of cellular responses and signaling cascades.

Using N-methyl-D-aspartate (NMDA)-lesioned organotypic hippocampal slice cultures (OHSCs) treated with 100 µg/mL mycophenolate mofetil (MMF) within specific time frames, we determined the number of propidium iodide (PI) positive degenerating neurons and isolectin B4 positive microglial cells. The role of microglia and astrocytes in MMF – mediated effects was investigated after depletion with the bisphosphonate clodronate in NMDA lesioned OHSC. Furthermore, the involvement of further targets of MMF was identified in the cell culture.

MMF treatment after excitotoxic damage significantly reduced both microglial and astroglial proliferation rates without affecting apoptosis. A crucial time-frame of significant neuroprotection was identified between 12 and 36 hours after injury.

Our data indicates that MMF significantly reduces the extent of neuronal cell death in a specific crucial time frame after injury. Therefore, long term immunosuppression seems unnecessary. Currently, the mechanism of MMF action in glial cells is still unexplored; however, it seems to be an interesting target to understand the interactions in proliferation between microglia and astrocytes.

Poster 110

Titel:
Hidden clinical anatomy and imunology of Myasthenia Gravis

Autoren/Adressen:
Paul Salahoru (University of Medicine and Farmacy Grigore T. Popa), Marius Hinganu (University of Medicine and Farmacy Grigore T. Popa), Marius Hinganu (University of Medicine and Farmacy Grigore T. Popa), Cristina Grigorescu (University of Medicine and Pharmacy "Gr. T. Popa" Iasi);
hanganu.marius@yahoo.com

Abstract:
The objectives of this study are to highlight the role of thymus in the pathogenesis of myasthenia gravis.

We used a group of 14 patients diagnosed with myasthenia gravis who required timectomy. Patients were explored both pre- and postoperatively by dosing specific antibody titres, CT and electromyographic.

The results of our study demonstrate a functional and pathological correlation between thymus and myoneural junction, demonstrated by improvement of clinical manifestations of myasthenia gravis following timectomy. This is based on the postoperative reduction in anti-acetylcholine antibody titre.

Our study contributes to reveal the role of thymus in the pathogenesis of myasthenia gravis. The decrease in AChR titre was recorded after each timectomy. We consider timectomy as a therapeutic indication in the control of myasthenia gravis. The results of the study open up new insights into understanding this condition.

Poster 111

Titel:
Glial cells transfer ribosomes to axons in response to nerve injury

Autoren/Adressen:
Christina F. Vogelaar (University Medical Center of the Johannes Gutenberg University Mainz);

Abstract:
Neurons are highly specialized and polarized cells with long axons that function partially independent of the cell body. Axonal mRNA transport and local protein synthesis are crucial for axon regeneration, guidance and retrograde signaling. To date, it remains unclear how ribosomes localize to axons. They may be co-transported with mRNAs or, as suggested by recent studies, transferred from Schwann cells.

We generated transgenic “RiboTracker” mice expressing tdTomato-tagged ribosomal protein L4 in specific cell types when crossed with Cre lines. We made use of neuronal CamKIIa and Advillin Cre lines to express L4-tdTomato in dorsal root ganglion (DRG) neurons, and CNP Cre and inducible GFAP ERT2 Cre lines to express L4-tdTomato in Schwann cells. We analyzed axonal ribosomes in naive and injured sciatic nerves via immunohistochemistry and immuno-electron microscopy (EM).

Neuronal RiboTracker-Cre lines displayed extremely low levels of axonal L4-tdTomato-positive ribosomes, which were unaltered after sciatic nerve injury. In contrast, glial RiboTracker-Cre lines revealed numerous tagged ribosomes in sciatic nerve axons with increasing amounts after injury. In vitro experiments with RiboTracker Schwann cells in combination with wild type DRGs confirmed the transfer. Immuno-EM showed numerous axonal ribosomes and suggests transfer via vesicles.

These data indicate Schwann cells transfer ribosomes to injured sciatic nerve axons and suggest that glial cells are the predominant source of axonal ribosomes in the peripheral nervous system.

Poster 112

Titel:
Modulation of neuronal plasticity in response to sexual hormones

Autoren/Adressen:
Verena Theis (Institute of Anatmy, Ruhr-University Bochum), Frederique Wach (Institute of Anatomy, Ruhr-University Bochum), Annika Eickhoff (Institute of Anatomy, Ruhr-University Bochum), Carsten Theiss (Institute of Anatomy, Ruhr-University Bochum); verena.theis@rub.de

Abstract:
The sexual hormone progesterone is synthesized de novo from cholesterol in the central (CNS) nervous system to provoke neuroregenerative and neuroprotective potential. Progesterone induces morphological changes in neonatal and mature neurons, with enhanced somato- and dendritogenesis in Purkinje Cells (PC) (Wessel et al., 2014). These effects, mainly mediated by the classical progesterone receptor (PR) A and B, seem to be age-related regulated. By means of microRNA profiling we identified miR-26a-5p (Pieczora et al. 2017) that seems to minimize functionality of PR-A/B and sensitivity for progesterone. Additionally, we demonstrated the increase of functional spines after progesterone treatment, which indicates an intensive reorganization of synaptic plasticity in PCs.

1. With aid of microinjection the influence of miR-26a-5p mimics and inhibitors on progesterone receptor expression and at least on somato- and dendritogenesis were tested in organotypic slice cultures.
2. To analyze the impact of T-Type Ca2+ channels on synaptic plasticity highly purified PCs were cultivated (Tjaden et al. 2018, under revision). The Ca2+ flux after progesterone stimulation was measured by using electrophysiology in oocytes (xenopus laevis).

1. Whereas miR-26a-5p mimics trigger the loss of functional PR-A/B, the injection of miR-26a-5p inhibitors promotes the transcription of functional PR-A/B in mature PCs.
2. In the PC-culture the number of T-Type Ca2+ channels did not change, whereas the activity of these channels is increased.

Progesterone is a highly potent regulator of neuronal and synaptic plasticity. To overcome age-depended differences during maturation the modulation of miR-26a5p expression might lead to inducible regeneration after brain injury.

Poster 113

Titel:
Modulation of the proteoglycan receptor PTPσ improves recovery after compressive spinal cord injury (SCI) in rats

Autoren/Adressen:
Svenja Rink (University of Cologne), Aliona Wöhler (Bundeswehrzentralkrankenhaus), Dominik Arnold (University of Cologne), Marilena Manthou (Aristotle University), Theodora Papamitsou (Aristotle University), Levent Sarikcioglu (Akdeniz University), Doychin Angelov (University of Cologne); angelov.anatomie@uni-koeln.de

Abstract:
SCI is followed by a dramatic upregulation of chondroitin sulfate proteoglycans (CSPGs) which limits axonal regeneration, oligodendrocyte replacement and remyelination. The recent discovery of specific CSPGs signaling receptor protein tyrosine phosphatase sigma (PTPσ) provides the opportunity to refine the therapeutical approach against the CSPGs inhibitory actions. Thus, subcutaneous (s.c.) delivery of 44 µg/day from the peptide mimetic of PTPσ called intracellular sigma peptide (ISP), which binds to PTPσ and blocks CSPG-mediated inhibition, facilitates recovery. Since this result could be of interest for clinical trials, we repeated this study applying 44 µg/day intraperitoneal (i.p.) and 500 µg/day s.c. of ISP (CSBio Co., Menlo Park, CA 94025 USA).

Following SCI at Th10-segment 40 rats were distributed in 4 groups (each of 10 animals). Rats in groups 1 and 3 (SCI only) received no treatment. Rats in group 2 were treated with intraperitoneal (i.p.) injections of 44 µg/day ISP (SCI+ISP44) and animals of group 4 with s.c. injections of 500 µg/day ISP (SCI+ISP500) for 7 weeks after SCI. Recovery was analyzed at 1, 3, 6, 9 and 12 weeks after SCI. We determined: (i) BBB-locomotor score, (ii) foot-stepping angle, (iii) rump-height index, (iv) number of correct ladder steps, (v) bladder score and (vi)sensitivity (withdrawal latency after thermal stimulus).

ISP therapy results in improved locomotor and sensory recovery as assessed for the BBB score, rump-height index, bladder hypertrophy, bladder score, and withdrawal latency.

Our results show that, compared to SCI alone, SCI combined with ISP therapy has a certain beneficial effect.

Poster 114

Titel:
US-guided perineural injection in ulnar neuropathy at the elbow

Autoren/Adressen:
Ulrike M. Hamscha (Medical University Vienna), Ines Tinhofer (Medical University Vienna), Stefan Heber (Medical University Vienna), Wolfgang Grisold (KFJ Hospital), Wolfgang J. Weninger (Medical University Vienna), Stefan Meng (KFJ Hospital); ulrike.hamscha@meduniwien.ac.at

Abstract:
Ulnar neuropathy at the elbow (UNE) is a common peripheral compression neuropathy and in most cases occurs either in the retroepicondylar groove or the cubital tunnel. With regard to a potential therapeutic approach with perineural corticosteroid injection the aim of this study was to evaluate the distribution of injection fluid applied at a standard site.

We performed US-guided perineural injections of india ink at the ulnar nerve halfway between the olecranon and the medial epicondyle in 21 upper limbs from 11 non-embalmed cadavers. In anatomical dissection we investigated the spread of the ink.

Ink was successfully applied selectively into the perineural sheath of the ulnar nerve in all 21 cases. Inside the perineural sheet it spread caudally into the cubital tunnel (21/21) and proximally into the retroepicondylar groove (19/21).

US-guided injection between the olecranon and the medial epicondyle is a feasible and safe method to spread injected fluids to both most commonly affected sites of ulnar nerve entrapment.

Poster 115

Titel:
Modulation of the phosphorylation state of the survival of motoneuron (SMN) protein: Impact on intracellular localization and stability

Autoren/Adressen:
Nora Tula Detering (Hannover Medical School), Sebastian Rademacher (Hannover Medical School), Inga-Maria Wefel (Hannover Medical School), Niko Hensel (Hannover Medical School); Detering.Nora@mh-hannover.de

Abstract:
Spinal muscular atrophy (SMA) is a neurodegenerative disease affecting alpha-motoneurons in the spinal cord resulting in muscle atrophy. SMA is characterized by low levels of the survival of motoneuron (SMN) protein due to deletion or mutations of the survival of motoneuron 1 (SMN1) gene. However, little is known about the potential role of SMN phosphorylation in SMN localization and stability. Phosphatase and tensin homologue (PTEN) directly binds and dephosphorylates SMN. In additional studies, PTEN knockdown by siRNA showed functional alterations of SMN, including inhibited neurite outgrowth and decreased numbers of SMN-positive nuclear bodies. Therefore, modulating the phosphorylation state of specific sites of SMN by PTEN might impact the stability of SMN. Accordingly, it is important to determine the PTEN-relevant phosphorylation sites of SMN to understand function and stability of SMN.

Here, we investigated the impact of SMN phosphorylation on localization by generating a library of non-phosphoryable SMN mutants based on known phosphorylation sites, our own mass spectrometry analyses and computational predictions. The localization of SMN mutants in nuclear bodies was quantified as it indicates both nuclear localization as well as protein stability of SMN.

We have successfully generated a library of SMN phospho-mutants. In the localization studies, we identified distinct mutants showing rescued numbers of SMN-positive nuclear bodies under PTEN knockdown.

The identified phosphorylation sites of SMN can be potentially relevant for regulation of localization and stability of SMN.

Poster 116

Titel:
The functional role of Itpka in the pathogenesis of autism spectrum disorder

Autoren/Adressen:
Wolniczak E1, Schumann S1, Roskoden T1, Windhorst S2, Schmeisser MJ1,3*
1 Division of Neuroanatomy, Institute of Anatomy, Otto-von-Guericke University Magdeburg, Magdeburg, Germany, 2 Department of Biochemistry and Signal Transduction, University Medical Center Hamburg-Eppendorf, Hamburg, Germany, 3 Leibniz Institute for Neurobiology, Magdeburg, Germany;
michael.schmeisser@med.ovgu.de

Abstract:
Autism spectrum disorder (ASD) is a complex neurodevelopmental condition primarily characterised by alterations in social interaction and communication combined with repetitive behaviour. Although the underlying pathological mechanisms are not yet fully understood, there is evidence that alterations in dendritic spine architecture are critical for the pathogenesis of ASD. Since the dendritic spine cytoskeleton mainly consists of actin filaments, spine shape and function can be controlled by actin binding proteins (ABP). The Inositol-1,4,5-trisphosphate-3-kinase-A (Itpka) is a neuron-specific ABP that regulates dendritic calcium transients and controls actin dynamics in dendritic spines. Functional and morphological investigations using Itpka mutant mice are now on the way to unravel the putative role of Itpka in the pathogenesis of neuropsychiatric phenotypes resembling ASD.

Poster 117

Titel:
Interactions of an antiserum to Streptococcus mutans with paralemmin1 correlates to reduced neurite length and impaired acetylcholine dependent calcium transients in SiMa neuroblastoma cells

Autoren/Adressen:
Bernhard Reuss (Inst. for Neuroanatomy, University Medical Center Göttingen);
breuss@gwdg.de

Abstract:
Prenatal bacterial infections during pregnancy, in the offspring result in an increased lifetime Schizophrenia risk.* We show here that antisera against the oral bacterium Streptococcus mutans (α-SMu) on a human first trimester prenatal brain multiprotein array (HexSelect, Engine, Berlin, Germany), interact with a set of 31 proteins, including Paralemmin1 (PALM), neuronal alpha-spectrin (SPTAN), Chromogranin-A (CHGA) and Seizure protein 6 (SEZ6). Interactions of α-SMu with PALM and CHGA, but not SPTAN and SEZ-6 could be confirmed by Western blotting with corresponding overexpression lysates in HEK493 cells. As revealed by immunocytochemistry in the human neuroblastoma cell line SiMa, α-SMu labels neurite growth cones, and on the functional level, treatment of these cells with 10µg/ml α-SMu leads to a significant reduction in neurite length as compared to untreated controls. Besides this, also acetylcholine dependent calcium transients are signficantly reduced in α-SMu-treated SiMa cells as compared to untreated cells. In contrast, an antiserum against the closely related respiratory tract bacterium Streptococcus pneumoniae (α-SPn) interacts with 6 different proteins, including the sterile alpha motif domain containing protein 14 and Tubulin beta-4, and is not able to interfere with both neurite outgrowth and acetylcholine dependent calcium transients in SiMa cells. These results demonstrate for the first time immunological interaction and functional interference of α-SMu with the neurite outgrowth promoting protein Paralemmin1 due to molecular mimicry, which could be of importance for a better understanding of changes in brain development, structure, and functioning in schizophrenic patients. * Sørensen et al., 2009, Schizophrenia Bull. 35, 631-637.

Reproductive Biology

Poster 118

Titel:
Experimentally induced Diabetes Melitus and obesity in rat affect steroidogenic capacity and cell populations in the testis

Autoren/Adressen:
Ekaterina Pavlova (IEMPAM - BAS), Emilia Lakova (Medical University - Pleven), Stilyanka Yochkova (Medical University - Pleven), Miroslav Donchev (Medical University - Pleven), Savelina Popovska (Medical University - Pleven), Konstantin Svechnikov (Karolinska Institutet), Nina Atanassova (IEMPAM - BAS); e_bankova@yahoo.com

Abstract:
Metabolic syndrome involves various abnormalities like obesity, insulin resistance/diabetes, hypertension, hormonal disorders being serious risk factor for male infertility.

The present study aimed to evaluate adult Leydig cell number (LC) and steroidogenesis in tandem with body (BW), testis weight (TW) and fat accumulation in experimental conditions of diabetes mellitus (DM) induced on day 1 (neonatally, NDM) or day 10 (prepubertally, PDM); short and long high-fat diet (HFD) applies since puberty.

Hyperglycaemia was confirmed by significant elevation of blood glucose levels in NDM and PDM rats. In NDM body weight and TW were increased but gonado-somatic index was decreased by 15%. PDM rats have normal BW but TW and gonado-somatic index were decreased by 30%. LC number and testosterone production was in normal range in adult NDM, whereas they were significantly decreased in PDM. Significant increase was established in BW, body fat in short and long HFD associated with decrease in relative TW in long HFD. Epididymal and inguinal adipocyte diameter was increased in the animals from both groups. HFD did not affect significantly serum testosterone and LH levels. Nevertheless expression of LC key steroidogenic factors and intratesticular testosterone levels were significantly lower in long HFD rats, associated with reduction of LC number.

All together, our data indicate that diabetes and obesity affect size of adult Leydig cell population and testicular steroidogenesis, associated with reduced absolute and relative testis weight.

Poster 119

Titel:
Prostatic ducts vs glands and what oxytocin has to say about it

Autoren/Adressen:
Beatrix Stadler (Justus-Liebig-University), Daniela Beyer (Justus-Liebig-University), Florian Wagenlehner (Justus-Liebig-University), Betty Exintaris (Monash Institute of Pharmaceutical Sciences), Ralf Middendorff (Justus-Liebig-University);
Beatrix.Stadler@anatomie.med.uni-giessen.de

Abstract:
Most recently we analysed for the first time separately the contractile pattern of prostatic glands and ducts (Kügler et. al. 2018, FASEB J), allowing to predict effects and local side effects of drugs used for the treatment of benign prostatic hyperplasia (BPH) by relaxing the smooth muscle cells in the prostate. Most of the experiments were performed in rodent tissue. Surprisingly, in the human prostate data on (i) structure and functional regulation of excretory ducts as well as (ii) the potential of targeting other receptors instead of adrenergic ones (as usually done) are missing. In the present study the duct system of the human prostate and oxytocin signalling were investigated.

Corrosion cast models, CLARITY and smooth muscle staining were utilised in visualizing the human prostatic duct system. Oxytocin effects were visualized by using Ca2+-imaging and video microscopy.

We were able to clarify the human prostatic ductal system and also got information about the organisation of the surrounding smooth muscle cells. Oxytocin significantly increased the frequency of spontaneous prostatic contractions. There also was a visible difference between oxytocin- and noradrenaline-induced contractions as well as between prostatic ducts and glands.

Revealing differences between ducts and glands in the human prostate extends our basic anatomical knowledge and might allow an even more targeted line of medications. These insights in combination with our novel oxytocin antagonists could open up new strategies in BPH treatment.

Poster 120

Titel:
Functionally relevant connective tissue septa of the epididymis already exist before the first sperm reach the epididymis

Autoren/Adressen:
Daniela Beyer (Justus-Liebig-University Giessen), Thorben Hau (Justus-Liebig-University Giessen), Andrea Mietens (Justus-Liebig-University Giessen), Ralf Middendorff (Justus-Liebig-University Giessen);

Abstract:
The epididymis is anatomically divided in caput, corpus and cauda. Furthermore, connective tissue septa (CTS) subdivide the epididymis into different segments. The number of segments differs among species (Turner et. al 2003, Thong et. al 2014, Stammler et al. 2015). In adults, these CTS are involved in creating a specific segmental interstitial milieu, which is important for the different steps during sperm maturation within the epididymal duct. In the first days after birth individual septa begin to develop and epithelial cells start differentiation (at postnatal day 14 in the rat). However, data about structural integrity and function of CTS as well as a segmental interstitial milieu before adulthood are missing.

The two- and three-dimensional structure of the epididymal CTS of 6-day-old – 40-day-old rats were visualized morphologically (serial sections, clarity). Interstitial and luminal perfusions with color tracer were used to characterize barrier functions.

The presence of CTS could be revealed at all investigated days in the postnatal rat epididymis. The known 19 segments in adult rats matched with the clearly visible 19 segments in postnatal rats. Infusion into the interstitial tissue resulted in a tracer distribution of one single segment restricted by sharp boundaries to the next non-infused segment. Luminal perfusion showed no restriction.

Our data showed CTS in the postnatal epididymis. These CTS created an interstitial segmental milieu already before the first sperm reach the epididymis or the differentiation of epithelial cells begins. This suggests an additional yet unknown function of the CTS during postnatal development.

Tissue Engineering

Poster 121

Titel:
In vitro and in vivo evaluation of Guiding Regenerative Gel (GRG) as a three-dimensional matrix filler for advanced peripheral nerve guides

Autoren/Adressen:
Nina Dietzmeyer (Hannover Medical School), Shimon Rochkind (Tel Aviv University, Tel-Aviv Sourasky Medical Center), Mara Almog (Tel Aviv University, Tel-Aviv Sourasky Medical Center), Zvi Nevo (Tel Aviv University, Sackler School of Medicine), Kirsten Haastert-Talini (Hannover Medical School); dietzmeyer.nina@mh-hannover.de

Abstract:
Recovery of sensorimotor function after peripheral nerve reconstruction with tubular nerve guides often remains incomplete. We are evaluating the properties of GRG alone and in combination with Fibroblast-growth-factor-2 over-expressing Schwann cells (FGF-2-SC) to serve as regeneration permissive luminal filler for chitosan nerve guides.

In vitro viability of neonatal rat Schwann cells (SC) was analyzed when cultured in 0.02%, 0.1%, 0.2%, and 0.4% GRG. An in vivo pre-study was performed to demonstrate the impact of the different GRG concentrations in collagen nerve guides in comparison to autologous nerve grafts (ANGs) and empty nerve guides (n=5 animals each) on rat sciatic nerve regeneration after reconstruction of an 8 mm gap.

In vitro the 0.2% GRG allowed three-dimensional distribution of SC and turned out to provide GRG in a viscosity suitable as luminal filler. Histomorphometrical analysis of distal nerve segments obtained from the in vivo pre-study also pointed to 0.2% GRG as most promising concentration. Neurite outgrowth assays from dorsal root ganglion preparations are performed to analyze the GRG properties alone and in combinations with FGF-2-SC in more detail. An in vivo study has been initiated where 15 mm rat sciatic nerve gaps were reconstructed with either ANGs, one-, or two-chambered chitosan nerve guides filled with 0.2% GRG alone or combined with FGF-2-SC.

This poster will present results from the in-depths in vitro analysis and first results from the in vivo study (functional sensory and motor recovery).

Poster 122

Titel:
Characterisation of the myogenic capacity of ovine muscle-derived stem cells (MDSCs) isolated from different muscle groups

Autoren/Adressen:
Kateryna Grom (Justus Liebig University of Giessen), Mohamed I. Elashry (University of Mansoura), Sabine Wenisch (Justus Liebig University of Giessen), Stefan Arnhold (Justus Liebig University of Giessen); elashry79@gmail.com

Abstract:
MDSCs possess a great promise for regenerative medicine. Evaluation of the stem cells population would impact on the regenerative ability of various muscles. Our aim was to evaluate the proliferation and differentiation potentials of ovine MDSCs isolated from different muscle groups.

MDSCs of four weeks old ovine newborn (n=3) were isolated from the hindlimb, diaphragm, extraocular and masseter muscles by the pre-plate technique. The cells were induced for myogenic differentiation up to day 7. The expression of stem cells markers and myogenic markers were evaluated by immunocytochemistry. The cell viability and protein contents were tested by using MTT and SRB assays. The cell migration and colony formation were assessed by the wound healing and colony forming unit assays. The expression of MyoD and Myogenin genes was measured using RT-PCR. The data were analysed by using a two-way ANOVA.

The MDSCs of all muscles showed positive immunostaining for alpha7-integrin, CD90 and Pax7. MDSCs of hindlimb, diaphragm and extraocular muscles were positive for MyoD and Myogenin during the course of myogenic differentiation. MDSCs of hindlimb and diaphragm displayed higher viability and cellular protein contents compared to the cells of extraocular and masseter muscles. MDSCs of hindlimb and diaphragm revealed superior migration and colony formation capacities compared to the cells of extraocular and masseter muscles. The expression of MyoD and Myogenin corresponded with the results of immunostaining.

The data point out that ovine MDSCs from hindlimb and diaphragm have a higher regenerative capacity compared to the cells of extraocular and masseter muscles.

Poster 123

Titel:
Long-term clinical results using a tissue-engineered pulmonary valve during the Ross procedure

Autoren/Adressen:
Alper Öner (Heart Center Rostock), Annick Mommsen (Charite Hospital), Axel Pruss (Charite Hospital), Hüssein Ince (Heart Center Rostock), Pascal Dohmen (Heart Center Rostock); pascal.dohmen@med.uni-rostock.de

Abstract:
The Ross procedure is mainly limited by the durability of a pulmonary homograft used to reconstruct the right ventricular outflow tract. This study was performed to collect prospective safety and effectiveness data of the Ross procedure using a tissue-engineered heart valve to reconstruct the right ventricular outflow tract.

Between May 2000 and February 2002, 11 patients received a tissue-engineered pulmonary heart valves. Two to four weeks before the Ross operation, a piece of forearm or saphenous vein was harvested to isolate, characterize, and expand endothelial cells. A pulmonary allograft was decellularized, coated with fibronectin, and seeded with autologous vascular endothelial cells, using a specially developed bioreactor. Follow-up was performed by clinical evaluation, transthoracic echocardiography, magnetic resonance imaging, and multi-slice computed tomography.

The patient mean age was 40 ± 10 years. Cell seeding density was 1.1 x 106 ± 0.5 x 106 cells/cm2, with a viability of 90.2% ± 8.9%. All patients survived the operation. Two patients died during follow-up (one non-cardiac death and one unknown), and 1 patient required reoperation. All surviving patients are currently in New York Heart Association functional class I. Transthoracic echocardiographic evaluation of the tissue-engineered heart valve showed a median flow velocity of 0.7 m/s (range 0.5 to 0.8 m/s) at 15 years. Multi-slice computed tomography showed no calcification postoperatively.

Tissue-engineered heart valves showed excellent hemodynamic performance during 15 years follow-up. Decellularization of heart valves and seeding with autologous vascular endothelial cells may prevent tissue degeneration and improve valve durability.