ANATOMISCHE GESELLSCHAFT
1886

29. Arbeitstagung der Anatomischen Gesellschaft in Würzburg

26.09.2012 bis 28.09.2012

Vortrag 1

Rubrik: Neuroanatomie: Glia und Entzündung

Titel: TGF-beta increases microglia-mediated engulfment of apoptotic cells - involvement of Itgb5/Mfg-e8 receptor-ligand pair

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Abstract:
Apoptotic cells are rapidly engulfed by phagocytes in order to prevent the release of noxious factors as well as immunogenic material from dying cells. In the central nervous system (CNS), microglia serve as phagocytes and have important functions in clearing apoptotic cells and cellular debris to maintain neuronal networks. The opsonin milk fat globule-EGF factor 8 (Mfg-e8) has been described to bind phosphatidylserine residues at the outer membranes of apoptotic cells, thereby presenting an "eat-me" signal for microglia expressing the Mfg-e8 receptor Integrin-beta 5 (Itgb5). Here we show that TGF-beta1 increases the expression of the Itgb5/Mfg-e8 receptor-ligand pair in microglia and, thus, promotes the phagoctosis of apoptotic cells. Using data from cDNA micro arrays and western blotting, we demonstrate that TGF-beta1 treatment of microglia resulted in upregulation of Mfg-e8 and its receptor Itgb5. Staurosporine-treated and fluorescently-labelled MN9D cells were used to analyse the rate of microglial phagocytosis under control conditions and after TGF-beta1 treatment. In BV2 and primary microglia TGF-beta1 significantly increased the phagocytosis of apoptotic MN9D cells. Moreover, we provide evidence that LPS-induced classical microglia activation is associated with downregulation of Mfg-e8. Together, our data introduce the receptor ligand pair Itgb5/Mfg-e8 as novel TGF-beta1-target genes in microglia and further underline the importance of TGF-beta1 as a regulator of microglia functions during the resolution and regeneration phase of neuroinflammatory responses.

Kategorie: Vortrag

Vortrag 2

Rubrik: Neuroanatomie: Glia und Entzündung

Titel: Myelin debris regulates inflammatory responses in an experimental demyelination animal model and multiple sclerosis lesions

Autoren: Clarner T.(1),Diederichs F.(1),Berger K.(1),Denecke B.(2),Gan L.(2),van der Valk P.(3),Beyer C.(1),Amor S.(3),Kipp M.(1),

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Abstract:
Background:
In multiple sclerosis (MS), grey matter pathology is characterized by less pronounced inflammation as compared to white matter lesions. Although regional differences in the cytoarchitecture may account for these differences, the amount of myelin debris in the cortex during a demyelinating event might also be contributory.
Methods:
To analyze the association between myelin debris levels and inflammatory responses, cortical areas with distinct and sparse myelination were analyzed for micro- and astrogliosis before and after cuprizone-induced demyelination in mice. In post-mortem tissue of multiple sclerosis patients, leucocortical lesions were assessed for the type and level of inflammation in the cortical and white matter regions of the lesion. Furthermore, mice were injected intra-cerebrally with myelin-enriched debris, and the inflammatory response analyzed in white and grey matter areas.
Results:
Our studies show that the magnitude of myelin loss positively correlates with microgliosis in the cuprizone model. In MS, the number of MHC class II expressing cells is higher in the white compared to the grey matter part of leucocortical lesions. Finally, direct application of myelin debris into the corpus callosum or cortex of mice induces profound and comparable inflammation in both regions.
Conclusion:
Our data suggest that myelin debris is an important variable in the inflammatory response during demyelinating events. Whether myelin-driven inflammation affects neuronal integrity remains to be clarified.

Kategorie: Vortrag

Vortrag 3

Rubrik: Neuroanatomie: Glia und Entzündung

Titel: Analysis of Schwann cell development along growing axons

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Abstract:
Analysis of Schwann cell (SC) development has been hampered by the lack of growing axons in many commonly used in vitro assays. As a consequence, the molecular signals and cellular dynamics of SC development along peripheral axons are still only poorly understood. Here we use a superior cervical ganglion (SCG) explant SC development assay, in which the development of endogenous SC can be investigated along growing axons. With this assay we identified Nrg1 type III ErbB signaling to be important for SC development in the sympathetic nervous system. Interference with the ErbB receptor lead to reduced SC proliferation, increased SC apoptosis and to reduced SC colonization of distal axonal segments. However, our data suggest that the effect of ErbB signaling on SC colonization of distal axonal segments is mediated mainly via supporting SC survival in proximal axonal regions rather than by directly altering SC motility.
Notably GDNF, also suggested to be involved in SC migration, seems to be dispensable for embryonal SC migration in the murine sympathetic nervous system and also along the sciatic nerve.

Kategorie: Vortrag

Vortrag 4

Rubrik: Neuroanatomie: Glia und Entzündung

Titel: The endocannabinoid system – an intrinsic site-specific system to regulate neuronal damage

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Abstract:
After focal neuronal injury the endocannabinioid system becomes activated and protects or harms neurons depending on cannabinoid derivates and receptor subtypes. The beneficial effects of exogenously applied endocannabinoids (eCBs) in processes of excitotoxic lesion, secondary damage and neuronal plasticity have previously been shown. We used Organotypic Hippocampal Slice Cultures (OHSC) to investigate the endogenous variations of the endocannabinoid system in neuronal damage over time. The spatial and temporal dynamics of eCB levels were analyzed after transection of perforant pathway (PPT). After PPT the responses of originating neurons (enthorhinal cortex, EC), areas of deafferentiation/anterograde axonal degeneration (dentate gyrus, DG) and of the synaptically linked cornu ammonis region 1 (CA1) were measured. We found a strong increase in N-arachidonoylethanolamide (AEA), N-oleoylethanolamide (OEA), N-palmitoylethanolamide (PEA) and 2-arachidonoyl glycerol (2-AG) levels in the denervation zone of the DG 24 hours post lesion (hpl). Additionally, in the CA1 region PEA and OEA levels were elevated. NAPE-PLD protein, responsible for biosynthesis of eCBs was increased early (1-6 hpl) and FAAH protein, a catabolizing enzyme as well as the CB1 receptor were up-regulated 48 hpl. The enzyme responsible for 2-AG hydrolysis (MAGL) underwent a re-distribution within neurons and astrocytes. Transection of long-range projections provides a strong time-dependent and anterograde mechanism of action of eCB, presumably to restrict neuronal damage. The data presented underline the importance of the eCB system in CNS pathologies and identifies a novel site-specific intrinsic regulation of eCBs after long-range projection damage.

Kategorie: Vortrag

Vortrag 5

Rubrik: Neuroanatomie: Glia und Entzündung

Titel: Oligodendrocytes modulate neuroinflammatory responses by Cxcl10

Autoren: Berger K.(1),Krauspe B.(1),Rickert M.(1),Clarner T.(1),Denecke B.(2),Heß F.(3),Neumann H.(4),Vallières L.(5),Beyer C.(1),Kipp M.(1),

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Abstract:
Multiple sclerosis (MS) is a demyelinating disorder of the central nervous system, classically discussed as a primary autoimmune disease. New neuropathological evidence, however, argues against the autoimmune hypothesis and suggests that MS is a primary oligodendrogliopathy disease in which the inflammatory response may be a mere epiphenomenon. Since in early MS lesions, activated microglia are in close vicinity to affected oligodendrocytes, we speculate that oligodendrocytes contribute to microglia activation and attraction. To address this, we used the toxic demyelination cuprizone model, which results in primary oligodendrocyte dysfunction, followed by oligodendrocyte apoptosis, microglia activation, astrocytosis and demyelination.
We were able to show that short-term cuprizone treatment induces a distal oligodendrogliopathy which is paralleled by early microgliosis but not astrocytosis. Genome-wide gene expression studies revealed induction of distinct chemokines, predominantly Cxcl10. By means of in situ hybridization we could demonstrate that oligodendrocytes express Cxcl10 mRNA, implicating that oligodendrocytes mediate early microgliosis. Subsequent in vitro experiments clearly showed that oligodendrocyte-derived Cxcl10 induces microglia migration, whereas early microglia activation was less severe in Cxcl10-deficient animals. Furthermore, oligodendrocyte-derived Cxcl10 induces a pro-inflammatory M1-phenotyp in cultured microglia, but does not influence phagocytosis activity.
Our results suggest that oligodendrocytes actively participate in the initiation of neuroinflammatory processes and thus might be important players in the regulation of MS lesion development and progression. Due to our findings, one should reconsider the pathogenesis of MS when developing more disease-specific treatments.

Kategorie: Vortrag

Vortrag 6

Rubrik: Neuroanatomie: Glia und Entzündung

Titel: Role of antimicrobial peptide CRAMP in inflammation and lethality after bacterial meningitis

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Abstract:
Antimicrobial peptides (APs) are an important part of the innate immune system of many organ systems, yet little is known about their expression and function in the brain. We showed the expression and secretion as well as bactericidal properties of a main antimicrobial family, the cathelicidins, in glial cells after bacterial infection. The expression of the antimicrobial peptide cathelicidin CRAMP/LL-37 is up-regulated in bacterial meningitis, but the consequence of cathelicidin expression and function for progression of inflammation and viability are far from clear. Therefore, we used CRAMP deficient mice to investigate the role of antimicrobial peptide CRAMP in inflammation and lethality after bacterial meningitis. Our results showed a higher lethality in vivo after bacterial meningitis for CRAMP-deficient mice compared to wildtype mice. The higher lethality correlated with decreased glial cell activation using realtime RT-PCR and immunohistochemistry. But the CRAMP-deficient mice showed significant increased endogen glial cell activation. Furthermore, the PCR array analysis of the hippcampus revealed increasing immune response after bacterial meningitis in CRAMP-deficient mice. Altogether, the results suggest that CRAMP produced by glial cells plays an important part in the innate immune response against pathogens in CNS bacterial infections.

Kategorie: Vortrag

Vortrag 7

Rubrik: Neuroanatomie: Glia und Entzündung

Titel: BBB-breakdown occurs independent of ruptures within endothelial tight junctions in the model of MCAO in rats

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Abstract:
The phenomenon of the blood- brain barrier (BBB) was first described by Paul Ehrlich in 1885 and relates to the ability of cerebral vessels to hold back hydrophilic molecules from entering the brain. This remarkable feature of the brain's vasculature was mostly attributed to the presence of endothelial tight junctions in the capillary segment and therefore, breakdown of the BBB is often regarded as an opening of tight junction complexes, which allows unhampered paracellular leakage of blood-derived molecules into adjacent compartments. This concept was also adapted to BBB breakdown associated with ischemic stroke, which enhances the risk of edema and hemorrhages thereby critically impacting on the clinical outcome. As there is a growing body of data suggesting essential tight junction constituents to be affected in this process, we used an embolic model of stroke in rats, to investigate the contribution of endothelial tight junctions to BBB breakdown by fluorescence and electron microscopy. Against our expectations, fluorescence microscopy revealed regular presence of critical tight junction proteins such as ZO-1, occludin and claudin-5 in areas of tracer extravasation. These findings were confirmed by ultrastructural analysis as affected vessels regularly showed established tight junctions. Contrary to the concept of a paracellular leakage, endothelial cells showed signs of enhanced transendothelial vesicle trafficking. We therefore question the established view of the criticial impact of tight junctions on BBB breakdown after ischemic stroke as we are able to demonstrate novel evidence indicating a transcellular, not paracellular leakage pattern.

DFG FOR1336 (to I.B.)

Kategorie: Vortrag

Vortrag 8

Rubrik: Neuroanatomie: Glia und Entzündung

Titel: Altered lipid signaling at the synapse induces endophenotype of psychiatric disorders

Autoren: Vogt J.(1),Mobascher A.(2),Kirischuk S.(3),Luhmann H.(3),Nitsch R.(4),

Adressen:

Abstract:

Kategorie: Vortrag

Vortrag 9

Rubrik: Klinische Anatomie, Metabolismus, Didaktik

Titel: Alteration of coxal bones' elasticity by anatomical fixation

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Abstract:
Introduction: Biomechanical tests and validations of virtual computer models of the human locomotive system are frequently accomplished in anatomically fixed tissues. It is well known that ethanol and formaldehyde fixation cause minor alterations of the anorganic bone matrix, but their influence on organic matrix has not been evaluated yet. We hypothesized that fixation alters bone elasticities and therefore leads to different results in biomechanical testing, as compared to unfixed material.

Material and methods: Six human hemipelves were investigated in the fresh condition, after ethanol (n=3) and formaldehyde fixation (n=3), and after rinsing. Bone masses were recorded in each state and eigenfrequency as an indicator of elasticity was determined using 3D laser vibrometry.

Results: As compared to the fresh condition (100%), masses decreased (85%) and eigenfrequencies increased (110%), using ethanol fixation. Subsequent rinsing caused a mass increase (91%) and eigenfrequency decrease (104%). Formaldehyde fixation caused a mass reduction (97%) and a loss of eigenfrequency (98%); subsequent rinsing caused a mass increase (98%), while the eigenfrequency remained on a constant level (98%).

Discussion: In the ethanol fixed condition and after rinsing, masses and eigenfrequencies were inversely related. Both effects were partly reversible after rinsing. In contrast, formaldehyde fixation irreversibly decreased eigenfrequencies, even after mass regain due to rinsing. Both fixation techniques cause changes in bone elasticity, which can be attributed to the organic matrix. We therefore recommend that biomechanical validation should only be accomplished in an anatomically unfixed condition to guarantee reliable results.

Kategorie: Vortrag

Vortrag 10

Rubrik: Klinische Anatomie, Metabolismus, Didaktik

Titel: T-tubular damage and membrane repair in skeletal muscle of non-myopathic patients undergoing statin therapy

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Abstract:
Skeletal muscle complaints are a common consequence of cholesterol-lowering statin therapy. In patients with clinically-diagnosed statin-associated myopathy, discrete signs of structural damage predominantly localize to the T-tubular region and are suggestive of a calcium leak. Concomittant with these structural changes, genes for the expression of proteins which regulate Ca2+ homeostasis and membrane repair were found to be significantly upregulated in myopathic patients

Kategorie: Vortrag

Vortrag 11

Rubrik: Klinische Anatomie, Metabolismus, Didaktik

Titel: Are brain arteries anatomical or functional end arteries – and what controls their development?

Autoren: Kurz H.(1),Förster E.(2),

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Abstract:
One of the most famous anastomotic vascular arrangements in humans is found at the base of their brains, the arterial circle of Willis: connecting (or not) the two internal carotids with each other and, via the basilar artery, with the two vertebral arteries. While some authors still maintain the circle might, as a rule, provide functional redundancy in case of occlusions, the enormous variability of these interconnecting vessels raises some doubt if functional redundancy rather might be the exception. Far less conspicuous and therefore almost unkown is the frequency of anastomotic arteries in the pial vasculature, before true anatomical end arteries are sent down radially into the brain parenchyma. But are these microvascular anastomoses of functional significance? A relationship of cortical arteries to the columnar organization of cortical neurons is only emerging, and the question of mutual developmental control between neurons, glia and vascular cells is far from settled. We provide a review of the literature, including some aspects of hippocampal neurovascular development. Lindhorst T, Kurz H, Sibbe M, Meseke M, Förster E. Congruence of vascular network remodeling and neuronal dispersion in the hippocampus of reelin-deficient mice. Histochem Cell Biol 2012.

Kategorie: Vortrag

Vortrag 12

Rubrik: Klinische Anatomie, Metabolismus, Didaktik

Titel: *Staphylococcus aureus* and *Pseudomonas aeruginosa* express and secrete human surfactant proteins

Autoren: Bräuer L.(1), Schicht M.(1), Worlitzsch D.(2), Bensel T.(2), Paulsen F.(1)

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Abstract:
Surfactant proteins (SP), originally known from human lung surfactant, are essential to proper respiratory function in that they lower the surface tension of the alveoli. They are also important components of the innate immune system. The functional significance of these proteins is currently reflected by a very large and growing number of publications. The unexpected and surprising finding revealed in our investigations is that different strains of *Staphylococcus aureus* and *Pseudomonas aeruginosa* express and secrete proteins that react with currently commercially available antibodies to known human surfactant proteins. Our results strongly suggest that the bacteria are either able to express 'human-like' surfactant proteins on their own or that commercially available primers and antibodies to human surfactant proteins detect identical bacterial proteins and genes. These findings may reflect the existence of a new group of bacterial surfactant proteins and DNA currently lacking in the relevant sequence and structure databases. At any rate, our knowledge of human surfactant proteins obtained from immunological and molecular biological studies may have been falsified by the presence of bacterial proteins and DNA and therefore requires critical reassessment.

Kategorie: Vortrag

Vortrag 13

Rubrik: Klinische Anatomie, Metabolismus, Didaktik

Titel: Local macrophage proliferation contributes to the adipose tissue inflammation in obesity

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Abstract:
Obesity is highly correlated with a low grade inflammation within the adipose tissue. It has been suggested that this inflammation might be a response to adipocyte death in obese individuals. Dying adipocytes get surrounded by macrophages, lymphocytes and mast cells to form characteristic crown-like structures (CLS). Nevertheless, the reason for the dramatic increase in pro-inflammatory immune cells within adipose tissue is still unknown and has been attributed to an enhanced recruitment from the blood stream.
We here report that macrophages and lymphocytes proliferate on site and that this proliferation of macrophages preferentially occurs within CLS. In contrast, lymphocytes tend to proliferate near CLS in follicle-like accumulations. Further, the proliferation was independent of leptin, because leptin-deficient ob/ob and leptin receptor-deficient db/db mice exhibit the same phenotype as diet-induced obese mice. Proliferating macrophages were also found in human adipose tissue of obese individuals and expression of the proliferation marker Ki67 correlated with body mass index and insulin resistance in these patients. Additionally, we perform live-imaging on CLS to observe macrophage activation, migration and phagocytosis in situ. In this set up, phagocytotic activity of macrophages can be studied directly by measuring the area of the lipid remnants of the dying adipocyte. Postfix immunofluorescence revealed proliferating macrophages even after 48h in culture.
We conclude that proliferation of leukocytes contributed to the increase in adipose tissue immune cells in obese mice and patients. Hence, local proliferation of macrophages and lymphocytes should be considered in adipose tissue inflammation.

Kategorie: Vortrag

Vortrag 14

Rubrik: 8.Neuroregeneration/Neurodegeneration

Titel:Ultrastructural studies of demyelination and axonal damage in mog35-55-induced experimental autimmune encephalomyelitis in c57bl/6 mice

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Abstract:
While ultrastructural analysis of the patterns and kinetics of demyelination and axonal pathology in myelin oligodendrocyte glycoprotein peptide 35-55 (MOG:35-55)-induced EAE could further extend our understanding of the disease as well-suited animal model of MS, such studies have not been carried out so far. To this end, transverse segments of the lumbar spinal cord of MOG:35-55-immunized mice were obtained at the peak of acute EAE, three months after EAE onset (chronic EAE) and six months after EAE onset (long-term chronic EAE), EPON-embedded and evaluated by electron microscopy. The extent of myelin pathology in CNS lesions was evaluated by measurement of the g-ratio (axon diameter divided by nerve fiber diameter) and increased progressively over the course of time. Conversely, axonal pathology was present already at disease onset and could be observed constantly in the chronic stages of the disease. Features of axonal damage covered axonal loss, axolysis, mitochondrial swelling and decrease in nearest neighbour neurofilament distance (NNND). This renders axonal damage the morphologic-structural correlate of irreversible clinical impairment in accordance with the chronic disease course of the mice in this model, strongly supporting the concept that has been proposed for MS patients. Furthermore, we were able to show the occurrence of isolated axonal damage without concurrent myelin damage already early on in the disease course, a finding which demonstrates that axonal pathology can occur, at least partly, independently of myelin pathology. These data clearly point towards the need for alternative therapeutic strageties that are not only immune modulatory, but also neuroprotective.

Kategorie: Vortrag

Vortrag 15

Rubrik: Klinische Anatomie, Metabolismus, Didaktik

Titel: Test anxiety among medical students – an approach to solve the problem

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Abstract:
Test anxiety is an underestimated and widespread problem among students. Approximately 15-20% of all university students suffer from test anxiety of different degrees. The persons concerned can`t effect sufficient performance although they prepare intensively for their exams. Test anxiety often leads to psychiatric and somatic symptoms and disorders like insomnia, panic attacks, depression, substance abuse and to suicidal tendency.
2012, we started a multidisciplinary project in which medical students with test anxiety were supervised by lecturers of the Department of Anatomy, the Clinic of Psychosomatic Medicine as well as by psychologists of the Psychological Counseling Service of the University of Erlangen-Nuremberg. Along with lectures, students were attended by tutorials in which they underwent exam simulations and also were skilled in cognitive restructuring and relaxation techniques.
Altogether, lectures were attended by approximately 100 students of all semesters. The tutorials, offered only to undergraduate students, were attended by 60 students. In a first evaluation, students appreciated the possibility of such a project and stated that they now had the motivation to face with the problem. The psychological counseling service reported from several students, which sought for help at the service after they attended the courses.
In the next term the tutorials are to be expanded, so that also graduate students can participate. Additionally, courses for lecturers will be offered, in which they can practice how to treat students with test anxiety. The authors hope that they can help concerned students via this project and thereby also contribute reducing duration of studies.

Kategorie: Vortrag

Vortrag 16

Rubrik: Neuroanatomie/vegetatives Nervensystem

Titel: Egfl7: a novel modulator of neural homeostasis

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Abstract:
In the adult brain the formation of new neurons is tightly controlled at the molecular level to keep the system in homeostasis. In this context, Notch signaling has a key regulatory function. Epidermal Growth Factor-like domain 7 (EGFL7) is a newly described non-canonical Notch ligand that stimulates the formation of neurons from neural stem cells (NSCs) by inhibition of Notch activation through Notch ligands of the Jagged-type. Recently, we demonstrated how EGFL7 reduces the self-renewal capacity of subventricular zone (SVZ)-derived NSCs to promote neuronal differentiation (Schmidt et al, Nat. Cell. Biol, 2009). Data from our lab now implicate EGFL7 in the regulation of neurogenesis in the adult hippocampus (HC): EGFL7 is expressed in mature hippocampal neurons in humans and mice, is absent from immature granule cells and reduces the self-renewal potential of HC-derived neurospheres. We propose a negative feedback mechanism by which mature granule cells regulate their own renewal in order to keep the total number of granule cells in a homeostatic range. Exploration of such self-regulatory processes in adult neurogenesis maylead to the development of new strategies to enable a successful incorporation of new neurons in existing networks.

Kategorie: Vortrag

Vortrag 17

Rubrik: Neuroanatomie/vegetatives Nervensystem

Titel: Regulation of the cholinergic gene locus by 17-beta-estradiol in NSC-34 cells and in the mouse spinal cord

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Abstract:
Alpha-motoneurons in the spinal cord are exceedingly affected in neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS). The neuronal dysfunction is particularly accompanied by alterations of cholinergic metabolism and signaling documented by a decreased expression of choline acetyltransferase (ChAT) and vesicular acetylcholine transporter (VAchT). 17-beta-estradiol (E) is generally accepted as a neuroprotective factor in the brain under neurodegenerative conditions and also appears to exert a protective role for motoneurons. In the present study, we attempted to analyze the effects of E and the role of classical and non-classical steroid signaling on cholinergic gene expression in the motoneuron-like cell line NSC-34 and in the mouse spinal cord. In a first step, we demonstrated the presence of estrogen receptor (ER) alpha and beta in NSC-34 cells as well as in the spinal cord. The application of E significantly increased the transcription rate of ChAT and VAchT in NSC-34 cells and in vivo. In NSC-34 cells, treatment with E and estrogen receptor agonists PPT (ERa) and DPN (ERb) selectively activate non-classical signaling via the MAPK pathway. Treatment with the estrogen receptor antagonist ICI 182,780 or the induction of p42/44 MAPK signaling via the MEK1/2 pathway resulted in an inhibition of E-induced CHAT expression. Our results suggest that E regulates the cholinergic function of alpha-motoneurons under physiological conditions. Furthermore, the close interaction of classical and non-classical E-dependent pathways is required to mediate steroid effects on the spinal cord cholinergic system.

Kategorie: Vortrag

Vortrag 18

Rubrik: Neuroanatomie/vegetatives Nervensystem

Titel: Impaired anxiety level and depressive like symptoms in humans and in a rodent model of chronic kidney disease

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Abstract:
Chronic kidney disease (CKD) is an increasing common chronic illness. Although kidney function is partially replaced by dialysis, patients develop depression-like symptoms, cognitive insufficiency and reduced physical activity. To systematically investigate the psychological deficiencies, 22 control persons and 27 dialysis patients were investigated with a standardized test battery to evaluate parameter of depression, apathy, and current mental state. Results show a significantly higher depression scale of dialysis patients as compared to the control group. Analyses showed for the first time that the depression is related to physical impairment. Social integration, mood and life orientation are only slightly altered as compared to the control group.
In the second part of the study male Sprague-Dawley rats (n=54), were randomly assigned to three groups receiving either isotonic saline or ADMA (an endogenous NOS inhibitor, which is markedly increased in CKD patients) via osmotic mini pumps or underwent 5/6 nephrectomy (Nx). After 28 days several behavioral tests (e.g. Holeboard Test) were performed. Both, 5/6 nephrectomy and ADMA infusion, lead to a significant decrease in exploratory behavior and increased anxiety levels. Immunohistological analyses of the hippocampi showed significant increase of Spermin-Synthase, in both experimental groups. This could be traced back to a decreased Spermidine-concentration. Spermidine plays an important role in aging of neuronal cells and is used for treatment of depression. The results of the present study show that humans and rats with CKD develop a similarly affected behaviour. In rats the behavior is accompanied by altered Spermin-Synthase expression in the brain.

Kategorie: Vortrag

Vortrag 19

Rubrik: Neuroanatomie/vegetatives Nervensystem

Titel: Neural lineage differentiation from human pluripotent stem cells

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Abstract:
To make use of stem cells as a source for biomedical approaches, we need to better control their vast proliferation and differentiation potential. While insight into key transcriptional regulators has grown, the classic embryological question of how cells interacting during development give rise to their progeny in a temporally and spatially adequate manner remains unsolved.

We set out to identify molecules and mechanisms that regulate the critical transition from neural stem cell to neuron on a microenvironmental level. Exploiting a range of genetic, biochemical, and screen-based methods, we study neural cultures derived from human embryonic and induced pluripotent stem cells, primary cortical tissue and human neural tumor cell lines.

We find that members of the recently described Hippo signaling pathway, a highly conserved sensor of cell-density and organ size control, appear to be tightly regulated in human neural proliferation versus differentiation. Moreover, we identify mitogen-activated protein kinases as intersection points with other signaling networks and as a potential link to as of now elusive upstream diffusible modulators.

Resulting from a parallel comprehensive characterization of the surface molecular signature of human neural lineage specification, we identify a panel of candidate membrane-bound factors that contribute to cell-cell signaling during neural development. While functional analyses are ongoing, the resulting cluster of differentiation (CD) marker codes enable the flow cytometric isolation of purified neuronal subsets derived from human pluripotent stem cells.

The insights gained will feed into continued efforts aimed at deriving neural cells for biomedical applications in disease modeling and regeneration.

Kategorie: Vortrag

Vortrag 20

Rubrik: Neuroanatomie/vegetatives Nervensystem

Titel: Site-specific gene expression of the Gdnf system in diverticular disease

Autoren: Barrenschee M.(1),Wedel T.(1),Harde J.(1),Hellwig I.(1),Egberts J.(2),Becker T.(2),Böttner M.(1),

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Abstract:
Background and aims:
Glial cell line-derived neurotrophic factor (GDNF) promotes the survival of enteric neurons, since mice lacking either GDNF or its corresponding receptors GDNF family receptor alpha 1 (GFRa1) and RET proto-oncogene (RET) exhibit total intestinal aganglionosis. As diverticular disease (DD) is associated with intestinal hypoganglionosis, alterations of the GDNF system were studied by analyzing site-specific mRNA expression profiles of GDNF and its receptors in DD.
Material and methods:
Samples of tunica muscularis of the sigmoid colon from patients with DD and controls were assessed for mRNA expression of GDNF by qPCR. Laser-microdissected (LMD) control samples isolated from circular and longitudinal muscle and myenteric ganglia were analyzed by qPCR for site-specific expression of GDNF, GFRa1 and RET. Based on these results, mRNA expression levels of GDNF receptors were assessed in microdissected myenteric ganglia obtained from patients with DD and controls.
Results:
GDNF mRNA expression was down-regulated in the tunica muscularis of patients with DD.Site-specific gene expression analysis identified the main source of GDNF in intestinal muscle layers, while its receptors were predominantly expressed in myenteric ganglia. In myenteric ganglia of patients with DD, GFRa1 and RET gene expression were decreased.
Conclusions:
Since mRNA expression of both GDNF receptors is down-regulated in myenteric ganglia of patients with DD, a lack of GDNF-responsiveness might contribute to the intestinal hypoganglionosis previously reported in DD. Moreover, this study approach is a “proof of principle” that LMD-assisted isolation of enteric ganglia is a valuable tool for site-specific gene expression analysis in enteric neuropathies.

Kategorie: Vortrag

Vortrag 21

Rubrik: Neuroanatomie/vegetatives Nervensystem

Titel: Alterations of intestinal smooth muscle in patients with diverticular disease

Autoren: Hellwig I.(1),Böttner M.(1),Harde J.(1),Barrenschee M.(1),Egberts J.(2),Becker T.(2),Wedel T.(1),

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Abstract:
Background and aims:
Diverticular disease (DD) is associated with intestinal motor disturbances characterized by increased intracolonic pressure. The pathogenesis of DD is considered to be multifactorial including evidences for an underlying enteric neuromuscular pathology. Although the intestinal smooth muscle is the effector tissue mediating intestinal motility, studies of muscular alterations in DD are limited.
Material and methods:
Full-thickness sigmoid specimens obtained from patients with DD (n=20) and controls (n=19) were processed for morphological and molecularbiological studies. Morphometric analysis was performed to evaluate the thickness of the circular and longitudinal muscle layers and the ratio of connective tissue area within the tunica muscularis. Structural alterations were determined and mRNA profiles of components of the smooth muscle apparatus were assessed by qPCR. Altered gene expression levels were confirmed at protein level by immunohistochemistry.
Results:
Compared to controls, patients with DD showed (1) increased thickness of the circular and longitudinal muscle layers, (2) focal architectural alterations with connective tissue replacement, (3) increased connective tissue amount in the longitudinal muscle layer, (4) specific down-regulation of smooth muscle myosin heavy chain (SMMHC) and caldesmon mRNA levels, (5) decreased immunoreactivity of caldesmon and SMMHC.
Conclusions:
The results show that DD is associated with structural alterations and fibrosis of the intestinal musculature and a deficit of selected proteins of the smooth muscle apparatus. The data support the hypothesis that the histopathology of DD includes an enteric myopathy. Further studies have to elucidate the impact and time course of smooth muscle alterations on the pathogenesis of DD.

Kategorie: Vortrag

Vortrag 22

Rubrik: Neuroanatomie/vegetatives Nervensystem

Titel: Interstitial cells of Cajal: a crucial factor in the development of megacolon in human chagas disease?

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Abstract:
Chagasic megacolon is accompanied by an extensive myenteric neuron loss least affecting the nitrergic, inhibitory neurons and intramuscular nerve fibres. This disproportional excitatory denervation may explain the chronic dilation. However, the most anal, transitional zone of the surgically resected chagasic segments, although non-dilated, displayed the most pronounced preponderance of remaining inhibitory nerves.
Applying quadruple immunohistochemistry on cryosections, we focused on the balance of nerves (marker: synaptophysin) with other structures in megacolonic specimens: musculature (smooth-muscle actin), glia (S100) and interstitial cells of Cajal, ICC (c-kit). Area measurements of stained profiles in the oral (non-dilated), the megacolonic and the anal (non-dilated) zones of seven resected chagasic specimens were compared with data from seven age and region matched control specimens.
Gut wall thickening, observed in all chagasic segments, was due to muscular hypertrophy mostly in the anal segment (muscle area related to measurement surface: 68% controls, 54% oral, 42% mega, 48% anal). Related to the muscle area each, nerve-fibres (6.3% controls; 2.7% oral; 1.9% mega; 1% anal) and glia (2.7% controls; 2.4% oral; 1.9% mega; 1.4% anal) were reduced in the chagasic segments, being lowest in the anal zones. In contrast, ICCs were lowest in the dilated, megacolonic part (2.6% controls; 2.0% oral; 1.0% mega; 1.8% anal).
The density of ICCs corresponds to the amount of dilation of the three zones (lowest in dilated, higher in non-dilated oral and anal zones). We suggest that their degeneration may be crucial for the development of dilation in chagasic megacolon.

Kategorie: Vortrag

Vortrag 23

Rubrik: Neuroanatomie/vegetatives Nervensystem

Titel: Gene expression and localization study of estrogen receptors in the human and rat intestine – putative impact on gastrointestinal motility disorders

Autoren: Böttner M.(1),Hellwig I.(1),Barrenschee M.(1),Harde J.(1),Jarry H.(2),Wedel T.(1),

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Abstract:
Background and aims:
Several gastrointestinal motility disorders (e.g. slow-transit constipation, irritable bowel syndrome) exhibit a pronounced gender prevalence suggesting an involvement of sex steroids in the regulation of intestinal motility. Thus, we monitored the expression and localization of estrogen receptors (ERs) in the human colon, investigated their mRNA expression in the intestine during rat ontogenesis and assessed the effects of estradiol (E2) on enteric sex steroid receptors and neurotransmitters.
Material & methods:
Human colonic samples were assessed for mRNA expression levels of ERa and ERb and localization of ERa. The time course of ER expression in the intestine of rats of postnatal day 0, 3, 6, 21 and adult age was investigated by qPCR. The effects of E2 on intestinal gene expression of sex steroid receptors and components of the cholinergic system were monitored by qPCR in ovariectomized (OVX) and E2 treated rats.
Results:
ERa and ERb are expressed in the human colon and ERa localizes to enteric ganglia and smooth muscle cells. In rats, ERa and ERb mRNA expression increases during ontogenesis in the small and large intestine. E2 treatment of OVX rats modulates ERa, ERb, and progesterone receptor expression and increases mRNA expression of components of the cholinergic system.
Conclusions:
Our study reveals that the intestine is an E2-responsive organ expressing all relevant female sex steroid receptors. Modulation of sex steroid receptors and the cholinergic system by estrogen treatment indicates an involvement in regulating gastrointestinal motility and may account to obvious gender-specific epidemiologic differences of gastrointestinal motility disorders.

Kategorie: Vortrag

Vortrag 23a

Rubrik: Klinische Anatomie, Metabolismus, Didaktik

Titel: Alcohol and ENS: an unhappy couple

Autoren: Schäfer K.(1),Kolbe P.(1),Schnepf J.(1),Schwab T.(1),

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Abstract:
Ethanol is a toxic agent which, as part of alcoholic beverages, is responsible for severe damage in different parts of the body. Whereas brain and liver are in the focus of alcohol research, little is known about its effects on gut motility and especially its intrinsic gastrointestinal innervation: the enteric nervous system (ENS). Ethanol does have acute and chronic effects, and does also interfer with developmental processed. In the presented study, both acute and developmental influences of ethanol upon the ENS was investigated.
Myenteric plexus in vitro was exposed to ethanol and investigated using multielectrode array (MEA)approaches and calcium imaging. To investigate ethanol effects during development, ethanol was injected into the yalk sak of fertile chicken eggs. After 72h the embryo was removed and the gut dissected, fixed and stained for migrating neural crest cells. Enteric neural stem cells and postnatal myenteric plexus were cultured under the influence of increasing ethanol concentrations. Neurosphere number, respectively neurite outgrowth was assessed.
Myenteric neurons responded differentially to increasing doses of ethanol. Both MEA experiments and calcium imaging revealed neurons which showed an increased, respectively a decreased activity, or no change at all. Ethanol changed both migration and neurite outgrowth in both pre- and postnatal ENS. Migration patterns were changed in the chicken egg model. Neural crest derived stem cells showed an increased proliferation rate at several ethanol concentrations. Neurite outgrowth, as well as neurite density were reduced with increasing ethanol concentrations. So, ethanol interferes with both the pre- and postnatal ENS.

Kategorie: Vortrag

Vortrag 24

Rubrik: Entwicklungsbiologie, Reproduktion, Zellbiologie

Titel: Impact of di-ethylhexylphthalate (dehp) exposure on metabolic programming in p19-ecc

Autoren: Schädlich K.(1),Fischer B.(1),

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Abstract:
The plasticizer DEHP is a ubiquitous environmental contaminant. DEHP to crosses the placenta and is primarily known to impair gonadal development and fertility especially in the male. Our analyses focused on expression and DNA methylation of key metabolic marker genes (PPARs, GLUT4) in an in-vitro model for early embryonic development. To investigate the effects of DEHP on early embryonic cells during different developmental stages, we exposed murine P19 embryonic carcinoma cells (P19-ECC) to DEHP (5, 50, 100 µg/ml) during cardiomyogenesis. The P19 cells were exposed at the undetermined and undifferentiated stage for four days and subsequently differentiated to beating cardiomyocytes. At different developmental stages the expression of key genes in fatty acid and glucose metabolism (Pparg1, Fabp4, Slc2a4) as well as epigenetic marker genes (Dnmts, Hdac1) were analyzed by qRT-PCR. The methylation status of Pparg1, Ppara and Slc2a4 was investigated by pyrosequencing. We found that DEHP significantly increased the expression of Dnmt3a and Dnmt, and altered the expression of Pparg1, Fabp4 and Slc2a4. Promoter associated CpG islands within Ppara, Pparg1 and Slc2a4 were hypomethylated. Exposure to DEHP led to small but statistically significant increases in methylation of specific CpGs within Ppara and Pparg1 but not Slc2a4. Some of the differentially methylated CpGs were associated with transcription factor binding sites of known Pparg regulating transcription factors. We conclude that early DEHP exposure can alter the expression of genes associated with cellular metabolism. However, the mechanism directing the long-lasting effects of DEHP exposure on cellular metabolism (DOHaD paradigm) remains to be established.
Supported by EU (FP7-REEF N°212885) and the Wilhelm Roux Programme of the MLU Faculty of Medicine

Kategorie: Vortrag

Vortrag 25

Rubrik: Entwicklungsbiologie, Reproduktion, Zellbiologie

Titel: Dermomyotomal lip sustainment is differently regulated during epaxial and hypaxial muscle development

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Abstract:
During vertebrate embryonic development, diverse derivatives like skeletal muscle, connective tissue and endothelia arise from the dermomyotome, the dorsal part of the somite. The dermomyotome is a pseudostratified epithelial cell layer with a flat central domain and marginal infoldings, the dermomyotomal lips. These dermomyotomal lips are known as blastema-like epithelial growth-zones, which give rise to the first embryonic muscle cells in the myotome. Whereas the central dermomyotome soon dissociates into mesenchymal progenitor cells, the dermomyotomal lip epithelium persists until the end of somite development, thus contributing to myotomal muscle growth.
Both, formation of the epithelial somites, and maintenance of the dermomyotomal epithelium during sclerotome mesenchymalization, are known to depend on Wnt signaling. While these early events are thought to be regulated via canonical, beta-catenin dependent signaling, the signaling pathway sustaining the epithelial dermomyotomal lips during later stages of myotome formation has been unknown.
Here, we present evidence that the dorsomedial and ventrolateral dermomyotomal lips are maintained by different mechanisms. The dorsomedial lip, which is giving rise to epaxial back muscles, is regulated by canonical Wnt signaling, thus maintaining the early somitic epithelialization mechanism. However, the ventrolateral lip, which is giving rise to ventrolateral trunk muscles, is regulated by a non-canonical, PCP-like signaling pathway.
We discuss our findings with respect to different prerequisites during early muscle development in the epaxial and hypaxial compartment, respectively.

Kategorie: Vortrag

Vortrag 26

Rubrik: Entwicklungsbiologie, Reproduktion, Zellbiologie

Titel: Time-lapse imaging of the 'in-out' mechanism during pectoral girdle muscle formation

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Abstract:
In vertebrates, all skeletal muscles develop from the somite. At the forelimb level, migrating myogenic precursors delaminate from the ventrolateral lip (VLL) of the dermomyotome and migrate into the developing limb bud. Whereas most of the myogenic precursors remain in the limb bud, to form the forelimb muscles, several cells migrate back towards the trunk to give rise to the superficial pectoral girdle muscles. This mechanism is referred to as the 'In-Out' mechanism. Until now the molecular mechanisms of the retrograde migration of the pectoral girdle muscle precursors are insufficiently studied.

The aim of the present study is to investigate the mechanism of the 'In-Out' migration of the pectoral girdle muscle precursors.

Using in ovo electroporation of a chicken embryo, we labeled the ventrolateral part of the somites adjacent to the forelimb bud with a Tol2-EGFP construct. With the aid of fluorescence microscopy we traced the migration of the labeled cells in ovo and confirmed their distribution to the pectoral girdle muscles. Confocal laser scanning microscopy allowed us to perform live-cell imaging in slice cultures of electroporated chicken embryos. We observed the migrating cells on their pathways to the target locations and demonstrated their behavior during this period. In order to depict the role of the chemokine receptor CXCR4 and its ligand SDF for the pectoral girdle muscle formation, we performed microsurgical implantations of CXCR4-inhibitor beads in the proximal forelimb region of chicken embryos. Additionally, we examined MyoD expression in CXCR4-mutant mice embryos by using in situ hybridization.

Kategorie: Vortrag

Vortrag 27

Rubrik: Entwicklungsbiologie, Reproduktion, Zellbiologie

Titel: Crosstalk between arylhydrocarbon receptor (ahr) and peroxisome proliferator-activated receptor (ppar) signaling pathway in human granulosa cells

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Abstract:
There is much evidence that environmental contaminants binding to the AhR and/or to PPARs might contribute to adverse effects on reproduction.
DEHP (di-(2-ethylhexyl) phthalate, common plasticizer) is known as ovarian toxicant in rodents. It alters gene expression predominantly by activating PPARs. These are transcription faktors that are critical for several metabolic pathways but also for normal ovarian function by mediating steroidogenesis.
TCDD (tetracholordibenzodioxin) as a strong ligand of the AhR. The AhR is a widespread nuclear transcription factor with an important role in many tissues and in ovarian physiology too. AhR activation leads to altered gene expression of gonadotropin and estrogen receptors as well as to a decreased estradiol synthesis in the human granulosa cell line KGN. The human granulosa cell line KGN, expressing PPARs as well as AhR, offers the opportunity to study regulatory mechanisms of the receptor crosstalk independently from overriding endocrine control.
Expression analyses show a PPAR-dependent increase of AhR and CYP1B1 transcription under DEHP exposure. A TCDD-DEHP mix shows no additive effects in expression of these genes. Expression of the PPARalpha and PPARgamma target genes 17beta-HSD and PEPCK were unaffected by TCDD exposure. ERalpha, FSH-R and LH-R expression was upregulated under DEHP exposure. Upregulation did not occur when specific PPARalpha and PPARgamma antagonists were simultaneously applied.
In conclusion, in the human granulosa cell line KGN DEHP exposure does induce an activation of AhR and AhR target genes and expression of gonadotropin and estrogen receptors via the PPAR signaling pathway. An activation of PPAR signaling downstream of the AhR pathway was not detectable.

* Supported by EU (FP7-REEF N°212885)

Kategorie: Vortrag

Vortrag 28

Rubrik: Entwicklungsbiologie, Reproduktion, Zellbiologie

Titel: Visualizing cGMP effects on spontaneous contractility of seminiferous tubules and the epididymal duct

Autoren: Mietens A.(1),Tasch S.(1),Schneider-Hüther I.(1),König P.(2),Eichner G.(3),Feuerstacke C.(1),Müller D.(1),Middendorff R.(1),

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Abstract:
The transport of immature spermatozoa relies on the well-orchestrated function of contractile cells (myofibroblasts, smooth muscle cells) surrounding seminiferous tubules in the testis and the epididymal duct. Contractile cell function influences maturation processes of spermatozoa and therefore contributes to ensure male fertility. The second messenger cGMP plays a major role in contractility of smooth muscle cells and accordingly components of the cGMP pathway have been described in contractile cells of testis and epididymis.
Visualization of cGMP effects in seminiferous tubules and epididymal duct, however, is missing.
Elements of cGMP pathways like sGC (soluble guanylate cyclase), PKGI (cGMP-dependent protein kinase I), PDE5 (cGMP-specific phosphodiesterase 5) could be localized to myofibroblasts and smooth muscle cells by immunohistochemistry and RT-PCR analyses of laser-dissected tissue samples. cGMP assays proved enzyme activity.
Using a novel ex vivo approach, spontaneous contractile function of isolated seminiferous tubules from rat and men was investigated by time lapse video microscopy and shown to be sensitive to cGMP. Tracking of wall movements revealed a slow irregular contraction pattern associated with passive movement of luminal contents. Wall movements could be characterized by a spectrum of frequencies. Enhancing cGMP signalling modified the frequency spectrum towards slower frequencies.
In contrast, in isolated epididymal duct segments, video microscopy revealed a regular spontaneous contraction pattern that was sensitive to cGMP correlating to our results in organ bath studies.
In the present study, we could directly show that spontaneous contractions of seminiferous tubules and epididymal duct affect movement of sperm and are regulated by cGMP pathways.

Kategorie: Vortrag

Vortrag 29

Rubrik: Entwicklungsbiologie, Reproduktion, Zellbiologie

Titel: Okt3 and ucht-1 act differently on human t-lymphocytes

Autoren: Singer B.(1),Koldehoff M.(2),Dreesen J.(1),

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Abstract:
Monoclonal antibodies (mAbs) directed against CD3 are able to induce proliferation in human T-lymphocytes. Though it has been reported that for proper mitogenic T-cell stimulation the CD3 antibody needs to be immobilized and accompanied by a co-stimulatory mAb against e.g. CD28. It was also shown that monocytes and interleukin-2 (IL-2) are required for this T-cell stimulation. In the present study we have investigated the effect of two soluble applied CD3 mAbs, namely UCHT-1 (IgG1) and OKT3 (IgG2a) on T-lymphocytes. Treatment of PBMCs with OKT3 resulted in a strong T-cell activation. In contrast T-cells of just 38% of healthy donors responded with proliferation to UCHT-1. At this 65% of males and only 13% of females appeared to be UCHT-1 responders. In case of patients treated for leukemia the frequency of UCHT-1 responders increased to 64% with a slight decrease of male (53%) and a significant increase of female (77%) responders. Again, OKT3 activated T-cells of all donors. Further analyzes revealed that monocytes as well as IL-2 played a central role for the OKT3 induced T-cell proliferation. However, the UCHT-1 effect seemed to be less dependent on monocytes and only in some cases on IL-2. Other not yet identified cytokines seemed to be crucial for the UCHT-1 triggered T-cell proliferation. These cytokines must be present in some of the PBMC subpopulation of responders maybe answer to priming of the immune system. Thus, the knowledge about patients being UCHT-1 responders or non-responders may help to select the right therapeutic approaches applied to patients.

Kategorie: Vortrag

Vortrag 30

Rubrik: Entwicklungsbiologie, Reproduktion, Zellbiologie

Titel: Nrf2 initiates the resolution of inflammation

Autoren: Fragoulis A.(1),Greiber A.(1),Rosen C.(1),Pufe T.(1),Wruck C.(1),

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Abstract:
Introduction: The switch between pro-inflammatory (M1) and anti-inflammatory (M2) states of macrophage polarization allows the resolution of inflammation. Little is known about the molecular mechanisms responsible for this class switch. Recent studies have demonstrated that Nrf2 signalling is crucial for the abating inflammation-associated tissue damage.

Methods: The human monocytic cell line THP-1 and the murine macrophage cell line RAW296.7 as well as bone marrow derived macrophages from various transgenic mouse strains were cultured for in vitro experiments and analysed by realtime-PCR, western blot and promoter studies. Expression of the Nrf2 target gene heme oxygenase-1 (HO-1) and the M2-macrophage marker Ym-1 were analysed in tissues from an in vivo model of aseptic inflammation using immunofluorescent stainings.

Results: TNF-alpha activated Nrf2 in monocytes and macrophages in an ERK1/2- and p38-kinase depended manner. Interestingly, redox signalling via NADPH-oxidases was a prerequisite for Nrf2 activation in this scenario. Activated Nrf2 induced the production of carbon monoxide (CO) via up regulation of HO-1 expression. Increased HO-1/CO concentrations induce a M2 genetic program and diminish M1 phenotype. In agreement with this in vitro data, HO-1 expression in inflamed muscle tissue was Nrf2 dependent and resulted in more Ym-1 positive macrophages in Nrf2-WT mice compared to their Nrf2-KO littermates.

Conclusion: These data suggest that Nrf2 is important for M2-polarization of macrophages. Thus, Nrf2 activity initiates the resolution of inflammatary processes.

Kategorie: Vortrag

Vortrag 31

Rubrik: Entwicklungsbiologie, Reproduktion, Zellbiologie

Titel: Genetic variants of human atp-binding cassette (abc) transporter abcg2: analysis of interaction with the at1 receptor antagonist telmisartan

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Abstract:
Background: The ATP-binding cassette (ABC)-transporter breast cancer resistance protein (BCRP/ABCG2) affects the pharmacokinetics of numerous drugs and plays a role in stem cell biology. We have previously demonstrated that telmisartan, an AT1 receptor antagonist, interacts with BCRP, thereby inhibiting its transport capacity. The aim of this study was to evaluate whether single nucleotide polymorphisms (SNPs) or somatic mutations in the BCRP-Gen affect the interaction of telmisartan with the efflux transporter.
Methods: For this purpose, we first established a cellular system for the conditional (doxycyclin-dependent) expression of BCRP. Next, we generated via site-directed mutagenesis several BCRP variants (G34A, C421A, T742C; T1291G, T1444C, T1465C), expressed them in HEK293-Tet On cells, and subsequently investigated the interaction of telmisartan with these BCRP variants using the pheophorbide A efflux assay. Moreover, we analysed the membranous localization and expression levels of the BCRP variants using confocal laser scanning microscopy and Western Blot.
Results: All BCRP variants showed a membranous localization pattern. Nevertheless, the expression levels of the C421A and especially the T1465C variant were drastically reduced. Moreover, telmisartan-induced inhibition of BCRP-mediated pheophorbide A transport was almost abolished in cells expressing the R482G variant, whereas interaction of telmisartan with BCRP was not significantly affected by the other mutations.
Conclusion: Largely reduced BCRP expression levels in subjects carrying the C421A or T1465C mutations may affect drug pharmacokinetics and stem cell biology in these individuals. Moreover, the arginine residue at position 482 of the BCRP molecule appears to be critical for the interaction of telmisartan with the ABC transporter.

Kategorie: Vortrag

Vortrag 32

Rubrik: Zellbiologie

Titel: Inverse signalling – a novel concept to understand transmembrane chemokine signalling

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Abstract:
Chemokines are small chemotactic peptides that are involved in cell migration and proliferation during inflammation, tumourigenesis, but also in normal tissue development. Of the about 50 chemokines, two are synthesized as transmembrane (tm) precursor from which a soluble (s) chemotactic from is released ("shedded") by cell-surface proteases. Investigating the role of tm-chemokines in cancer, we detected remarkable high synthesis of CXCL16 in several types of tumour cells without an appropriate expression of its classical receptor CXCR6. Therefore, we looked for alternative functions and receptors. Serendipitously, we found that cells expressing high levels of tm-CXCL16 also responded to s-CXCL16 without expressing the previous known G protein-coupled receptor. Further detailed investigations revealed that tm-chemokines themselves in fact responded to s-chemokines by a novel mechanism by which they bind them and subsequently transduce signals. We term this mechanism "inverse signalling", because the soluble forms are produced from the transmembrane forms by proteolytic shedding and thereafter generate auto- or paracrine signals in their synthesizing cells. Inverse signalling was proved by (1) binding of fluorescence- or gold-labelled s-CXCL16 to tm-CXCL16, (2) signal transduction and biological effects were observed in tm-CXCL16-positive cells or in negative cells only after tm-CXCL16-transfection, (3) effects were insensitive to Pertussis toxin (affecting G protein-signalling), (4) mutagenesis experiments showed that an intracellular S-X-X-S-phosphorylation motif is essential for signalling, and (5) inhibition of shedding enhances inverse signalling. Further experiments to elucidate the biological role(s) of inverse signalling in tumour biology and inflammation, as well as its occurrence with other transmembrane ligands are ongoing.

Kategorie: Vortrag

Vortrag 33

Rubrik: Zellbiologie

Titel: Mechanisms of tandem peptide-mediated protection in pemphigus vulgaris

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Abstract:
Loss of cell adhesion in the autoimmune blistering skin disease pemphigus vulgaris is mediated by autoantibodies targeting the desmosomal cadherins desmoglein (Dsg)3 and Dsg1. We recently demonstrated a tandem peptide (TP) to promote interaction of Dsg molecules and to protect against autoantibody-mediated cell dissociation and p38MAPK activation both in vitro and in vivo. Here we characterized the protective effect of TP in more detail in cultured human keratinocytes. An important mechanism for Dsg interaction is the insertion of a tryptophan residue to a hydrophopic pocket of the opposing Dsg molecule. Loss of Dsg interaction, cell dissociation as well as p38MAPK activation by both, excess of tryptophan and AK23, a monoclonal Dsg3 antibody from a pemphigus mouse model, were prevented by TP incubation, indicating loss of Dsg binding to be a trigger for downstream signaling. Although TP did not prevent depletion of Dsg3 in response to AK23, it promoted oligomerization of desmosomal Dsg3. Furthermore, upon incubation with AK23, immunoprecipitation and proximity ligation assays revealed that phosphorylated p38MAPK rapidly associated with Dsg3, which was blocked by TP incubation. In line with this, TP blocked AK23-induced cytokeratin filament retraction, a hallmark of pemphigus. Interestingly, loss of cell adhesion in response to direct activation of p38MAPK by anisomycin also was largely abrogated by TP incubation, however without inhibition of p38MAPK phosphorylation. Together, these data indicate that TP both prevents signaling by Dsg molecules to p38MAPK and inhibits cell dissociation by stabilizing Dsg binding.

Kategorie: Vortrag

Vortrag 34

Rubrik: Zellbiologie

Titel: The transporter regulator hrs1 controls differential plasma membrane insertion of na+-d-glucose cotransporter sglt1 and concentrative nucleoside transporter cnt1

Autoren: Veyhl-Wichmann M.(1),Kipp H.(1),Gorboulev V.(1),Koepsell H.(1),

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Abstract:
The intracellular 67 kDa-protein hRS1 encoded by gene RSC1A1 was initially identified as a transcriptional and posttranscriptional down-regulator of the human Na+-D-glucose cotransporter hSGLT1. Previously we reported that posttranscriptional down-regulation of hSGLT1 by hRS1 occurs at the trans-Golgi-network (TGN), is stimulated by PKC and is modulated by intracellular D-glucose (Veyhl et al., Am J Physiol Renal Physiol 291, 2006). In the present study we demonstrate that RS1 is involved in the differential regulation of the membrane insertion of hSGLT1 and the human concentrative nucleoside transporter hCNT1.
Employing oocytes of Xenopus laevis as expression system we identified at the NH2-terminal regulatory domain of hRS1 different oligopeptides which were capable to down-regulate hSGLT1 and/or hCNT1 with nanomolar affinity. By the peptides different pathways of trafficking were inhibited. Posttranscriptional down-regulation of hSGLT1 by the NH2-terminal domain was enhanced after stimulation of phosphorylation whereas down-regulation of hCNT1 was enhanced after blockage of phosphorylation. Mimicking different phosphorylation states by amino acid exchange in the NH2 terminal domain analogous results were obtained.
Our data suggest that hRS1 at the TGN contains a complex regulatory interaction surface that is modulated by phosphorylation/dephosphorylation and that this region is responsible for blocking exocytosis of vesicles containing either hSGLT1 or hCNT1.

Kategorie: Vortrag

Vortrag 35

Rubrik: Zellbiologie

Titel: Overexpression of connective tissue growth factor in the mouse eye effects Tgf-β and Bmp signaling

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Abstract:
Purpose: Overexpression of connective tissue growth factor (CTGF) in the mouse eye causes glaucoma. Since patients with glaucoma show an activation of TGF-beta signaling in the eye, we analyzed the effects of CTGF on expression and signaling activity of different members of the TGF-beta superfamily in the mouse eye and in cultured ocular cells in vitro.

Methods: In the eyes of CTGF overexpressing mice, expression patterns and the activity of canonical signaling pathways for TGF-betas and BMPs were analyzed by RT-PCR, immunoblotting, immunohistochemistry and microscopy. In vitro, trabecular meshwork cells (HTM) and retinal ganglion cells (RGC) were treated with recombinant CTGF. The viability of CTGF treated RGC cells were analyzed by WST-1 assay under starving conditions. In vivo the effect of CTGF on RGC cells was analyzed after intravitreal NMDA injections.

Results: In the anterior eyes of CTGF overexpressing mice, the expression of various BMPs and the activity of BMP signaling is significantly reduced while the expression and activity of TGF-betas is enhanced. In the retina of the mice, BMP signaling is reduced in RGC. In vitro analysis of CTGF-treated HTM and RGC cells confirmed the negative effects on BMP signaling. RGC cells with reduced BMP signaling showed a lower resistance to stress both in vivo and in vitro.

Discussion: CTGF disturbs the homeostatic balance between BMPs and TGF-betas, an effect that may significantly contribute to its role in the pathogenesis of glaucoma.

Support: DFG FOR 1075

Kategorie: Vortrag

Vortrag 36

Rubrik: Zellbiologie

Titel: Lack of endothelial diaphragms in fenestrae and caveolae of mutant plvap-deficient mice

Autoren: Herrnberger L.(1),Seitz R.(1),Kuespert S.(1),Bösl M.(2),Fuchshofer R.(1),Tamm E.(1),

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Abstract:
Purpose: Plasmalemmal vesicle-associated protein (PLVAP) is specifically expressed in endothelial cells in which it localizes to diaphragms of fenestrae, caveolae, and transendothelial channels. To learn about its function, we generated mutant mice that lack PLVAP.
Methods: To disrupt gene function, an IRES:lacZ trapping cassette was inserted between exons 1 and 2 of Plvap. Deletion of Plvap was confirmed by RT-PCR, Western blot analysis and immunohistochemistry. Promoter activity was assessed by β-galactosidase staining. Phenotype analysis was performed by light and electron microscopy.
Results: In a C57BL/6N genetic background, Plvap-/- embryos die before birth and suffer from subcutaneous edema, hemorrhages, and defects in the vascular wall of subcutaneous capillaries. In wild-type embryos, PLVAP and caveolae with a stomatal diaphragm are present in endothelial cells of subcutaneous capillaries and endocardium, while a diaphragm is missing in caveolae of Plvap-/- littermates. Plvap-/- mice in a mixed C57BL/6N/FVB-N background are born and survive at the most for 4 weeks. Capillaries of exocrine and endocrine pancreas and of kidney peritubular interstitium were investigated as examples of fenestrated capillaries. In these vascular beds, Plvap-/- mice show a complete absence of diaphragms in fenestrae, caveolae, and transendothelial channels, findings which are associated with a substantial decrease in the number of endothelial fenestrae.
Conclusion: Functional analyses of Plvap-/- mice will help to clarify the specific role of endothelial fenestrae and their contribution to passage of water and solutes in different organs. In addition, they are expected to provide an animal model to clarify the specific function of stomatal diaphragms

Kategorie: Vortrag

Vortrag 37

Rubrik: Zellbiologie

Titel: Phosphodiesterase-4 inhibition as a therapeutic approach to treat endothelial barrier disruption in systemic inflammation

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Abstract:
In sepsis and systemic inflammation, increased microvascular permeability and consecutive breakdown of microcirculatory flow significantly contribute to organ failure and death. Evidence points to a critical role of cAMP levels in endothelial cells to maintain capillary endothelial barrier properties in acute inflammation. However, approaches to verify this observation in systemic models are rare. Therefore, we tested here whether systemic application of the phosphodiesterase-4-inhibitors (PD-4-Is) rolipram or roflumilast to increase endothelial cAMP was effective to attenuate capillary leakage and breakdown of microcirculatory flow in severe lipopolysaccharide (LPS)-induced systemic inflammation in rats. Measurements of cAMP in mesenteric microvessels demonstrated significant LPS-induced loss of cAMP levels which was blocked by application of rolipram. Increased endothelial cAMP by application of either PD-4-I rolipram or roflumilast led to stabilization of endothelial barrier properties as revealed by measurements of extravasated FITC-albumin in postcapillary mesenteric venules. Accordingly, microcirculatory flow in mesenteric venules was significantly increased following PD-4-I treatment and blood gas analyses indicated improved metabolism. Furthermore application of PD-4-I after manifestation of LPS-induced systemic inflammation and capillary leakage therapeutically stabilized endothelial barrier properties as revealed by significantly reduced volume resuscitation for haemodynamic stabilization. Accordingly microcirculation was significantly improved following treatment with PD-4-Is. Our results demonstrate that inflammation-derived loss of endothelial cAMP contributes to capillary leakage which was blocked by systemic PD-4-I treatment. Therefore these data suggest a highly clinically relevant and applicable approach to stabilize capillary leakage in sepsis and systemic inflammation.

Kategorie: Vortrag

Vortrag 38

Rubrik: Zellbiologie

Titel: Interplay between Nrf2 and amphiregulin - implications for ventilator induced lung injury

Autoren: Siegl S.(1),Fragoulis A.(2),Pufe T.(2),Uhlig S.(2),Wruck C.(2),

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Abstract:
Rationale: Oxidative stress plays a critical role in the development of Ventilator Induced Lung Injury (VILI), an adverse effect in mechanically ventilated patients. We hypothesized that the transcription factor Nrf2, the major regulator of the cellular antioxidant defence, protects against VILI.
Methods: Nrf2 activity was detected in isolated perfused mouse lung. Precision cut lung slices were prepared and stimulated and Nrf2 activity was measured. Cells with inactivated Keap1, the cellular inhibitor of Nrf2, were used in a dual luciferase reportergene assay utilizing an amphiregulin promoter containing luciferase vector. Nrf2 knockout and wild type mice were in vivo ventilated with high volume ventilated over a time period of six hours. Physiological and inflammatory parameters, lung mechanics and gene expression of Nrf2 target genes were measured.
Results: Nrf2 is activated in a ventilation strategy dependent manner. Nrf2 induced the cellular antioxidant defence of the lung. Intratracheally applicated amphiregulin and amphiregulin stimulated PCLS showed significant Nrf2 activation and Nrf2 leads to amphiregulin expression and promoter activation.
Conclusions: Mechanical ventilation leads to Nrf2 activation, which increases the expression of the cellular antioxidant defence and amphiregulin. Amphiregulin, in turn, is able to activate Nrf2 and leads to a positive feedback loop between Nrf2 and amphiregulin during lung ventilation. This Interplay between Nrf2 and Amphiregulin may protect against VILI.

Kategorie: Vortrag

Vortrag 39

Rubrik: Zentrales Nervensystem/Signaltransduktion und Verschaltung

Titel: Degenerative Changes at the Rod Photoreceptor Synaptic Ribbon in Aging DBA/2J Mice

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Abstract:
The DBA/2J mouse is a commonly used inbred strain for glaucoma research. The eyes of DBA/2J mice show severe age-related changes that include iris stroma atrophy and pigment dispersion, increase of intraocular pressure (IOP) with age, and degenerative changes of retinal ganglion cells and the optic nerve. The relevance of increased IOP as a diagnostic risk factor for glaucoma is beyond controversy, however, correlations of long term IOP progression with axon loss in individual eyes show that in DBA/2J mice ocular hypertension cannot be considered as the only causative factor for the onset of glaucomatous changes. Recent electroretinogram studies identified functional deficits, which suggest that also photoreceptor cells are involved in the pathological processes occurring in the DBA/2J mouse retina. In a comparative study, we examined anatomical and molecular changes with light and electron microscopy in the retinae of DBA/2J and C57BL/6 mice. Our data show age-dependent and progressive degenerative structural changes at rod photoreceptor ribbon synapses in the retina of the DBA/2J mouse, accompanied by a thinning of the outer plexiform layer. Here, at the first synaptic layer of the retina, the photoreceptor cells transfer the visual signals to the post-receptoral retinal network, and malfunctioning ribbon synapses will inevitably lead to impaired vision or even total blindness. The structural ribbon changes represent a photoreceptor synaptic phenotype that has not yet been described in this animal model of secondary angle-closure glaucoma.

Kategorie: Vortrag

Vortrag 40

Rubrik: Zentrales Nervensystem/Signaltransduktion und Verschaltung

Titel: Norrin interacts with Tgf-β1 signaling *in vitro* and *in vivo*

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Abstract:
Purpose: Norrin is a growth factor that activates canonical Wnt/beta-catenin signaling. A mutual interaction has been shown between TGF-beta and Wnt/beta-catenin signaling pathways by several studies. Moreover, Norrin is an important initiator for retinal angiogenesis whereas TGF-beta1 is known to have anti-angiogenic properties during retinal development. Therefore we wondered if there is a possible retinal cross-talk between both growth factors. To this end we analyzed the interaction of Norrin and TGF-beta1 in cell culture experiments as well as in transgenic mouse lines.

Methods: A luciferase reporter cell line for TGF-beta1 signaling (MLEC/PAI/L) and human microvascular endothelial cells (HMEC) were used for in vitro studies. Both cell lines were incubated with Norrin and/or TGF-beta1 to analyze luciferase activity, and the expression of different mediators of TGF-beta1 signaling on mRNA and protein level. In parallel experiments, transgenic mice with an ocular overexpression of Norrin (betaB1-Norrin) or TGF-beta1(betaB1-TGF-beta1) were mated and double transgenic mice were examined by light microscopy, immunohistochemistry, western blot analyzes and real-time RT-PCR.

Results: In cell culture experiments an inhibitory effect of Norrin on TGF-beta1 signaling was observed. In addition, the retinal phenotype of betaB1-TGF-beta1 mice such as lack of retinal capillaries and a pronounced number of apoptotic cells during development was attenuated by cross-breeding with animals with an ocular overexpression of Norrin. As likely mediator of this inhibitory effect, Smad 7 was identified.

Conclusion: Norrin has inhibitory effects on TGF-beta1 signaling in vitro and vivo. The inhibition is mediated, at least partially, by Smad7.

Kategorie: Vortrag

Vortrag 41

Rubrik: Zentrales Nervensystem/Signaltransduktion und Verschaltung

Titel: The role of the protooncogene ski in cortical development

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Abstract:
First insights into the molecular programs orchestrating the progression from neural stem cells to cortical projection neurons are emerging. The transcriptional regulator Ski is linked to the human 1p36 deletion syndrome, which includes brain abnormalities. Here, we report critical roles for Ski in the maintenance of the neural stem cell pool and the specification of callosal projection neurons. Ski-deficient mice revealed altered cell cycle characteristics of neural progenitors and disturbed timing of neurogenesis. Mutant neurons of the superficial cortical layers ectopically express the deep-layer marker Ctip2 and largely fail to form the corpus callosum. We identify Satb2 as a novel partner of Ski, and show that both proteins are required for transcriptional repression of Ctip2 in upper-layer neurons. We propose a model in which Satb2 recruits Ski to the Ctip2 locus, and Ski in turn attracts histone deacetylases, thereby enabling the formation of a functional NURD repressor complex.

Kategorie: Vortrag

Vortrag 42

Rubrik: Zentrales Nervensystem/Signaltransduktion und Verschaltung

Titel: Repetitive magnetic stimulation of hippocampal neurons in organotypic slice cultures induces functional and structural plasticity of excitatory postsynapses

Autoren: Vlachos A.(1),Müller-Dahlhaus F.(2),Rosskopp J.(2),Lenz M.(1),Ziemann U.(2),Deller T.(1),

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Abstract:
Repetitive transcranial magnetic stimulation (rTMS) is a non-invasive brain stimulation technique, which can alter cortical excitability in human subjects for hours beyond the stimulation period. It thus has potential as a therapeutic tool in neuro-psychiatric disorders associated with altered cortical excitability. However, the effects of rTMS remain insufficiently understood at the cellular level. Here, we used repetitive Magnetic Stimulation (rMS) of mouse entorhino-hippocampal slice cultures and assessed the outcome of a single high frequency (10Hz) rMS protocol on functional and structural properties of excitatory synapses in mature hippocampal CA1 pyramidal neurons. Whole-cell patch-clamp recordings, immunohistochemistry, and time-lapse imaging techniques revealed that rMS induces a long-lasting increase in glutamatergic synaptic strength which is accompanied by structural remodeling of dendritic spines. Furthermore, our data indicate that rMS interferes with the molecular machinery which controls the NMDA-receptor dependent accumulation of AMPA-receptors at synaptic sites. These results provide first experimental evidence that rMS induces coordinated functional and structural plasticity of excitatory postsynapses.

Kategorie: Vortrag

Vortrag 43

Rubrik: Zentrales Nervensystem/Signaltransduktion und Verschaltung

Titel: Disrupted molecular architecture of glutamatergic synapses and autistic-like behaviors in prosap/shank mutant mice

Autoren: Schmeisser M.(1),Ey E.(2),Wegener S.(3),Bockmann J.(1),Janssen A.(1),Grabrucker A.(1),Schmitz D.(3),Kreutz M.(4),Bourgeron T.(2),Gundelfinger E.(4),Böckers T.(1),

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Abstract:
Various studies from recent years strongly support the hypothesis that synaptic imbalances contribute to the development of autism spectrum disorders (ASDs). In this context, genetic alterations of synaptic proteins such as PROSAP/SHANK play a major role. Our group has generated ProSAP1/Shank2 and ProSAP2/Shank3 mutant mice, which are analysed in collaborative studies (Bourgeron lab, Paris, Gundelfinger lab, Magdeburg, Schmitz lab, Berlin). Interestingly, we primarily found molecular rather than morphological alterations of glutamatergic synapses in these models. We demonstrate that genetic deletion of ProSAP1/Shank2, for example, leads to an early, brain region specific up-regulation of ionotropic glutamate receptors such as the NMDAR. Predominantly affected regions include the hippocampus and striatum. These mutants further exhibit imbalanced glutamatergic neurotransmission and show autistic-like behavior. Based on these findings, our studies suggest that mutations in genes causing ASD in humans can indeed alter glutamate signaling and at the same time lead to abnormal social interaction and communication in mice. ProSAP1/Shank2 and ProSAP2/Shank3 mutant mice will thus provide a comprehensive framework to identify new knowledge-based treatments of ASD.

Kategorie: Vortrag

Vortrag 44

Rubrik: Zentrales Nervensystem/Signaltransduktion und Verschaltung

Titel: Short-term psychosocial stress protects against light-induced damage of photoreceptors via a corticosterone-mediated activation of the Akt pathway

Autoren: Ohlmann A.(1),Forkwa T.(1),Reber S.(2),Neumann I.(2),Tamm E.(1),

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Abstract:
Purpose: Glucocorticoids have repeatedly been shown to be neuroprotective in animal models of retinal degenerations. Since glucocorticoid release is one of the hallmarks of the classical stress response, we wondered if psychosocial stress has protective effects on photoreceptors after light-induced damage. In addition, the roles of the hypothalamic–pituitary–adrenal (HPA) axis, of Müller cell gliosis and of AKT pathway activation in stress-mediated neuroprotection was investigated.
Methods: Psychosocial stress was induced via chronic subordinate colony housing (CSC) of mice either for 10 h (short-term stress) or 19 d (chronic stress) followed by an illumination with 5000 lux. Apoptosis of photoreceptors was assessed by TUNEL staining. To investigate the role of the HPA axis, adrenalectomy was performed. Retinal expression of neuroprotective growth factors and phosphorylation of AKT was analyzed by real-time RT-PCR and western blotting.
Results: Short-term psychosocial stress protected photoreceptors from light induced damage when compared to single-housed controls (SHC), an effect that was lost after 19 d of CSC housing. As opposed to those of sham-operated mice, photoreceptors of adrenalectomized mice were not protected from light damage after 10 h CSC. Retinal expression of GFAP but not of neuroprotective factors was increased after 10 h CSC. After short-term psychosocial stress, a significant higher level of AKT phosphorylation was observed when compared to SHC. Finally, inhibition of AKT phosphorylation by Triciribine blocked the stress-mediated protective effects on photoreceptors after illumination.
Conclusions: Short-term psychosocial stress protects photoreceptors against light-induced damage. This effect is most likely mediated via an increase in plasma corticosterone which in turn activates the AKT signalling pathway.

Kategorie: Vortrag

Vortrag 45

Rubrik: Zentrales Nervensystem/Signaltransduktion und Verschaltung

Titel: Receptorarchitectonic mapping of four new areas in the inferior frontal sulcus of the human brain

Autoren: Bradler S.(1),Palomerso-Gallagher N.(1),Schleicher A.(1),Zilles K.(1),Amunts K.(1),

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Abstract:
Functional imaging studies frequently reported activations in the inferior frontal sulcus (ifs), e.g., in sentence comprehension and verbal working memory , which cannot be attributed to any known cytoarchitectonic area. Furthermore, we recently found, that Broca's region and its surrounding are more complex than previously thought [1]. The aim of this study was to analyze the segregation of the ifs using quantitative receptor autoradiography [2].
Seven hemispheres were cut coronally into 20µm thick slices. Alternating sections were labeled with tritiated ligands for receptor binding sites [2]. Seventeen receptor types of different transmitter systems were analyzed. Autoradiographs were digitized, and the gray value information was converted into absolute binding sites densities (fmol/mg protein).
The results proved a receptorarchitectonical differentiation of the ifs from dorsally and ventrally adjacent areas (BA46/9 and 45/44). Four new areas were identified: ifs1-4. They differed in mainly with respect to AMPA, GABAA, M1, α1, and 5HT1A receptors. The hierarchical cluster analysis showed that ifs1 and 2 were more similar to each other than both areas to ifs3/4. Furthermore, ifs1-4 were arranged in a rostro-caudal order along the ifs.
Four new areas, ifs1-4, were mapped in the human inferior frontal sulcus. Areas ifs1-4 differed in their receptorarchitecture regarding mean binding site densities and laminar distribution patterns. Furthermore ifs1/2 (and ifs3/4) were more similar to each other than to neighboring areas 46, 9 and 45p, indicating a functional distinction of the ifs-areas from neighboring dorsal prefrontal areas.

References:
[1] Amunts, et al. (2010), PLoS Biology, 8(9): e1000489.
[2] Zilles, et al. (2004), J. Anat., 205(6):417-432.

Kategorie: Vortrag

Vortrag 46

Rubrik: Zentrales Nervensystem/Signaltransduktion und Verschaltung

Titel: Magnetic resonance microscopy of the murine brain

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Abstract:
Magnetic resonance imaging (MRI) using 7 Tesla animal scanners for small animals is well-suited for comparative investigation of treated (either genetically altered rodents or rodent models of disease) versus untreated rodents. However, the resolution is somewhat limited for the analysis of brains of mice, due to the small structure of the brain. Moreover, motion of living animals introduces considerable artifacts and anaesthetized animals can usually only be scanned for about 90 minutes. Therefore, the resolution is quite low and small brain areas can not be visualized in detail. To overcome this problem, formaline-fixed animals have been used. Aside from shrinkage artifacts, relatively little structural details are preserved. To overcome this problem, contrast enhancing agents have been introduced, resulting in a considerable gain of resolution. However, this method is very time consuming, since it takes several days or weeks for these substances to penetrate the tissue. We will present a quick method that enables high resolution magnetic resonance imaging of the murine brain without the need of any contrast enhancing agents or other tissue manipulation that could induce artifacts. Nevertheless, it is possible to obtain resolutions in the range of 100 µm (in the z-axis) and 25 µm (in the x- and y-axis) and therefore this method can be used even for the analysis of fine structures within the murine brain.

Supported by the „Forschungsverbund Neurowissenschaften Greifswald“.

Kategorie: Vortrag

Vortrag 47

Rubrik: Zentrales Nervensystem/Signaltransduktion und Verschaltung

Titel: Aromatase inhibition abolishes ltp generation in female but not in male mice

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Abstract:
Inhibitors of aromatase, the final enzyme of estradiol synthesis, are suspected of inducing memory deficits in women. In previous experiments, we found hippocampal spine synapse loss in female mice that had been treated with letrozole, a potent aromatase inhibitor. In this study, we therefore focused on the effects of letrozole on long-term potentiation (LTP), which is an electrophysiological parameter of memory and is known to induce spines, and on phosphorylation of cofilin, which stabilizes the spine cytoskeleton and is required for LTP in mice. In acute slices of letrozole-treated female mice with reduced estradiol serum concentrations, impairment of LTP started as early as after 6 h of treatment and progressed further, together with dephosphorylation of cofilin in the same slices. Meanwhile, 1 week of theta-burst stimulation failed to induce LTP. The effects were confirmed in vitro by using hippocampal slice cultures of female mice. The sequence of effects in response to letrozole were similar in ovariectomized female and male mice, with, however, differences as to the degree of downregulation. The differences seen between males and females, the results from additional in vitro studies, and our finding that ovariectomy does not influence LTP, all point to a role of hippocampus-derived estrogen, rather than to a role of estrogen from peripheral sources, for the LTP and spine synapse stability.

Kategorie: Vortrag

Vortrag 48

Rubrik: Zentrales Nervensystem/Signaltransduktion und Verschaltung

Titel: Dual functions for bcl11b/ctip2 in hippocampal neurogenesis

Autoren: Simon R.(1),Venkataramanappa S.(1),Baumann L.(1),Fischer J.(1),Seigfried F.(2),Andratschke J.(1),Schwegler H.(3),Britsch S.(1),

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Abstract:
Hippocampal structures play an important role in memory and learning. The dentate gyrus, in particular contributing to the formation of new memories, is one of only two brain regions where adult neurogenesis occurs. The development of the dentate gyrus is characterized by distinct phases establishing a durable stem cell pool required for postnatal and adult neurogenesis. Previously we have shown that forebrain-specific ablation of Bcl11b uncovers dual phase-specific functions of Bcl11b demonstrated by feedback control of the progenitor cell compartment as well as regulation of granule cell differentiation, leading to impaired spatial learning and memory in mutants (EMBO J, 2012, 13: 2922-2936). Surprisingly, we identified Desmoplakin, as a direct transcriptional target of Bcl11b. Similar to Bcl11b, postnatal neurogenesis and granule cell differentiation are impaired in Desmoplakin mutants. Re-expression of Desmoplakin in Bcl11b mutants rescues impaired neurogenesis suggesting Desmoplakin to be an essential downstream effector of Bcl11b in hippocampal development. Bcl11b expression occurs also throughout adulthood raising the question of its function in adult neurogenesis. Generating an inducible mouse line using the Tet-off system under the control of the CaMKIIa promoter we further determined a similar role of Bcl11b in adult neurogenesis. Taken together our data define an important novel regulatory pathway in hippocampal development and adult neurogenesis. Moreover, our data presented here establish an essential function for the transcription factor in the maintenance of adult hippocampal function.

Kategorie: Vortrag

Vortrag 49

Rubrik: Neuroanatomie/Neurobiologie

Titel: Neuronal migration and upper layer formation is disturbed in Bcl11a mutant mice

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Abstract:
Our group is interested in the functions of B cell lymphoma 11 genes, namely Bcl11a/Ctip1 and Bcl11b/Ctip2, in the developing central nervous system (CNS). We have previously shown that Bcl11a plays an essential role for morphogenesis of dorsal spinal neurons and also is involved in spinal circuitry formation through the direct regulation of Frzb, a component of the Wnt signaling pathway (John et al., 2012). Moreover, Bcl11a is expressed in the neocortex during development and at postnatal stages. Conditional ablation of Bcl11a in progenitor cells or postmitotic projection neurons leads to a clear decrease of cell numbers in cortical layers 2-4 at postnatal stages. The observed hypoplasticity of upper cortical layers is not due to changes in neurogenesis or an increase of early apoptosis. By using BrdU labeling and in utero electroporation, we instead show that Bcl11a mutant neurons fail to migrate properly into the cortical plate. We observe, that mismigrating neurons show an abnormal morphology, displayed by supernumerary processes, but are still correctly specified. At postnatal stages, apoptosis is dramatically increased in the upper cortical layers of Bcl11a mutant mice suggesting that mutant neurons fail to integrate and are thus eliminated. Collectively, our findings point to an essential role of the Bcl11a gene during migration and integration of cortical projection neurons.

Kategorie: Vortrag

Vortrag 50

Rubrik: Neuroanatomie/Neurobiologie

Titel: Functional involvement of oligodendrocyte specific endoplasmatic reticulum stress in the cuprizone model for multiple sclerosis

Autoren: Victor M.(1),Goldberg J.(1),Krauspe B.(1),Clarner T.(1),Beyer C.(1),Kipp M.(1),

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Abstract:
Oligodendrogliopathy with concomitant microglia activation is a hallmark of Multiple sclerosis (MS) histopathology. Results from our lab suggest that the cuprizone model is a unique tool to study the relation of oligodendrogliopathy and early microglia activation. However, the underlying mechanisms of cuprizone-induced oligodendrocyte loss are still unknown.

The aim of the study was to elucidate pathways involved in this toxin-induced cellular stress response.

Genome wide expression analysis was performed after short term cuprizone intoxication (2 days). Early lesions were additionally analysed by RT-PCR and immunohistochemistry. In separate experiments, animals were injected (i.p.) with the endoplasmatic reticulum (ER)-stress response inhibitor Salubrinal, and oligodendrocyte apoptosis was analysed.

Gene array experiments revealed a dramatically reduced expression of oligodendrocyte specific genes in the corpus callosum after short-term cuprizone treatment. In contrast, the expression of many stress response genes such as the ER-stress related transcripts CHOP, ATF4, ATF3 and TRB3 is highly induced after short-term cuprizone treatment. Immunohistochemistry revealed that CHOP is selectively expressed by oligodendrocytes in this model. Inhibition of the ER-stress responses by Salubrinal, a drug which acts as a specific inhibitor of eIF2α phosphatase enzymes, led to a significant attenuation of cuprizone-induced oligodendrogliopathy.

Our results clearly demonstrate that the selective vulnerability of oligodendrocytes in the cuprizone model is linked to protein synthesis. Further studies will reveal the significance of ER stress in oligodenrocytes for MS lesion development.

Kategorie: Vortrag

Vortrag 51

Rubrik: Neuroanatomie/Neurobiologie

Titel: Overexpression of the small heat shock protein hspb5/alpha-b-crystallin in rat hippocampal neurons results in increased synapse number and enhanced dendritic branching

Autoren: Bartelt-Kirbach B.(1),Schmidt T.(1),Weller M.(1),Golenhofen N.(1),

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Abstract:
Sublethal cellular stress leads to the development of stress tolerance in the brain and other organs, meaning that the cells are more resistant to a second, potentially lethal insult. It is known that multiple effector groups contribute to this effect, one of them being the heat shock proteins.
To further characterize the function of HspB5/alpha-B-crystallin which is upregulated and phosphorylated in the brain after different kinds of stresses we investigated the subcellular localization of unphosphorylated and phosphorylated HspB5/alpha-B-crystallin in cultured hippocampal neurons. We also examined the effect of overexpression of HspB5/alpha-B-crystallin on neuronal morphology. Immunocytochemistry of cultured hippocampal neurons showed that while the unphosphorylated form was localized in the perikaryon and nucleus the phosphorylated forms were recruited into neuronal processes. pHspB5-Ser19 or -Ser45 localized to axons and dendrites with a filamentous-like staining pattern whereas pHspB5-Ser59 was found in dendrites especially along the plasma membrane and in spines. Overexpression of HspB5/alpha-B-crystallin in these neurons by lentiviral transduction led to significantly increased synapse number and enhanced dendritic branching.
These data show that dependent on its phosphorylation state HspB5/alpha-B-crystallin localizes to synapses and neuronal processes. In addition, it influences synapse formation and dendritic branching. Thus, one function of HspB5/alpha-B-crystallin might be to protect dendritic and synaptic structures during pathological situations.

Kategorie: Vortrag

Vortrag 52

Rubrik: Neuroanatomie/Neurobiologie

Titel: Mir-124-regulated rhog reduces neuronal process complexity via elmo/dock180/rac1 and cdc42 signalling

Autoren: Franke K.(1),Schumacher S.(1),Nitsch R.(2),Otto W.(1),Johannes S.(1),Baumgart J.(2),

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Abstract:
The small GTPase RhoG plays a central role in actin remodeling during diverse biological processes such as neurite outgrowth, cell migration, phagocytosis of apoptotic cells, and the invasion of pathogenic bacteria. Although it is known that RhoG stimulates neurite outgrowth in the rat pheochromocytoma PC12 cell line, neither the physiological function nor the regulation of this GTPase in neuronal differentiation is clear. Here we identify RhoG as an inhibitor of neuronal process complexity which is regulated by the microRNA miR-124. We find that RhoG inhibits dendritic branching in hippocampal neurons in vitro and in vivo. RhoG also inhibits axonal branching, acting via an ELMO/Dock180/Rac1 signaling pathway. However, RhoG inhibits dendritic branching dependent on the small GTPase Cdc42. Finally we show that the expression of RhoG in neurons is suppressed by the CNS-specific microRNA miR-124 and connect the regulation of RhoG expression by miR-124 to the stimulation of neuronal process complexity. Thus, RhoG emerges as a cellular conductor of Rac1 and Cdc42 activity, in turn regulated by miR-124 to control axonal and dendritic branching.

Franke K et al (2012) EMBO J 31:2908-21

Supported by the DFG (grants SFB 665-A2 to StS and RN, and SCHU 1406/3-1 to StS)

Kategorie: Vortrag

Vortrag 53

Rubrik: Neuroanatomie/Neurobiologie

Titel: The difference between female and male neurons

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Abstract:
Our recent study (Vierk et al., 2012) revealed a clear cut sexual dimorphism in estrogen-induced synaptic plasticity. After inhibition of neuronal estradiol synthesis a dramatic LTP impairment and as a consequence spine synapse loss, in particular of mushroom spines, were found in females but in males. This result prompted us to investigate spine density and the frequency of spine phenotype in single female and male dissociated neurons after transfection with EGFP-plasmid to visualize spine morphology. Quantification of spine density along the proximal dendrites did not show any difference between dendrites that originated from male and female animals. Classification of spine phenotype, namely mushroom spines and thin spines, however, revealed significant differences between “female” and “male” neurons. “Female” neurons contain remarkably more mushroom spines, which are more stable in comparison to thin spines. Furthermore, they show more AMPA receptors, which make them functionally stronger. Mushroom spines are considered to be ‘memory spines’ (Tada and Sheng, 2006). In contrast, “male” neurons primarily show spines of filopodia-like phenotyp, considered to be immature and highly plastic. This sexual dimorphism under control conditions may account for differences between genders in the regulation of synaptic plasticity in general.

Kategorie: Vortrag

Vortrag 54

Rubrik: Neuroanatomie/Neurobiologie

Titel: Estradiol responsiveness of synaptopodin

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Abstract:
We have previously shown that estradiol regulates synaptic proteins in hippocampal neurons. Synaptopodin is an actin-associated protein, which mainly accumulates in the spine-apparatus of mature spines, characteristic of mature spines. In contrast to other synaptic proteins, synaptopodin is downregulated by estradiol application. In this study we show that inhibition of aromatase by letrozole and the associated reduction in estradiol synthesis in hippocampal neurons results in reduced immunoreactivity of synaptopodin, although to a lesser degree. To find out the underlying mechanism we found by using estrogen receptor (ER) agonists that estradiol-induced downregulation of synaptopodin is mediated by ER-beta. The blockade of ER-beta consistently upregulates synaptopodin. ER-beta, in turn, is upregulated in response to low level of intracellular estradiol, whereas ER-alpha is downregulated. Our data suggest that the autocrine regulation of ER subtypes by their ligands strongly contributes to estrogenic effects in hippocampal neurons.

Kategorie: Vortrag

Vortrag 55

Rubrik: Neuroanatomie/Neurobiologie

Titel: Mifepristone increases the spine density in developing Purkinje cells – new insights in progesterone receptor mechanisms

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Abstract:
Purkinje cells (PC) express the key enzymes of progesterone synthesis, cytochrome P450 side-chain cleavage enzyme, StAR protein as well as 3β-hydroxysteroid dehydrogenase and are able to synthesize progesterone de novo from cholesterol. Interestingly, progesterone reveals an age-dependent expression pattern with high concentrations only during the neonatal period of PCs. The correlation between physiologically high concentrations of intercellular progesterone during the neonate and the simultaneous development of the cerebellar circuit indicates an involvement of progesterone in the maturation process of the cerebellum. Indeed previous studies revealed a progesterone dependent induction of dendritogenesis, spinogenesis, and synaptogenesis in developing PCs, but most of the underlying molecular mechanisms still remain unclear.
By employing life-cell imaging in combination with FRAP experiments , electron microscopic techniques, PCR receptor studies, and blocking experiments we investigated the correlation between progesterone induced effects on the cerebellum and initial maturation grade of the PCs on the one hand and the underlying molecular processes of these effects on the other hand. Indeed we could demonstrate a diverging progesterone effect, depending on the maturation grade of PCs at the beginning of progesterone treatment. According to the physiological course of the progesterone concentration in PCs, treatment with progesterone achieves the highest effects on spine density and dendritic length during the early stages of development. Furthermore we gain new insights in underlying molecular mechanisms of the progesterone receptors, concentrating on well known progesterone receptors A und B (PR-A, PR-B), and the newly described progesterone receptor membrane component 1 (PGRMC1).

Kategorie: Vortrag

Vortrag 56

Rubrik: Neuroanatomie/Neurobiologie

Titel: Fast rearrangement of the neuronal growth cone's actin cytoskeleton following VEGF stimulation

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Abstract:
The neuronal growth cone plays a crucial role in the development of the nervous system. This highly motile structure guides the axon to its final destination and translates guidance cues into cytoskeletal rearrangements. Recently, vascular endothelial growth factor (VEGF), which is essential for angiogenesis and vascular sprouting, has also been found to exert a trophic activity on neurons, leading to an increased axonal outgrowth, similar to the well known nerve growth factor (NGF). The neurotrophic properties of VEGF are likely to be promoted via the VEGF receptor 2 (VEGFR-2) and neuropilin (NRP)-1. In the long term VEGF attracts and influences the growth cone velocity and leads to growth cone enlargement. Additionally the present study focuses on immediate VEGF effects using RFP-actin and GFP-NF-M microinjected chicken dorsal root ganglia for life cell imaging of the neuronal growth cone. We analyzed actin and neurofilament dynamics following VEGF- and NGF-treatment, thus comparing the effects. Additionally key signaling pathways of VEGF were investigated by specific blocking of VEGFR-2 or NRP-1. With aid of confocal laser scanning microscopy and STED-microscopy we showed for the first time that VEGF has an instantaneous effect on the actin-cytoskeleton, since actin rearrangements were identifiable within a few minutes, leading to a dramatically increased motion. Moreover, these effects were multiplied by adding both VEGF and NGF, however inhibited by blocking VEGFR-2. Therefore we propose that the immediate effects of VEGF on the actin-cytoskeleton are mediated through VEGFR-2.

Kategorie: Vortrag

Poster 1

Rubrik: Klinische Anatomie/Makroskopie

Titel: Case report „lusorian artery“ aberrant retrooesophageal right subclavian artery

Autoren: Hoermann R.(1),Fritsch H.(1),

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Abstract:
The clinical syndrome of dysphagia in association with the aberrant right subclavian artery was first described by Bayford in 1794 as lusus naturae, meaning a freak or jest of nature. Nowadays many vascular variations are found with different imaging techniques. During the educational dissection course 2010/11 for our students we detected an A. lusoria in an 85 years-old woman who died from right-heart failure. This ist the most common of the aortic arch anomalies (1-2 %). Having found this case of arterial anomaly we changed the dissection program to get a demonstration sample for this variation. We isolated the vascular situation and subsequently did HDR imaging to get a high qualitiy foto documentation. The lusorian artery starts dorso-caudally from the aortic arch and runs up behind the trachea and oesophagus and through the interscalene triangle for a distance of 82mm.
A non-recurrent laryngeal nerve was also observed, a common combination in such cases. For cervical surgery as well as for anaesthesia a profound knowledge of anatomical structures and their variations is absolutely essential.

Kategorie: Poster

Poster 2

Rubrik: Klinische Anatomie/Makroskopie

Titel: Is cerclage wiring in humeral fractures a safe procedure? An anatomical study

Autoren: Tanzer K.(1),Grechenig S.(1),Grechenig C.(1),Dreu M.(2),Dolcet C.(2),Tesch N.(2),

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Abstract:
The incidence of humerus fractures appears to be increasing as a result of patients longevity. Poor bone quality is the most common risk factor for fractures of the humerus. Also periprosthetic fractures during and after shoulder arthroplasty are becoming more often. Currently there is little information available on the treatment of these fractures with Cerclage wiring.
The fracture itself and the use of metal wires or other metal work is associated with damage of the radial nerve. Cerclage wiring is often needed for reduction or only just for fixation. The aim of our study was to identify the risk of potential damage of the radial nerve, when using cerclage wiring in humeral fractures There are no recent studies about the course of the radial nerve in relation to the optional placement of cerclages in the humeral shaft region and no literature about a standard procedure of setting cerclages in this anatomical region. This cadaver study assessed how often the radial nerve was trapped during setting cerclages on the humerus. Cerclages in 14 cadaveric arms were placed. We used the anterior approach to the humerus and a defined area and placement.
Dissection of the upper limbs showed radial nerve injury in only one of the 42 placed cerclages.
We recommend insertion of the Cerclage only after careful dissection down to the bone and remaining adjacent to the bone during the whole procedure.

Kategorie: Poster

Poster 3

Rubrik: Klinische Anatomie/Makroskopie

Titel: Anatomical evaluation of the anterior and posterior interosses nerve of forearm based on development of surgical procedures

Autoren: Dreu M.(1),Dolcet C.(1),Grechenig W.(2),Grechenig S.(3),Tesch N.(1),

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Abstract:
Background:
The anatomical evaluation of the anterior and posterior interosseus nerve and their correlation and course to muscles, arteries and the interosseus membrane of forearm have not been recorded in detail in the literature. Now the evolution of modern surgical techniques requires exact investigation in this field, to cite an example a procedure to reach both nerves for denervation with one surgical access.

Material and Methods:
Twenty upper limbs of human cadavers, embalmed with Thiel's method and in good axial alignment were used for measurement of specified points.
Main point of the investigation was the measurement of the distance of both nerves to the ulnar interosseus border. This was done by 2 cm steps along their course on the interosseus membrane. Starting point for axial measurement was the radial styloid process.

Results:
The distance of the ulnar interosseus border to the anterior interosseus nerve was between 0.4 to 1.4 cm (middle 0.96 cm), to the posterior interosseus nerve 0.3 to 1.4 cm (middle 1.0 cm). In 11 cases a congruent area of both nerves at their course on the interosseus membrane was found. Concerning the relation to muscles and arteries different groups of courses can be classified.

Conclusion:
The results suggest a frequent congruence of both nerves at a specific area of the interosseus membrane. So a simple locating of them should be possible.
However, an expansion of this investigation including more specimens is necessary to provide statistical significant statements.

Kategorie: Poster

Poster 4

Rubrik: Klinische Anatomie/Makroskopie

Titel: The course of the anterior and posterior interosseus nerve of forearm in relation to adjacent structures

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Abstract:
Background:
The course of the anterior and posterior interosseus nerve of forearm and their relation to muscles and arteries has not been examined in detail so far. A precisely description of these structures is required not only for the sake of completeness also for surgical use.

Material and Methods:
The ongoing examination involves twenty upper limbs of human cadavers, embalmed with Thiel's method and without obvious signs of interventions and pathologies in the area of interest.
The main points of data collection of the anterior interosseus nerve are: the relation to the anterior interosseus artery (AIA) and the crossing point with the proximal border of the pronator quadratus. The evaluation of the posterior interosseus nerve includes the point of exit of the supinator, the relations to the extensor muscles and the anterior interosseus artery.
Results:
The relation of the anterior interosseus nerve to the AIA shows three groups with one, two or no crossing points. In more than 50 % one crossing point was found.
The posterior interosseus nerve passes through the extensor compartment in two different ways. In 18 cases the nerve intersects the abductor pollicis longus and extensor pollicis brevis, in 2 cases it penetrates these muscles and in all cases extensor pollicis longus is crossed below. In all cases the AIA is located ulnar of nerve.

Conclusion:
A certain regularity in these nerves courses in relation to adjacent structure can be recognized. Though a higher number of specimens is required to provide statistical significance.

Kategorie: Poster

Poster 5

Rubrik: Klinische Anatomie/Makroskopie

Titel: Development of a new anatomically preformed osteosynthesis plate of the distal humerus by means of ct-aided measuring of the humerus – a new technique of implant development

Autoren: Grechenig S.(1),Tesch N.(1),Grechenig C.(2),Dreu M.(1),Dolcet C.(1),Pichler W.(2),

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Abstract:
Background:
The aim of this study was to develop an anatomically preformed plate for the osteosynthesis of the distal humerus with the aid of CT-scans of the human elbow joint. The project was carried out in conjunction with the company MEDARTIS® AG (Basel, Switzerland).
Method:
With the aid of a special software called MIMICS measurements of diverse points in the CT-scans of the distal humerus, that had been agreed upon beforehand, were taken.
The results were processed statistically and then divided into three groups based on the size of the original humerus (small, medium, large). From each group one humerus was selected, which was closest to the average. With the aid of MIMICS three-dimensional reconstructions of the humeri of each group were prepared. The outline and form of the bone surface that had direct contact to the plates was extracted. Based on these data CAD-files were calculated, which then served as models for the construction of plastic prototypes of the plates.
These prototypes where then tested on 100 human specimen to see how well the plates fit the bones.
Results:
The prototypes of the plates fit with high accuracy to over 90 % of the specimen in all three sizes. The prototypes of medium size showed the highest accuracy compared to the other two size types.
Due to this circumstance the medium sized prototype was selected as model for the anatomical preformation of the definitive plate system.
Conclusion:
This method of development of anatomically preformed osteosynthesis plates seems to be highly effective. The new elbow-plate system will be put on the market by the end of the year 2012.

Kategorie: Poster

Poster 6

Rubrik: Klinische Anatomie/Makroskopie

Titel: Is dorsal mipo plating of the tibia safe- an anatomic study

Autoren: Tesch N.(1),Grechenig S.(1),Grechenig C.(2),Dreu M.(1),Dolcet C.(1),

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Abstract:
INTRODUCTION: With the introduction of Locking Compression Plates (LCP), Minimally Invasive Plate Osteosynthesis (MIPO) has become widely used. The dorsal side of the tibia offers good soft tissue conditions and a possibility for plate fixation.

OBJECTIVES:The aim of this anatomic study was to develope a new and safe technique of minimal invasive plateosteosynthesis on the dorsal side of the tibia indicated for tibia shaft fractures.

METHODS: 10 paired adult lower limbs preserved with the method of Thiel [5] and the twelve hole LCP-plate (PHILOS) from Synthes were used. A five cm long skin incision over the proximal tibia was made for the insertion of the plate. Distaly a three cm long skin incision was made over the dorso medlial edge of the tibia and the soft tissue was mobilised. After that, the LCP-Philus plate was inserted proximally under direct bone contact. The plate was fixed with two self drilling and tapping screws and after that, the specimens were disected layer after layer to identify the relation of the neurovascular bundle to the plate.

RESULTS: The neurovascular bundle had an average distance to the plate at hole number six of 13mm(1-1.7cm) and at hole number ten of 11mm (0.6-2cm). The M.flexor digitorum longus had its origin along the hole plate (hole1-hole12) and was between the plate and the neurovascular bundle in all ten cases.

CONCLUSION: We conclude that this a safe and easy method of osteosynthesis whereas changed anatomical conditions due to severe tibia shaft fractures can make this procedure difficult.

Kategorie: Poster

Poster 7

Rubrik: Klinische Anatomie/Makroskopie

Titel: Shaft fractures of the radius – ideal positioning of fixation plates with regard to the anatomical situation

Autoren: Clement H.(1),Grechenig S.(2),Dolcet C.(2),Dreu M.(2),Grechenig C.(1),Tesch N.(2),

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Abstract:
Purpose:
In the treatment of shaft fractures of the radius in the central third of its extension the standard techniques applied are either the volar approach after HENRY or the dorso-lateral approach after THOMPSON. The usually applied standard implant (DCP-plate, 3,5 mm) is examined with special consideration of its fit to the anatomical situation.
Material and Method:
In 20 cadavers preserved with Thiel's method, the soft parts were removed to show the radius shaft and mounted plates. Photographs of the shaft were taken and the fit of the plates on the palmar and dorsal sides respectively was examined.
Results and Conclusion:
Due to the anatomy of the radius shaft a straight plate fits considerably better to the dorsal side than to the palmar side. This fact should be taken into consideration and the dorsal fixation should be favoured especially when using conventional systems.

Kategorie: Poster

Poster 8

Rubrik: Klinische Anatomie/Makroskopie

Titel: Femur lcp-df plates (liss);a safe method with regard to injuries of the fibular collateral ligament

Autoren: Freimoser F.(1),Grechenig S.(1),Grechenig C.(1),Dolcet C.(2),Dreu M.(2),Tesch N.(2),

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Abstract:
Introduction:
Shaft fractures of the thigh bone, distal fractures of the femur and periprothetic fractures are the decisive factors when considering the use of an LCP-DF plate by SYNTHES, which can be attached by means of the LISS osteosynthesis method.
Purpose:
The aim of this study was to determine whether the origin of the fibular collateral ligament could be injured owing to the attachment of a LCP-DF plate.
Material and Method:
For the study 14 lower extremities (7 right ones, 7 left ones) preserved with THIEL's solution were used.
A 9 hole LCP-DF plate by SYNTHES and self drilling locking screws were used in the osteosynthesis. Via a minimal invasive approach above the lateral condyle of femur the plate was fixed. Radiographical imaging (anterior-posterior and lateral) was used to visualize the correct positioning of the plates.
After the fixation of the plates with locking screws, the extremities were dissected layer by layer with special consideration of the origin of the fibular collateral ligament. The plates were then removed and the distance between the origin of the fibular collateral ligament and the most distal locking screw of the LCP-DF plate was measured by means of a slide caliper.
Results and Conclusion:
The shortest distance between the origin of the fibular collateral ligament and the most distal locking screw was 10 mm, the average distance being 14 mm. If the anatomical situation is taken into consideration while repositioning the femur and if the plates are attached in the correct position, osteosynthesis using LCP-DF plates does not lead to injuries of the origin of the fibular collateral ligament.

Kategorie: Poster

Poster 9

Rubrik: Klinische Anatomie/Makroskopie

Titel: Morphologic pattern of the superior mesenteric artery branches

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Abstract:
The anatomy of the superior mesenteric artery branches were examined in situ on 25 formolized human bodies, using macroscopic dissection. Only in 35% of cases the classic anatomic description was confirmed. Of equal frequency, the classic pattern of the superior mesenteric artery was that in which the ileocolic artery arose separately and the right and middle colic arteries arose together in a common trunk, in 29% of cases. The inferior pancreaticoduodenal artery was present in all 25 cases dissected. The small intestinal arteries (for ileum and jejunum), also were found in all cases in the same pattern. The ileocolic artery, which supplies the last part of ileum, caecum and appendix, was found in over 90% cases as arising separately. The right colic artery (that supplies the ascending colon) always was found as making a common trunk with others arteries and never being found alone. The middle colic artery (that supplies the transverse colon) also was found going together in a common trunk with the right colic artery. The literature data say that right colic is a single vessel in 78% of individuals, and arises independently from the superior mesenteric in only 28% of the population, but we did not find it alone. It could be absent in 16% of individuals, as the literature states, but either this we did not find it also.Keywords: middle colic artery, pattern, ileum.

Kategorie: Poster

Poster 10

Rubrik: Klinische Anatomie/Makroskopie

Titel: Liposarcoma - histopathological and anatomo clinical issues

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Abstract:
Liposarcoma is a tumour of adipose tissue rather unusual among soft tissue tumours, its variants accounting for approximately 1% of all tumours. Account for 15% of adult sarcomas, the prognosis is closely related to its location and histological type tumour as well. There are known several histological subtypes. One of them, more frequent, is myxoid liposarcoma. In two thirds of cases the tumour occurs in muscle, and it could be easily confused with other benign tumours that grow at this level. We present a 52-year old woman case that came to clinic with a tumour located in upper third of the right anterior thigh. Clinically the tumour was firm, adherent, painful, having 12/15/13 cm diameters. The arterial pulse was present on the pediosa artery and right posterior tibial artery. CT showed it looks like a solid tumour with mixed adipose dense structure in the medial compartment of the right thigh, anterior to the femoral artery, without changes in the adjacent bone structure. There were no thoraco-abdominal or pelvic metastases. Peritumoral vascular pedicles are ligated and the tumour was excised with oncologic safety limits. Histopathology shows a myxoid liposarcoma. Patient undergoes chemotherapy and radiation therapy.
Keywords: liposarcoma, tumour, chemotherapy.

Kategorie: Poster

Poster 11

Rubrik: Klinische Anatomie/Makroskopie

Titel: How to evaluate cellular and subcellular effects of ex vivo lung perfusion quantitatively in a large animal model? Technical procedures to obtain high quality samples for stereological analysis

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Abstract:
A major obstacle in lung transplantation is a serious shortage of donor organs since up to 80% of the potential donor lungs are considered unsuitable according to current selection criteria. For clinical evaluation and reconditioning of marginal donor lungs, techniques of ex vivo lung perfusion (EVLP) were developed (Wierup et al., Ann. Thorac. Surg. 2006; 81:460-466; Cypel et al., J. Heart Lung Transplant 2008; 27:1319-1325).
Here we describe a protocol of technical procedures we have developed in order to obtain unbiased measurements on the light and electron microscopical level to study the effects of EVLP in porcine lungs.
The lungs of 5 pigs (body weight 54 +/- 7 kg) were explanted. During surgery a protective ventilation mode was applied using defined ventilation parameters. Upon explantation, the trachea was blocked at an inflation pressure of 15 hPa. After a defined period of cold ischemia (24 h), the lungs were subjected to EVLP for 12 h. In the EVLP-circuit both ventilation (Vt, f, PEEP, Pmax) and perfusion parameters (Flow/min, pulmonary artery pressure, left atrium pressure) were tightly controlled. At the end of the EVLP period the trachea was blocked again with the lungs inflated at 15 hPa and the total organs were perfusion fixed with glutaraldehyde/paraformaldehyde using a perfusion pressure of 30 hPa. A cascade sampling design for stereology followed which included a slicing tool specifically devised for pig size lungs and size adapted sampling grids.
Both light and electron microscopical sections had a very high quality and ensured accurate stereological analysis.

Kategorie: Poster

Poster 12

Rubrik: Klinische Anatomie/Makroskopie

Titel: A comparison of the porcine and human glottis with emphasis on the elastic fibres

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Abstract:
Introduction
The porcine glottis contains two folds, a cranial (CraF) and a caudal (CauF) fold on each side. Often, the CauF is taken as the equivalent of the vocal fold of man; however, preliminary studies have revealed distinct histological differences. In this paper, the local distribution of elastic fibres within both folds shall be described in order to challenge the pig's suitability as an animal model.

Methods
Histological cross sections of the glottis of 8 minipigs (female, approx. 1 year old; resorcin-fuchsin stain) were studied histomorphometrically by use of standardised colour values in a semi-automatic process.

Results
CraF and CauF had one feature in common: directly next to the epithelium, there was a distinct, approx. 50 µm wide elastic layer. Here, very densely packed elastic fibres made up to 15% of the respective tissue area. Underneath this distinct layer, elastic fibres decreased markedly: In the CraF, the values went down from 10% to 3%. In the CauF, the values were very heterogeneous without any stratigraphical order; they ranged around 7%, sometimes with highest values (10%) in deeper regions of the CauF, and 5% in upper regions of the CauF, or vice versa.

Discussion
Apart from the subepithelial elastic layer, no distinct elastic stratification was seen either in CraF or in CauF. There was no indication of a marked elastic fibre concentration in terms of what is seen in the human as a so-called free, upper edge of the Conus elasticus. In other words, there was no predominantly elastic vocal ligament.

Kategorie: Poster

Poster 13

Rubrik: Klinische Anatomie/Makroskopie

Titel: Variant location of fork of median nerve with concomitant arterial variations of axillary or brachial artery

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Abstract:
Variations in the architecture of the brachial plexus not seldom concern the location of the fork of the median nerve. Here we report on forks of median nerve which cover variant branches of axillary or brachial artery. Among 31 arms, evaluated during the gross anatomy course of Rostock Anatomical Institute in summer term 2012, variant locations of the fork of median nerve were detected in 7 specimen (23%).
In the right arm of a 94-year-old female cadaver, axillary artery divided into three main branches. Branch 1 continued into the brachial artery. Branch 2 showed a behaviour like a superior ulnar collateral artery. This branch was covered by the fork of the median nerve. Branch 3 was a common trunk giving origin to posterior circumflex humeral artery and circumflex scapular artery. Surprisingly, at the left arm of this individual, a variant fork of the median nerve covered an artery which also behaved like a superior ulnar collateral artery. However, here the variant artery took its origin from the brachial artery. Additionally, both arms were characterized by superficial ulnar arteries.
Thus, the knowledge of relationship of the brachial plexus with arterial variations of the axillary or brachial artery is paramount to ensure safe and successful regional anesthesia of upper extremity.

Kategorie: Poster

Poster 14

Rubrik: Klinische Anatomie/Makroskopie

Titel: Histological degeneration signs in temporomanibular joint discs

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Abstract:
Introduction:
The objective of this study was to evaluate the influence of dental status on degeneration of the articular disc of the temporomandibular joint (tmj)
Material and Methods:
30 tmj discs were harvested of 15 human body donors (mean age 79.4 years). Three groups were created: (1) fully toothed (n=6), (2) partially toothed (n=5) and (3) teeth less (n=4) and investigated in three regions (cranial, centre and caudal in axial plane) with twelve measuring points each region. Histological analysises were performed with hematoxylin-eosin staining. Chondrocytes, fibroblasts and tears were counted.
Results:
There were no difference in chondrocyte numbers in the three investigated regions and between the groups. Group 1 showed 2 fibroblasts each measuring point; however, there was an increase of fibroblasts (4 each measuring point) in the cranial and caudal region in group 2 and 3.
Tears occurred in 96 % and 95 % of partially tooth and tooth less discs. 78.1 % of fully toothed discs showed tears.
Conclusion:
An increasing tooth loss may predict destructions of the tmj discus.

Kategorie: Poster

Poster 15

Rubrik: Klinische Anatomie/Makroskopie

Titel: Comparative study regarding the angiogenesis in both tumoral and nontumoral tissues at the level of the female genital apparatus

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Abstract:
Angiogenesis represents the process through which new vascularisation is formed from the pre-existing vascularisation a process that begins during the foetal life, being the support for tissue development. Under normal conditions angiogenesis is initiated during certain physiological processes (ovulation, menstrual cycle, pregnancy, wound healing) or pathological processes such as inflammation, tumoral growth and metastasis.
The present study is a synthesis of a comparative analysis of the angiogenic process on 25 cases of endometrial adenocarcinoma and 55 cases of placentas in different maturity stages that came from births where the newborns didn’t have macroscopically detectable development disorders.
The technique used was the immunohistochemical technique that used the CD34 monoclonal antibody as a marker. The afore mentioned antibody belongs to the second class of monoclonal antibodies that recognize a CD34 epitope neuraminidase resistant, glucoprotease and chymopapain sensitive. Although it was initially described a hematopoietic stem cell specific antigen CD34 is a specific marker of the endothelial cell in vascular placental proliferation but also noticed in different types of cancers.
Our study confirms the utility of the CD34 marker for the positive diagnosis in tumoral pathology through distinguishing the neovascularisation the CD34 maker also being expressed in nontumoral tissue distinguishing the vascular cytoarchitectural changes at placental level.
Key words: anti CD 34, angiogenesis, placenta, adenocarcinoma

Kategorie: Poster

Poster 16

Rubrik: Klinische Anatomie/Makroskopie

Titel: Study of the incidence of the avascular necrosis of the femoral head

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Abstract:
Introduction
Avascular necrosis of the femoral head (AVN) becomes a frequent cause of musculo-skeletal disability, which raises a lot of diagnostic and treatment problems. Initially, the patients are asymptomatic, but the progression of the avascular necrosis of the femoral head is to the destruction of the joint, and the patients usually needs total hip replacement before the 5th decade of life.
Patients and method
The study was elaborated on 71 patients (62 men and 9 women) from the UKT Hospital in Tuebingen, between 2005 and 2008, which presented vascular necrosis of the femoral head and were investigated through x – ray and magnetic resonance imaging.
Discussion
Study group is not homogeneous for an extended population area. For the result to be relevant for an important population area there would be necessary similar studies in Central and Eastern Europe.
Necrotic lesion is situated on the superior, medial and anterior areas of the femoral head. In these cases there is an intact part of healthy bone which protects the femoral head by destruction by his own weight. These hips have a better future then the hips where the lesion is included in the acetabulum limits.
Conclusions
Magnetic Resonance Imaging (MRI) is the best investigation for the patients who have risk factors associated (steroid therapy, fracture of the femoral head) and simptoms that can lead to AVN, but normal radiographs. For this reason we suggest a MRI screening to all these patients.

Kategorie: Poster

Poster 17

Rubrik: Neuroanatomie/Neurobiologie

Titel: Cytoarchitecture and mapping of the dorsal striatum of the human brain

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Abstract:
The dorsal striatum, composed by the putamen and the caudate nucleus, is involved in psychomotor behavior and decision making, through the integration of sensory, motor, cognitive and emotional information. Brockhaus [1] suggested a detailed structural segregation of the dorsal striatum into different components. This segregation, however, is not reflected in the current neuroimaging literature although it can be expected that these components are functionally relevant. In addition, there is currently no data available describing the localization of these components in 3D and their inter-subject variability. We examined therefore serial histological sections of ten adult post-human brains, which were stained for cell bodies. Twelve striatal components, five for the caudate nucleus, four for the putamen, and three for the caudoventral striatum were mapped based on differences in the density of the neurons. The ventral striatum with the fundus differed from the dorsal part mainly by a higher density of neurons.
The cytoarchitectonic analysis revealed a highly complex architecture and multiple components of the human dorsal striatum. The borders of these components were traced in images of histological sections using in-house software, spatially normalized to the MNI reference space, and 3D-probabilistic maps were generated [2]. Striatal components do not follow macroscopic landmarks and are not visible in routine MR images. Thus, our 3D-probalistic maps provide a new and promising tool to localize data from in vivo MR imaging of the healthy and pathologically altered brain.
[1]Brockhaus (1942) J Psychol Neurol 51:1-56
[2] Amunts et al. (2005) Anat Embryol 210: 343

Kategorie: Poster

Poster 18

Rubrik: Neuroanatomie/Neurobiologie

Titel: Pannexin1 expression in the developing mouse embryo

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Abstract:
Pannexins are similar in structure to gap junction proteins, connexins. The pannexin family has three members (Panx1, 2 and 3). Pannexin1, a protein with four transmembrane domains, forms channels in single membranes and thereby allows the passage of ions and signaling molecules between the cytoplasm and extracellular space. Panx1 shows a broad expression in the nervous system and other tissues.

We are interested in Panx1 function during development. Thus, we investigated Panx1 mRNA and protein expression in the mouse embryo, using whole mount in situ hybridization and immuno-histochemistry, respectively. In addition, quantitative analysis of Panx1 protein was performed by Western blot.

Our results show Panx1 mRNA and protein expression in the central and peripheral nervous system from embryonic day (E) 9 onwards. Expression sites include the mantle layer of the neural tube, the otic vesicle, as well as cranial and dorsal root ganglia.

Thus, Panx1 expression was demonstrated at distinct sites of the mouse embryonic nervous system and provide a first hint of Panx1 function during development.

Kategorie: Poster

Poster 19

Rubrik: Neuroanatomie/Neurobiologie

Titel: Expression of neurotransmitter markers in the brainstem of an Alzheimer mouse model

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Abstract:
Introduction: Changes in cholinergic and monoaminergic neurotransmission in Alzheimer's disease (AD) patients may contribute to the clinical manifestation of this most common form of dementia. The early occurrence of apathy and depression suggests the involvement of catecholaminergic and serotoninergic brainstem nuclei in the initial pathogenesis of AD. To address the question whether P301L tau-transgenic pR5 mice model the degeneration of neurotransmitter systems observed in AD brainstem, the pattern of cholinergic and monoaminergic neurons was investigated.

Material and Methods: Coronal brainstem sections of 20-months-old P301L tau-transgenic pR5 mice and gender- and age-matched non-transgenic littermates were assessed semiquantitatively regarding localization and number of cholinergic, catecholaminergic and serotonergic neurons, visualized by means of immunohistochemistry.

Results: We found no obvious differences in the distribution and number of neurons immunoreactive for choline acetyltransferase, tyrosine hydroxylase and serotonin between tau-transgenic and wild type animals. Moreover, the density of cholinergic and monoaminergic nerve cells in brainstem nuclei affected by pathological changes of the human tau protein appeared to be similar to the situation in non-transgenic littermates.

Conclusion: While tau pathology affected monoaminergic nuclei in the brainstem of AD patients show a distinct neuronal loss, the numbers of cholinergic and monoaminergic neurons in P301L tau-transgenic pR5 mice were unchanged. Therefore the influence of brainstem tau pathology on neurotransmitter systems and subsequent clinical features is an aspect for that the pR5 model seems to be less suitable.

Kategorie: Poster

Poster 20

Rubrik: Neuroanatomie/Neurobiologie

Titel: P75ntr-deficient mice display increased cholinergic innervation of the amygdala and subtle behavioral impairments

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Abstract:
The p75NTR is a low affinity receptor for all (pro-)neurotrophins and can influence a variety of intracellular signaling pathways, resulting in diverse possible effects including apoptosis, cell survival and differentiation. We analyzed two different lines of p75 knockout mice: p75ExonIII, which are still expressing s-p75NTR, a splicing variant of p75NTR missing the neurotrophin binding site, and p75ExonIV, representing a total knockout. Since p75NTR is highly expressed in cholinergic neurons, several studies investigated the effects of p75NTR deficiency on the basal forebrain, but reported inconclusive results. Therefore, we focused on one of the main projection targets of the basal forebrain by measuring the density of ChAT-positive fibres in the basolateral amygdaloid nucleus. We found that both p75ExonIII and p75ExonIV knockout mice show a significant increase in cholinergic innervation. To investigate whether p75NTR deficiency has an impact upon behavior, we designed a series of behavioral tests, including Open Field, Dark/Light Box, T-Maze, Holeboard, Morris Water Maze and Fear Conditioning. We only analyzed p75ExonIII mice, since p75ExonIV had to be precluded due to severe ataxia, which is familiar with this line. These knockout mice show increased locomotor activity, altered exploratory behavior and decreased anxiety in Open Field and Dark/Light Box. Performance in the Water Maze is impaired in the probe trial. Thus, structural alterations in the forebrain of p75NTR deficient mice (as e.g. increases in the cholinergic innervation) translate into behavioral peculiarities.
Supported by the DFG (BO 1971/5-1).

Kategorie: Poster

Poster 21

Rubrik: Neuroanatomie/Neurobiologie

Titel: Effects of prg-1 on sensory and motor skills - behavioral and morphological results

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Abstract:
Plasticity-related gene 1 (PRG-1) is a membrane-associated lipid phosphate phosphatase, which is located postsynaptically in glutamatergic synapses. It is involved in axonal outgrowth during development and regenerative sprouting. Deficit of this integral membrane protein causes epileptic seizure in young mice. Moreover hyperactivity and lower ability to handle sensory stimuli are discussed.
To test the hypothesis of hyperactivity and sensory processing disorder we behaviorally tested homozygous PRG-1 knockout (PRG1KO) animals in comparison to their wild type littermates (WT). Motor skills were analyzed by a Rota-Rod (increasing speed 4-40min-1 in 5 min) and beam walk on different beams (20, 14, 10, 8, 5mm width). Nociception was estimated using a hot plate test at 55°C. After the hot plate test we analyzed c-fos activation in selected brain areas by using immunohistochemistry.
The PRG1KO exposed a significantly higher Rota-Rod performance. This shows that they are adapted to higher motility and should support the thesis of hyperactivity. Furthermore PRG1KO a significantly slower running speed and higher number of slippings and changes of direction on the beam walk. Also this indicates a higher motility and a lower coordinating ability of PRG1KO. The hot plate test shows no striking differences, except for a shorter reaction time of WT at the fourth trial. The following morphological analysis of brain regions and spinal cord reveals a significant lower number of c-fos immunopositive cells in the cortex of PRG1KO mice. Hence PRG1KO do not have deficits in pain conduction, but that they have a delayed processing.

Kategorie: Poster

Poster 22

Rubrik: Neuroanatomie/Neurobiologie

Titel: Activity regulates axon initial segment development in rodent visual cortex in vivo

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Abstract:
During visual cortex development, neurons undergo sensory input-driven periods of plasticity. Previous studies have demonstrated that the neurochemical phenotype and soma size of visual system neurons remain plastic during early postnatal development. One fundamental cellular microdomain that has so far not been studied in this context is the axon initial segment (AIS), the site of action potential generation. Recent studies have unraveled its activity-dependent development. We hypothesized that the AIS is dynamically regulated during development and maturation of visual cortical neurons, and analyzed morphological changes in AIS length development in mice (E14 to P67) using confocal imaging of ankyrin-G, a key scaffolding membrane adaptor which plays a central role in AIS development and maturation.
We observed a continuous shortening of the AIS in cortical layers II/III and V between embryonic stages and adulthood. A significant step in this shortening occured between P7 and P28. To determine whether visual activity is required for AIS maturation, we deprived vision by keeping age-matched mice in darkness for one week. Interestingly, upon sensory deprivation, the developmental AIS shortening occurred later (P28 to P67) in cortical layers II/III. However, sensory deprivation did not prevent the early AIS shortening in layer V neurons. This suggests that under sensory deprivation, the critical periods for influencing AIS length differ in these two cell populations, possibly because layer II/III neurons are ontogenetically younger. Our results indicate that neuronal activity influences AIS development, pointing to a novel mechanism of how this epigenetic factor potentially alters cellular excitability.

Kategorie: Poster

Poster 23

Rubrik: Neuroanatomie/Neurobiologie

Titel: CPB-K mice show differences in dopaminergic and serotonergic hippocampal innervation compared to BALB/cJ mice

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Abstract:
Schizophrenia is characterized by disturbances in social-, cognitive-and sensorimotor function, which may be caused by different transmitter systems. Beside alterations in cortical- and subcortical areas, reduced AMPA-, NMDA-, 5-HT2-receptor densities and increased 5-HT1-receptor densities are found in the hippocampus. The two inbred mouse strains CPB-K and BALB/cJ display considerable differences in cognitive function, social interaction and prepulse inhibition, a stable marker of sensorimotor gating. Furthermore, CPB-K mice exhibit lower NMDA-, AMPA- and increased 5-HT-receptor densities in the hippocampus, compared to BALB/cJ mice. We investigated both mouse strains with immunocytochemical approaches for differences of dopaminergic and serotonergic parameters. CPB-K mice, compared to BALB/cJ, showed differences in the number of serotonin transporter-positive neurons and volume of raphe nuclei and a lower serotonergic fiber density in ventral and dorsal hippocampus. No alterations of dopaminergic markers in substantia nigra and ventral tegmental area were found. CPBK-mice displayed a significantly higher dopaminergic fiber density in dorsal hippocampus, ventral hippocampal CA1 and dentate gyrus. No differences in the amygdala were found. Based on our results and previous studies, CPB-K mice compared to BALB/cJ may serve as an important model to understand the interaction of the serotonergic and dopaminergic system and their impact on sensorimotor gating and cognitive function as related to neuropsychiatric disorders.

Kategorie: Poster

Poster 24

Rubrik: Neuroanatomie/Neurobiologie

Titel: Specific afferences of GABAergic neurons in hippocampus and amygdala in GAD67-GFP mice

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Abstract:
In the present study we looked for morphological differences between GAD67-GFP mice, which are characterized by replacement of one GAD67 allel by the green fluorescent protein gene and their wildtype littermates. The haplodeficiency results in a reduction of the GAD67 activity and may cause disturbances of closely related neurotransmitter systems i.e. the cholinergic, serotonergic and dopaminergic. Using quantitative immunohistochemistry against ChAT, 5-HT and TH we counted the numbers of serotonergic, dopaminergic and cholinergic neurons in their regions of origin and the densities of serotonergic and dopaminergic fibers in hippocampus and amygdala. Compared to wildtypes, GAD67-GFP mice showed a tendency to a lower density of choline acetyltransferase-positive neurons and a significantly higher volume of the medial septal region. GAD67-GFP mice displayed a lower density of serotonergic neurons in dorsal raphe nucleus, but only small differences in numbers of dopaminergic neurons in substantia nigra and ventral tegmental area. Furthermore, both genotypes displayed no striking differences in specific fiber densities in hippocampus and amygdala. In conclusion, we found that GAD67-GFP mice and wildtypes show a comparable morphology in their cholinergic, serotonergic and dopaminergic transmitter systems. Therefore, these mutants are a useful tool for studying the connectivity of GABAergic neurons in ascending modulatory fiber systems.

Kategorie: Poster

Poster 25

Rubrik: Neuroanatomie/Neurobiologie

Titel: Dcx, betaiii-tubulin, vimentin and gfap: immunohistochemical markers for neurogenesis and gliogenesis in the developing chicken optic tectum

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Abstract:
In this study we analyzed the distribution of DCX, Tuj-1, vimentin and GFAP between E3 and E20, thus covering the whole embryonic development of the chicken optic tectum (OT), including proliferation, migration and maturation of the neural cells. These immunohistological markers are known to be critical for distinct steps during neurogenesis (DCX, Tuj-1) and gliogenesis (vimentin, GFAP). Whereas DCX is expressed in neuronal precursors and young neurons, Tuj-1 is synthesized in committed intermediate precursors and postmitotic neurons. Vimentin is known to be expressed in radial glial cells (RGC) and later on during development also in astrocytes, in contrast to GFAP that is expressed in these cells at later time points.
In the chicken OT neurogenesis starts at E3 with a prominent DCX and moderate Tuj-1 expression. Typical RGC can be identified by a prominent vimentin signal from E6, which build the scaffold for migrating young neurons. At E12 vimentin is expressed in RGC and additionally in scattered, immature astrocytes. GFAP is also weakly expressed within these same cells. The decreasing immunohistological staining of DCX following E12 and the vanishing vimentin expression around E20 corroborate the assumption that neuronal proliferation mainly takes place between E3 and E6. This step is followed by migration, which ends around E20, when RGC have been replaced by GFAP positive astrocytes. This immunohistochemical data is verified by electron microscopic analysis of critical stages of OT development. Altogether these observations confirm and deepen our knowledge about neural development in the chicken optic tectum.

Kategorie: Poster

Poster 26

Rubrik: Neuroanatomie/Neurobiologie

Titel: Impact of the p75 neurotrophin receptor on age-related morphological alterations in the hippocampal formation

Autoren: Poser R.(1),von Bohlen und Halbach O.(1),

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Abstract:
P75NTR is capable of binding all known types of neurotrophins and can mediate diverse effects on cell differentiation (such as e.g. synaptogenesis), cell survival and apoptosis. To examine the roles of p75NTR, we used two different lines of p75NTR deficient mice: p75NTRExIII mice, lacking the full-length receptor and p75NTRExIV mice, lacking both, the truncated and full-length receptor. It is still a matter of debate whether there is a substantial loss of cholinergic fibers in the hippocampus during aging. Additionally, it has been reported that the density of cholinergic fibers is not altered in the outer molecular layer of the dentate gyrus of aged p75NTRExIII mice. In order to examine whether p75NTR deficiency affects hippocampal cholinergic innervation, we analysed the cholinergic fiber density (CFD) within the hippocampus of adult and aged p75NTR deficient mice and their controls. Comparison of the adult and aged wildtypes revealed no significant age-related decrease in CFD. On the contrary, in aged p75NTRExIII mice we found increased cholinergic innervation. Besides, our data indicate that both knock-out lines showed increased CFD which, in case of p75NTRExIV, persisted with aging. We further analyzed whether p75NTR deficiency affects hippocampal spine densities during aging, since p75NTR is discussed as a negative regulator of dendritic spines in adult mice. Spine densities were estimated on Golgi-impregnated material. This analysis revealed decreased spine densities in aged p75NTR deficient mice as compared to their wildtype littermates.
Supported by the DFG (BO 1971/5-1).

Kategorie: Poster

Poster 27

Rubrik: Neuroanatomie/Neurobiologie

Titel: Apoer2/vldlr are required for proper migration and correct positioning of mesencephalic dopaminergic neurons

Autoren: Sharaf A.(1),Spittau B.(1),Bouché E.(2),Bock H.(2),Krieglstein K.(3),

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Abstract:
Migration of mesencephalic dopaminergic (mDA) neurons from the subventricular zone to the substantia nigra compacta (SNc), ventral tegmental area (VTA), and retrorubral field (RRF) are controlled by a plethora of neurotrophic factors, cell adhesion molecules (CAMs) and extracellular matrix molecules (ECM). Reelin and the cytoplasmic adaptor protein disabled 1 (Dab1) have been shown to play important roles for the migration and correct positioning of mDA neurons. Mice lacking Reelin and Dab1, both display phenotypes characterised by migration failure of mDA neurons. ApoER2 and VLDLR are the signalling receptors of Reelin and, thus, involved in signal transduction to intracellular adaptor such as Dab1. Here, we describe the roles of ApoER2 and VLDLR on normal positioning and migration of mDA neurons in mice. Our results demonstrate that VLDLR and ApoER2 mutant mice show a reduction and malpositioning of mDA neurons. This phenotype was more pronounced in VLDLR mutant mice. Moreover, we provide evidence that ApoER2/VLDLR double-knockout mice show a phenotype comparable with the phenotypes observed for Reelin and Dab1 mutant mice. Taken together, our results clearly demonstrate that the Reelin receptors ApoER2 and VLDLR play essential roles in Reelin-mediated migration and positioning of mDA neurons.

Kategorie: Poster

Poster 28

Rubrik: Neuroanatomie/Neurobiologie

Titel: Fgfr1 trafficking in human glioma cells

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Abstract:
Fibroblast growth factor receptor 1 (FGFR1) is a receptor tyrosine kinase playing an important role in glioblastoma proliferation. Ligand binding leads to activation of several signaling pathways and internalization of receptor/ligand complexes. The internalized receptor remains active until being absorbed into multivesicular bodies, followed by transfer of the receptor to the lysosome for final degradation. The receptor can escape this fate via recycling back to the cell surface remaining signaling active or re-activatable. Our goal is to interfere with recycling and to shuttle the receptor into the degradation pathway to reduce tumor growth.
We have investigated the distribution of FGFR1 in different intracellular compartments by overexpressing fluorescently-tagged receptors in the human glioma cell line U373. These cells respond to ligand stimulation with increasing levels of phosphorylated FGFR1 and pERK and enhanced proliferation. Distribution analysis reveals that the receptor is mainly localized in the Lamp1-positive degradation compartment (40%), while 25% of the FGFR1-containing-vesicles represent early endosomes (EEA1-positive) and 15% recycling endosomes (transferrin-positive). Stimulation with FGF-2 increases the colocalization rates in each of these vesicle populations.
We are currently investigating the dynamic aspects of FGFR1-trafficking in U373 cells by live cell imaging of the overexpressed receptor and the fluorescently-labeled ligand FGF-2. Furthermore, we are aiming to influence FGFR1 trafficking by blocking receptor recycling with the ionophore Monensin or by interference with the Rab11 GTPase which is a key regulator for sorting into recycling endosomes. This may provide a basis for the development of new treatment strategies to reduce RTK-mediated tumor growth.

Kategorie: Poster

Poster 29

Rubrik: Neuroanatomie/Neurobiologie

Titel: Effects of clostridial c3 proteins on neuronal glutamate uptake

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Abstract:
Besides its metabolic function, glutamate is the main excitatory transmitter in the CNS. High extracellular concentrations of glutamate are known to cause neuronal damage by excitotoxicity. Therefore, it is crucial for the CNS to prevent the neurons from high extracellular glutamate concentrations and to maintain a high signal to noise ratio for this transmitter. Plasma membrane glutamate transporters are key players in lowering extracellular glutamate levels. Glial cells express GLT-1 and GLAST as transporter types whereas neurons recruit the neuronal subtype EAAC1 (excitatory amino acid carrier 1) to their surface. In previous works we could show that the glial subtypes are effectively regulated by Rho-dependent pathways. In the current work we investigated the expression and regulation of neuronal EAAC1 following treatment with enzyme-competent C3 transferase (C3bot) and enzyme-deficient C3bot- derived peptides to inhibit Rho signaling. In the neuronal cell line HT22 incubation with either C3bot or C3bot 26mer peptide resulted in the down-regulation of glutamate uptake by 25-30%. Furthermore, incubation with C3bot 26mer resulted in the activation of protein kinase C alpha (PKC alpha) as observed by translocation from the cytoplasma to sub-plasma membrane regions. Activation of PKC alpha with the potent phorbol ester PMA used as positive control confirmed down-regulation of glutamate uptake. In accordance with these observations, EAAC1 exhibited a reduced surface expression following incubation with C3 preparations. In conclusion, neuronal EAAC1 expressed in the cell line HT22 seems to be regulated by Rho- and PKC alpha-dependent pathways that result in the transporter down-regulation.

Kategorie: Poster

Poster 29a

Rubrik: 3.Neuroanatomie/Neurobiologie

Titel: Characterisation of the transport of active zone proteins to synapses
Autoren: Ghelani T.(1),Dresbach T.(1),Wittenmayer N.(1),

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Abstract:
The presynaptic cytomatrix is a meshwork of proteins in which synaptic vesicles are embedded. A specialized subcompartment of this meshwork is called the cytomatrix of active zones (CAZ). In electron microscopy the CAZ appears as a dense structure composed of filamentous material originating at the active zone plasmamembrane, i.e. the site of neurotransmitter release. Five CAZ-proteins have been identified, including Bassoon, Piccolo, RIM, Munc13 and CAST/ERC. In immature neurons, these proteins are associated with vesicles named Piccolo-Bassoon-transport vesicles (PTVs). PTVs are characterised by a dense core, a diameter of 80 nm and may deliver pre-assembled CAZ-precursors to synapses. By using fluorescence and electron microscopy we are investigating the transport of PTVs from the Golgi apparatus to synapses. Our results show that overexpression of GFP-Bassoon generates large aggregates of vesicles which migrate within the axon but the majority of these vesicles do not hold a dense core. The GFP-Bassoon vesicles colocalise with other active zone proteins such as Piccolo and Munc13-1.

Kategorie: Poster

Poster 29b

Rubrik: 3.Neuroanatomie/Neurobiologie

Titel:The role of the star family protein sam68, slm-1 and slm-2 in neural stem cells of the forebrain

Autoren: von Holst A.(1),Kirsch J.(1),Bertram B.(2),

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Abstract:
We have identified the signal transducer and regulator of RNA metabolism (STAR) family protein Sam68 as a target of the extracellular matrix glycoprotein Tenascin C (Tnc) by an induction gene trap screen in neural stem cells. The activity of Sam68 is regulated by phosphorylation and overexpression by nucleofection reduced the proliferation of cortical neural stem/progenitor cells. At the same time Sam68 in turn regulates the splicing of Tnc by favoring the inclusion of the alternative spliced fibronectin type III domains. Both, Tnc and Sam68 are expressed in the germinal layers of the developing neuroepithelium and their expression is maintained in the postnatal and adult subventricular zone of the lateral ventricle wall. This implies an auto-regulatory, oscillatory interplay between the neural stem cell niche microenvironment and cortical neural stem/progenitor cells, which may contribute to the precise timing of neurogenesis during development and in the adult neural stem cell niche. Interestingly, the two Sam68-related genes Slm-1 and Slm-2 are also expressed in the developing forebrain. Forced expression of Slm-1-EGFP and Slm-2-EGFP fusion constructs in cultured neural stem/progenitor cell revealed opposing functions. Slm-1 interfered with proliferation and appeared to favour neurogenesis while Slm-2 increased proliferation and promoted astrogliogenesis. So, Slm-1 is similar to Sam68 on the cell biological level, whereas Slm-2 appears to have opposing functions. We are currently investigating, if the role of the Sam68 family members is carried by the signal transducing function, the RNA binding and RNA splicing activity or both to define the molecular mechnism(s) that orchestrate maintenance, proliferation and differentiation of cortical neural stem and progenitor cells.

Kategorie: Poster

Poster 29c

Rubrik: 3.Neuroanatomie/Neurobiologie

Titel:Chondroitin sulfates as regulators of proliferation and neurogenesis in the neural stem cell niche

Autoren: von Holst A.(1),Harrach D.(1),Kirsch J.(1),

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Abstract:
Chondroitin sulfate proteoglycans (CSPGs) and their sulfation by chondroitin-sulfotransferases (Chsts) play a crucial role for the behaviour of neural stem cells (NSCs) in the embryonic neural stem cell niche during mouse forebrain development. It has been shown that the inhibition of the sulfation by sodium chlorate or the degradation of the CSPG glycosaminoglycans by chondroitinase ABC leads to less proliferation and altered cell fate decisions of cortical and striatal NSCs. These findings support the CS-code hypothesis that we have out to test be manipulating the degree of sulfation in the neural stem cell niche. The proliferation and differentiation of cortical neural stem cells from E13.5 mouse embryos upon forced expression of distinct Chst-EGFP constructs was examined by neurosphere forming assay and differentiation assay in vitro. Furthermore, the Chsts overexpression by CMV promotor vs. chicken ?-actin promotor was analyzed. The overexpression of distinct Chsts in the NSCs was functional as revealed by an increased signal for the complex sulfated CS-epitope detected by the monoclonal antibody 473HD. In the differentiation assay an increase in neurogenesis at the expense of gliogenesis was observed. Consistent with previous observations, the sulfation of the CSPGs plays a role in the commitment of the NSCs within the neural stem cell niche and could function as a possible communication platform between the NSCs and their extracellular surrounding in the neural stem cell niche.

Kategorie: Poster

Poster 29d

Rubrik: 3.Neuroanatomie/Neurobiologie

Titel: The *Drosophila* Adhesion-GPCR dCirl/Latrophilin controls the expansion of postsynaptic membranes

Autoren: Wagner N.(1), Gehring J.(1), Hartmann N.(1), Asan E.(2), Kittel R. J.(1), Langenhan T.(1)

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Abstract:
Targeted membrane addition has a major impact on the development, function and plasticity of neuronal synapses. Despite its fundamental importance, this process is poorly understood. The larval neuromuscular junction (NMJ) of the fruitfly *Drosophila melanogaster* is an excellent model system to study synaptic membrane expansion, because the area and complexity of the postsynaptic membrane increases dramatically during larval development, resulting in the formation of a highly convoluted and multilayered membrane specialization known as subsynaptic reticulum (SSR). We have studied the *Drosophila* adhesion class G protein-coupled receptor (Adhesion-GPCR) dCirl/Latrophilin, which has been implicated in neuronal development and the control of synaptic transmission, using the *Drosophila* larval NMJ as model system. Applying confocal and transmission electron microscopy, we documented while normal morphology of neurotransmitter release sites is not affected, quantitative analysis of larval boutons reveals a striking expansion of the postsynaptic SSR at dCirl mutant synapses. Thus, our results show that dCirl is involved in controlling the expansion of postsynaptic membranes during this developmentally dynamic period.

Kategorie: Poster

Poster 30

Rubrik: Zellbiologie

Titel: Anaphylatoxine receptors and complement regulatory proteins in human articular and non-articular chondrocytes: interrelation with cytokines

Autoren: Schulze-Tanzil G.(1),Kohl B.(1),John T.(1),Stoelzel K.(2),Ertel W.(3),

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Abstract:
Tissue trauma induces an inflammatory response associated with a cytokine release which may engage complement pathways. Hence, we analysed the complement expression profile in primary articular and non-articular chondrocytes and its interrelation with cytokines.
The gene expression of the anaphylatoxine receptors (C3aR and C5aR) and the complement regulatory proteins (CPRs) CD35, CD46, CD55 and CD59 was studied in cultured primary articular, auricular and nasoseptal chondrocytes. The complement profile of leukocytes (peripheral blood mononuclear cells) was opposed to the expression in joint cartilage-derived chondrocytes. The time-dependent regulation (6 and 24 h) of these complement factors was assessed in response to the cytokines TNFalpha, IL-10 or TNFalpha combined with IL-10 (each 10 ng/mL).
C3aR, C5aR, CD46, CD55, CD59 but not CD35 mRNA was expressed in all studied chondrocyte types. The expression of anaphylatoxine receptors was lower in chondrocytes compared with PBMCs, whereas that of CD46, CD55 and CD59 was higher. Despite mostly not significant, the studied factors were generally at a lesser level expressed in nasoseptal chondrocytes and except for CD46 and CD59, also lesser in auricular compared with articular chondrocytes. TNFá increased significantly the C3aR expression in chondrocytes after 6 and 24 h, but had no significant effect on the CRP expression. TNFalpha combined with IL-10 downregulated C5aR and IL-10 inhibited CD46 and CD55 gene expression after 24 h significantly. Anaphylatoxine receptors and CRPs are expressed in chondrocytes and regulated differentially by TNFalpha and IL-10. Whether cytokine-induced complement activation occurs in response to cartilage trauma has to be further identified.

Kategorie: Poster

Poster 31

Rubrik: Zellbiologie

Titel: Influence of in-vitro injury on human tenocytes complement expression

Autoren: Girke G.(1),Kohl B.(1),Ertel W.(2),John T.(2),Schulze-Tanzil G.(1),

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Abstract:
Traumatic injury of tendons is characterized by a retarded regeneration compared to other connective tissues. Hereby, the activity of the complement system might influence the healing process. To investigate the effect of mechanical injuries on human tenocytes complement expression profile we designed a new cell scratch plotter system. In this context we analyzed the gene expression of complement regulating proteins and a possible proinflammatory response of mechanically injured tenocytes. Gene expression analysis of two different time points post cell injury was assessed using qRT-PCR, which revealed an elevated expression of C3a receptor after 24h, whereas the C5a receptor was marginally lower expressed. The complement inhibitory proteins CD46 and CD55 and the matrix metalloprotease MMP1 were induced 24h post injury. In contrast the transcription of the proinflammatory cytokine genes for TNFalpha and IL-1beta was not affected by this treatment. To validate the gene expression data we performed immunofluorescence staining for the C5a receptor, TNFalpha and MMP1.
The increased expression of the C3a receptor due to mechanical injury suggests an elevated sensitivity of tenocytes to the complement cleavage product protein C3a, but not to C5a. Furthermore, it was supposed that the upregulated gene expression of the cytoprotective proteins CD46 and CD55 impairs the complement activation cascade. Hence, the chemotactic attraction of immunocompetent cells might be reduced. The concurrent heightened expression of MMP1 could reflect the beginning of the reorganization of tendon tissue post injury, which is not attended by an inflammatory reaction in this in-vitro cell scratch assay.

Kategorie: Poster

Poster 32

Rubrik: Zellbiologie

Titel: Dsg2-interaction is crucial for cardiomyocyte cohesion *in vitro*

Autoren: Schlipp A.(1),Ebel C.(1),Waschke J.(1),

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Abstract:
Impaired cell-cell-cohesion between cardiomyocytes is an important factor in the development of Arrhythmogenic cardiomyopathy (AC), an autosomal-dominant heritable heart disease with variable phenotype expression and a high incidence of sudden cardiac death as the first symptom presentation. AC primarily is a disease of the desmosomal part of the intercalated disc, which was ascribed to mutations in genes encoding for desmoglein 2 (Dsg2) and desmocollin 2, plakoglobin, plakophillin 2 and desmoplakin. To investigate the pathogenesis of AC in the future, we started to characterize the functional relevance of Dsg2, the only desmoglein present in the intercalated disc, for cardiomyocyte cohesion. Therefore, we used the mouse cardiomyocyte cell line HL-1. In a collagenase-based adhesion assay we demonstrated that EDTA treatment increased the number of fragments indicating that cadherin-mediated adhesion is crucial for cell-cohesion. Knock-down of Dsg2, application of a peptide inhibiting Dsg homophilic interactions or excess tryptophan, an amino acid involved in Dsg transinteraction, also led to a loss of cell cohesion. The effect of tryptophan was prevented by co-incubation with a tandem-peptide linking desmogleins between adjacent cells. Similarly, overexpression of Dsg2 increased cell-cell adhesion. In conclusion, our results state an important role of Dsg2 for cardiomyocyte cohesion, at least in vitro.

Kategorie: Poster

Poster 33

Rubrik: Zellbiologie

Titel: Extracorporeal shock wave effects on equine adipose tissue-derived mesenchymal stem cells in vitro

Autoren: Raabe O.(1),Damm A.(2),Crispens C.(3),Scheiner-Bobis G.(4),Wenisch S.(1),Arnhold S.(1),

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Abstract:
Extracorporeal shock wave therapy (ESWT) is used as a standard method for treatment of urolithiasis and musculoskeletal disorders in human and veterinary medicine. In equine medicine, the therapy is almost exclusively applied to orthopaedics. However, mesenchymal stem cells are promising as therapeutic aids in the repair of musculoskeletal damages in the horse. In equine veterinary practice stem cell therapy is very often combined with the ESWT.
Therefore, the aim of this study was to investigate the influence of focused ESWT on the viability, proliferation, and differentiation capacity of adipose tissue-derived mesenchymal stem cells (ASCs) and to explore its effects on gap junctional communication and the activation of signalling cascades associated with cell proliferation and differentiation. The ASCs were treated with different pulses of focused ESWT: the control group did not receive any extracorporeal shock wave treatment, while Experimental Group 1 (EG 1000/9) received 9 pulses of 1000 shock waves and Experimental Group 2 (EG 2000/3) received 3 pulses of 2000 shock waves.
Treated cells showed increased proliferation and expression of Cx43, as detected by means of qRT-PCR, histological staining, and immunocytochemistry. At the same time, cells responded to ESWT by significant activation (phosphorylation) of Erk1/2, detected in western blots. No significant effects on the differentiation potential of the ASCs were evident. Taken together, the present results show significant effects of shock waves on stem cells in vitro.

Kategorie: Poster

Poster 34

Rubrik: Zellbiologie

Titel: Dsg3-mediated p38mapk regulation: role of extradesmosomal dsg3?

Autoren: Hartlieb E.(1),Partilla M.(1),Spindler V.(1),Waschke J.(1),

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Abstract:
The integrity of the epidermis is ensured by desmosomes, composed of desmosomal cadherins including four desmoglein (Dsg1-4) and three desmocollin (Dsc1-3) isoforms, as transmembrane adhesion molecules. Dsg 1 and 3 are known targets of autoantibodies in the blistering skin disease pemphigus, highlighting their role for cell-cell cohesion. Furthermore, desmosomal cadherins are increasingly recognized to be involved in signaling events such as p38MAPK activation. We previously showed by siRNA depletion that Dsg2 in contrast to Dsg3 is dispensable for keratinocyte cohesion and that Dsg3 but not Dsg2 regulates the p38MAPK pathway. Here, this was confirmed by the protective effect of both additional p38MAPK depletion and the specific p38MAPK inhibitor SB202190 on the loss of cell cohesion in Dsg3-depleted cells. It is known that desmosomal cadherins are located also on the plasma membrane outside of desmosomes, i.e. extradesmosomal. However, it is unclear which pools of desmosomal cadherins are involved in signaling. Therefore, we evaluated the distribution of Dsg2, Dsg3 and activated p38MAPK in human keratinocytes by Triton-X-100-mediated cell fractionation. Desmoplakin served as an indicator to identify the insoluble fraction as the desmosomal pool. Accordingly the Triton-X-100 soluble pool represented extradesmosomal proteins. The ratio of insoluble/soluble pool of Dsg2 was five times higher than that of Dsg3 suggesting a more pronounced extradesmosomal localization of Dsg3. Interestingly, phosphorylated p38MAPK was present only in the soluble fraction. This result is consistent with the idea that signaling in keratinocytes is primarily initiated by extradesmosomal cadherins such as Dsg3.

Kategorie: Poster

Poster 35

Rubrik: Zellbiologie

Titel: Short term characterisation of mesenchymal stem cells derived from two rat osteoporosis models

Autoren: Goergen J.(1),Hempel U.(2),Raabe O.(1),Heiß C.(3),Wenisch S.(1),Arnhold S.(1),

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Abstract:
Osteoporosis is a disease characterized by low bone mass and micro architectural deterioration of bone tissue, resulting in an increased fracture risk. Since this disease is restricted to humans, there is a great need for valuable animal models to be able to test new therapeutic approaches or new prosthetic devices for the osteoporotic bone. Thus, the goal of this study is to get a further understanding of the role of rat bone marrow derived mesenchymal stem cells (BM-MSCs) after induction of osteoporosis and thereby to be able to draw comparisons between the situation in human osteoporosis and the rat model. In that regard we compare two different ways of induction of osteoporosis in rats overlooking the characteristics of BM-MSCs. Induction of osteoporosis is accomplished by ovariectomy in combination with either a calcium-deficient diet or application of a steroid. A sham-operated group is established as a control. MSCs are isolated from the bone marrow three months after surgery and characterized regarding their morphology and their proliferation, migration and differentiation capacity.
After isolation the MSCs of ovariectomized rats have a different morphology compared to those from control animals. They are more broad and flattened as that is the case in aged MSCs. Furthermore, the proliferation and migration potential of these MSCs is decreased compared to those of the control group. In contrast MSCs of the ovariectomized rats show an even greater osteogenic differentiation potential than MSCs of the control group.
This work is supported by the DFG / Collaborative Research Center Transregio 79.

Kategorie: Poster

Poster 36

Rubrik: Zellbiologie

Titel: Organ-cultured mice cornea as a model for donor corneas: endothelial and epithelial cell survival strongly depends on organ culture media composition

Autoren: Götze D.(1),Knels L.(1),Valtink M.(1),Funk R.(1),Engelmann K.(2),

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Abstract:
Purpose: Human donor corneas are stored in organ cultivation for several days before transplantation, but 20% of corneal grafts experience severe endothelial cell loss during organ cultivation, rendering them unsuitable for keratoplasty. We investigated the influence of culture media on corneal endothelial and epithelial cell viability in a mice corneal organ cultivation model.
Methods: Mice cornea were excised and cultured for 4 days in standard corneal organ culture medium MEM+2% fetal calf serum (FCS) or serum-free Human Endothelial-SFM, each supplemented with either 6% Dextran T500 or 7,5% hydroxyl-ethyl starch (HES) as deswelling supplements. Treatment with staurosporine (0.5 mmol/l, at day 3) was used as apoptosis control. Samples were then fixed, paraffin-embedded and stained with HE or with antibodies against apoptotic / anti-apoptotic proteins (caspase-3 and -8, Bcl-2, HSP-32).
Results: Only corneas with intact epithelial and endothelial layers at day 1 were examined further. After 4 days, endothelia and epithelia of SFM-cultured corneas appeared normal and regularly architectured. The nedothelia showed strong staining for HSP-32, while the ir epithelia had a regular architecture with 1 basal layer of prismatic cells, 2 layers of cuboidal cells and 2 layers of suprabasal squamous cells. Endothelia and epithelia of MEM cultured corneas were lost or showed morphological signs of apoptosis like caspase-3 and -8 positivity and reduced HSP-32 positivity. Caspase-3 staining was slightly higher in HES-supplemented media than in Dextran-supplemented media.
Conclusions: Cultivation in SFM supported corneal cell viability, while cultivation in MEM led to endothelial cell loss and epithelial and stromal cell reduction.

Kategorie: Poster

Poster 37

Rubrik: Zellbiologie

Titel: Blocking of organic cation transporter oct1 by preventing access of substrate to the innermost part of the outward-open binding pocket

Autoren: Gottlieb N.(1),Egenberger B.(1),Gorbunov D.(1),Gorboulev V.(1),Koepsell H.(1),

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Abstract:
Organic cation transporters are critical in drug absorption, distribution and excretion (1) Employing extensive mutagenesis and homology modeling, outward-open and inward-open substrate binding pockets containing several amino acids which are involved in binding of different substrates and inhibitors have been identified (2). For example, replacement of aspartate 475 in the eleventh transmembrane alpha-helix (TMH) of rat Oct1 rOct1) by glutamate changed the affinities for binding of tetraethylammonium (TEA) and of 1-methyl-4-phyenylpyridinium (MPP) to the outward-open binding pocket. Whereas the affinity for TEA translocation by rOct1 was increased in this mutant, the affinity for MPP translocation remained unchanged (2). In the present study we observed that the cationic sulfhydryl reagent [2-(trimethylammonium)ethyl]methanethiosulfonate (MTSET) is transported by rOct1. When glycine 478 which is located one alpha-helix turn of TMH11 above aspartate 478, was exchanged by cysteine, covalent modification of cysteine 478 with MTSET blocked binding of MPP+ to rOct1 and thereby prevented rOct1 mediated translocation of MPP. The data demonstrate that binding of MPP to aspartate 475 is required for translocation and suggest that aspartate 475 triggers a transport-related conformational change of rOct1. Refernces: 1.Koepsell, H., Lips, K., and Volk, C. (2007) Pharm. Res. 24, 1227-1251; 2. Koepsell, H. (2011) Biol. Chem. 392, 95-10

Kategorie: Poster

Poster 38

Rubrik: Zellbiologie

Titel:Functional role of ceacam1 in the lymphogenic metastasis of the prostate carcinoma

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Abstract:
Prostate carcinoma is one of the most common cancer types in men above 50 years of age. It metastasizes often via the lymphatic system resulting in a poor prognosis for the patient. For the metastatic process adhesion molecules were described to be essential. Recently it has been shown that CEACAM1 is down-regulated in prostate carcinoma, whereas tumor-associated blood and lymph vessels showed an induction of the CEACAM1 expression. CEACAMs are homophilic, low affine cell-cell adhesion molecules of the carcinoembryonic antigen (CEA) family known to trigger an integrin-mediated adhesion of leukocytes to endothelial cells. Following this idea we investigated the role of CEACAMs during the lymphogenic metastatic spread utilyzing various murine and human prostate cancer and lymphendothelial cell lines (LECs). We identified low expression of CEACAMs in prostate cancer cell lines (human PC-3, murine AD-Ca and ADI-Ca) and high expression in LECs (human AS-M.5, murine bEnd.3). Interaction studies of GFP-labeled CEACAM1-overexpressing prostate cancer cell lines and control cells with endothelial monolayers emphasized the role of CEACAM1 in inducing cell-cell interaction. However, this adhesion was not mediated by CEACAM1 itself. Thus, we have established a model system that will allow us to analyze the cellular and biochemical processes underlying the CEACAM1-mediated metastasis.

Kategorie: Poster

Poster 39

Rubrik: Zellbiologie

Titel: Deoxynivalenol increases the pore number in the basement membrane in the jejunum

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Abstract:
The intestinal basement membrane contains numerous pores through which immune cells can migrate from the lamina propria into the epithelium. Deoxynivalenol (DON) as an important mycotoxin is known to regulate the immune function of the intestine – probably based on the cell migration between epithelium and lamina propria. Therefore, we analysed gut segments of jejunum and ileum with the focus of pore size and their number. In control pigs (n=5) and in pigs fed a DON containing diet (n=5; initial weight of all pigs=35.1 ± 3.2kg) the structure of the basement membrane was examined. An anti-laminin antibody was used to label the basement membrane and thus to visualise the pores.
The jejunum showed a significant lower pore number /1000µm in the basement membrane (6.4 pores/1000µm) in comparison to ileum (11.26 pores/1000µm; $p \leq 0.001$) but no differences were found with the focus on pore size (jejunum=3.8µm; ileum=3.4µm). DON exposition resulted in an increase of the number of pores only in the jejunum (control=6.4 pores/1000µm; DON=9.71 pores/1000µm; $p=0.055$). On the other hand, no difference between control and treatment group was detected in the ileum (control=11.26 pores/1000µm; DON=11.33 pores/1000µm; $p=0.997$).
Due to the increase of pore number in the DON-treatment group we suggest that DON may trigger the cell traffic at the basement membrane in the jejunum but not in the ileum. This is possibly the basis for changed immune reaction in DON exposed animals.

Kategorie: Poster

Poster 40

Rubrik: Zellbiologie

Titel: Down-regulation of sirt-1 by antisense oligonucleotides induces apoptosis in human tenocytes in vitro

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Abstract:
Objective: Protein deacetylase Sirt-1, has been shown to play a central role in modulating transcriptional silencing, thereby differently affecting cell proliferation, inflammatory and apoptotic signaling in various cell types. The objective of this study was to clearer define the molecular mechanisms involved in the pathogenesis of tendon overuse injuries and tendinitis by using antisense oligonucleotides (ASO) against Sirt-1.
Methods: Primary human tenocytes were transiently transfected with different concentrations of ASO alone or in combination with resveratrol (Sirt-1 activator), inflammatory cytokine interleukin-1beta (IL-1beta) or Sirt-1 inhibitor nicotinamide (NA) and examined by immunofluorescence, electron microscopy, cell viability assay and western blotting.
Results: ASO against Sirt-1 induced mitochondrial degradation and apoptosis in a dose-dependent manner. Activation of NF-kappaB signaling pathway and increased expression of NF-kappaB-regulated gene products were found to the same extent as in treatment with IL-1beta or NA. ASO also increased acetylation of tumor suppressor p53, expression of Bax protein, and cleavage of caspase-3 and PARP. Interestingly, immunoprecipitation revealed that Sirt-1 and p53 interacted with each other. In contrast, Sirt-1 activator resveratrol inhibited IL-1beta - and NA-induced NF-kappaB-mediated inflammatory signaling, including Akt activation and suppression of tenogenic transcription factor scleraxis, but could not abolish catabolic effects of ASO against Sirt-1, thereby highlighting the crucial role of this enzyme.
Conclusion: Our results demonstrate, that down-regulation of Sirt-1 activated inflammatory and apoptotic signaling mediated by NF-kappaB and p53 and significantly reduced cell survival in human tenocytes. This could provide a novel role for resveratrol as an anti-inflammatory agent in the treatment of tendinitis.

Kategorie: Poster

Poster 41

Rubrik: Zellbiologie

Titel: A point mutation in an intracellular loop of organic cation transporter oct1 changes the configuation of outward-open substrate binding pocket

Autoren: Gottlieb N.(1),Rehman S.(1),Gorboulev V.(1),Roth U.(1),Ganscha A.(1),Koepsell H.(1),

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Abstract:
Organic cation transporters mediate absorption, distribution and excretion of many drugs (1). Extensive mutagenesis in rat OCT1 , detailed functional characterization of mutants, and homology modelling allowed the identification of amino acids in transmembrane alpha-helices (TMHs) 2 (F160), 4 (W218), and 11 (D475) which are directly involved in the recognition of transported and inhibitory cations (2). In addition amino acids in the outward-open substrate binding pocket were identified which interact with bulky inhibitors but not with substrates. Previously we observed that the replacement cysteine 451 by methionine in the short intracellular loop between TMH10 and 11 changed the affinity for choline transport (4). Since homology modelling of the outward-open conformation of rat Oct1 suggested sterict hindrance of the intracellular part of TMH11 by methionine 451, we investigated whether effects of D475E exchange on cation interactions were different if the mutation was performed on the rOct1 wildtype on Oct1(C451) background. Dramatic effects where observed. Examples are that the Km value for tetraetylammonium and the Ki values for tetrabutylammonium were decreased when the D475E exchange was performed in rOct1 wildtype background whereas the values were increased when the D475E exchange was performed in the OCT1(C451M) background. Differential effects in the wildtype and C451M background were also observed when Phe160 was replaced by thyrosine or when Tryp218 was replaced by phenylalanine. The data indicate that the structure of substrate binding region is altered by a mutation in the intracellular loop between TMH10 and 11.
References:
1. Koepsell, H., Lips, K., and Volk, C. (2007) Pharm. Res. 24, 1227-1251; 2. Koepsell, H. (2011) Biol. Chem. 392, 95-10.

Kategorie: Poster

Poster 42

Rubrik: Zellbiologie

Titel: Visualizing the mouse lung via scanning laser optical tomography (slot)

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Abstract:
Scanning Laser Optical Tomography (SLOT) is a new rapid scanning technique, which exploits intrinsic contrast mechanisms like fluorescence, light scattering and absorption to visualize the internal structure of previously cleared lungs. Via back projection algorithms data stacks are generated that allow three-dimensional imaging of specimens.
Using perfusion fixed and inflated lungs from 10 week old mice SLOT enables visualization visualization of whole lobes ex vivo with a resolution down to the level of single alveoli. Airways, blood vessels and parenchyma from optically cleared mouse lung lobes using absorption and autofluorescence scan modes can be analyzed in three-dimensional datasets in any preferred planar orientation. Virtual reconstructs of the blood vessel system, the airways in general and individual acini are presented. Furthermore the procedure preserves the microscopic structure of the lung and allows for subsequent correlative histologic studies.
In summary, SLOT is a highly efficient three dimensional fluorescence microscopy technique to study the internal structure from whole mouse lung lobes with a resolution down to the level of single alveoli. In addition to currently available technologies a valuable tool for e.g. quantitative phenotype analysis in different mouse models of lung diseases.

Kategorie: Poster

Poster 43

Rubrik: Zellbiologie

Titel: Pten is nuclear-colocalized with p-akt1/pkb in a subpopulation of human odontoblasts

Autoren: Korkmaz Y.(1),Klinz F.(2),Neiss W.(2),Schneider K.(1),Bloch W.(3),Raab W.(1),Addicks K.(2),

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Abstract:
The subcellular localization site of phosphatase and tensin homolog deleted on chromosome 10 (PTEN) could be helpful to elucidate its cellular functions. PTEN dephosphorylates phosphatidylinositol 3,4,5-trisphosphate to phosphatidylinositol 3,4-bisphosphate and thus mediates its function by inactivating downstream Akt/protein kinase B (PKB)-mediated signals for growth, proliferation and differentiation. The conditional deletion of PTEN in osteoblasts induces increased formation of bone matrix indicating a PTEN-dependent regulation of PI3K-Akt/PKB signaling in matrix formation. PTEN is regulated in dependence of its subcellular localization in tumour cells. But, the subcellular localization of PTEN and its subcellular localization with phosphorylated-Akt/PKB in terminally differentiated odontoblasts that produce dentin matrix, remains unclear. Implying immunohistochemistry to decalcified, frozen-sectioned, free-floating sections of human molars, we detected colocalization of PTEN with Akt1/PKB and with phosphorylated-Akt1/PKB at Thr308 and Ser473 in the nuclei of a subpopulation of odontoblasts. PTEN colocalized with Thr308 in all but with Ser473 only in a subset of odontoblast nuclei indicating that nuclear localization of PTEN and p-Akt1/PKB may be linked to the differentiation state of odontoblasts. In a subpopulation of odontoblasts, PTEN was nuclear-localized whereas p-Akt/PKB was detected only in cytosol suggesting PTEN has some PI3K-Akt/PKB-independent nuclear function in odontoblasts.

Kategorie: Poster

Poster 44

Rubrik: Zellbiologie

Titel: Granulocytes of the ostrich (struthio camelus): an ultrastructural and histochemical study

Autoren: Rodler D.(1),Scholz J.(1),Sinowatz F.(1),

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Abstract:
Different to mammals, the ostrich (Struthio camelus), like most other avian species, has two morphologically different types of granulocytes with eosiniphilic granules, termed heterophilic granulocytes and eosinophilic granulocytes. The aim of this study was to characterize these two cell types and to contribute to the comparative morphology of leucocytes.

For light microscopical studies, conventional staining methods (Diff-Quick, Giemsa, May-Grünwald, Pappenheim) and several substrate-histochemical techniques (PAS, Toluidine-blue, Alcian-blue at pH 1 and pH 2.5, and Sirius Red) were applied. For the histochemical characterization of the granules, additionally a panel of 14 different lectins, specific for certain carbohydrate residues, was used. For peroxidase detection, blood smears were fixed in ethanol-formol solution and incubated with 0.05% ortho-toluidine and 0.6% hydrogen peroxide in distilled water for 15 min. For ultrastructural studies, blood cells were fixed in Karnovsky´s solution, embedded in Araldit and sectioned using a Reichert ultramicrotome. The ultrathin sections were evaluated using a Zeiss EM 902 electron microscope.

With Sirius-Red and May-Grünwald staining, heterophils and eosinophilic granulocytes can clearly be discerned. Basophilic granulocytes are best characterized by Toluidine-blue and Alcian-blue, which selectively stain the basophilic granules. Heterophils show two types of granules, which can easily be discerned at the ultrastructural level. Eosinophilic granulocytes possess a kidney shaped nucleus and contain only round, homogenous granula without any crystalline inclusions. Contrary to the elliptical granules of the heterophils, the eosinophilic granulocytes were peroxidase-positive

The results obtained in our study will help in the diagnosis of infectious blood diseases of this interesting huge bird species.

Kategorie: Poster

Poster 45

Rubrik: Zellbiologie

Titel: Trans-differentiation of vascular wall-resident progenitor and stem cells into cardiomyocytes

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Abstract:
Introduction: Cardiovascular diseases including myocardial infarction remain one of the leading causes of mortality worldwide. Recent studies showed that adult vascular adventitia serves as a niche for stem and progenitor cells named vascular wall-resident stem cells (VW-SCs). We hypothesized that these cells might serve as an endogenous source for cardiomyocytes and studied the potential of human and mouse VW-SCs to differentiate into cells with cardiomyocyte properties.
Material and Methods: VW-SCs (CD34+ and CD44+) have been isolated using magnetic cell sorting (MACS) from human internal thoracic artery (hITA) and mouse thoracic aorta. Purity of isolated cells was confirmed by immunocytochemistry and FACS analysis for hematopoietic and mesenchymal stem cell markers. Generation of cardiomyocytes from VW-resident progenitor/stem cells *in vitro* was performed by stimulation of VW-SCs with specific factors (e.g. 5-Azacytidine, TGF-β1) and modifying cell culture conditions. Expression of cardiac markers in differentiated cells from hITA and mouse thoracic aorta was investigated by immunocytochemistry, electron microscopy and RT-PCR.
Results: VW-SCs exhibited cardiomyocyte-like contractile capacity *in vitro*. Immunostaining of CD44(+)hITA cells stimulated with 5-Azacytidine and TGF-β1 revealed that these cells are apparently capable to differentiate into cardiomyocyte-like cells by expressing the cardiomyocyte marker Troponin I, while CD44(-)hITA cells exhibited only a weak Troponin I staining.
Discussion: Our data demonstrate for the first time that VW-SCs from human adult artery as shown here for hITA and from mouse aorta have the capacity to differentiate into cardiomyocyte-like cells with properties of spontaneous beating and expression of some cardiac-specific markers. Thus, blood vessel themselves might contribute to cardiac regeneration not only by angiogenesis but probably also by serving as a cellular niche for cardiomyocyte generation.

Kategorie: Poster

Poster 46

Rubrik: Zellbiologie

Titel: Surfactant proteins are expressed in articular joints and are regulated during osteoarthritis

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Abstract:
Surfactant proteins (SP) are well known from human lung. The proteins assist the formation of a monolayer of surface-active phospholipid at the liquid-air interface of the alveolar lining, play a major role in lowering surface tension of interfaces, and have functions in innate and adaptive immune defense. During recent years it became obvious that SPs are also part of other tissues and fluids such for example tear fluid, gingiva, saliva, the nasolacrimal system, kidney and several other. By means of different morphological, molecular biological and cell culture methods we tested the hypothesis whether SPs are also expressed in articular joints as well as joint related diseases such as osteoarthritis. All four SPs are expressed by articular chondrocytes and cells of synovial membrane of human and mice and are also present in synovial fluid. Inflammatory mediators regulate the SP expression and in case of osteoarthritis there is a significant increase in the SP expression of all four SPs. Our results reveal that SPs are a physiological part of articular joints probably having functions in lowering friction and immune defense.

Kategorie: Poster

Poster 46a

Rubrik: 4.Zellbiologie

Titel: Brain Tumor Angiogenesis: A versatile Organotypic ex vivo Assay

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Abstract:
The blood vessels are the bodies‘ highways delivering nutrients and satisfying all the needs of the tissues and organs of the human body. The process of angiogenesis is highly connected with the development of organs and tissues itself. Further, during tissue maintenance and regeneration supply of oxygen and nutrients for cell function and survival is required. In particular proliferating cells within a tissue depend strongly on continuous blood supply and thus create a microenvironment which encourages the generation and growth of blood vessels. In the past, a variety of assays has been established to study angiogenesis, but very few allow investigations in a model with organotypic cellular and microenvironmental composition. Previously, we established an Organotypic Glioma Invasion Model which allows to monitor in real-time tumor invasion, metastasis and angiogenesis in an in-vitro experiment with in-vivo conditions. Therefore, we extended this ex-vivo system by tracking the distribution of genetically marked glioma cells (RFP, GFP) and vascular components. In this model, we found that the blood-vessel architecture is altered drastically in and around the tumor-bulk in comparison to normal brain tissue without tumor contact. Within the tumor, the blood vessels show ranging diameters with erratic alterations of vessel types and course. The peritumoral region is characterized by the absence of large diameter vessels, or metarterioles whereas unaffected brain regions display an even and regular distribution of vessels from capillaries, arterioles and metarterioles. We classified these alterations in physiological vessel architecture with large diameter vessels at the pial surface and smaller vessels radiating into the cortex. In contrast, vessels in the peritumoral area show a heterogeneous and diffuse architecture. Vessels in the tumor core range from „bigger than normal“ to „capillary size“ diameter and represent an altered architecture in terms of variations, diameter and distribution. Thus, our novel model system represents a versatile system to study tumor – brain – blood vessel interaction and thus represents a bridging assay for purely cell based in-vitro assays and in-vivo animal experiments.

Kategorie: Poster

Poster 47

Rubrik: Entwicklungsbiologie

Titel: Epithelial and mesenchymal regionalization of the human utero-vaginal anlagen. prenatal development and lifelong clinical relevance

Autoren: Fritsch H.(1),Hörmann R.(1),Bitsche M.(1),Elisabeth P.(1),Reich O.(2),

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Abstract:
Literature on the development of the human vagina is abundant, however, contributions about the prenatal development of the entire utero-vaginal anlagen (UVA) are rare or carried out in rodents. The primary epithelial characteristics in the adult vagina and uterus are determined during prenatal development and depend on epithelio-mesenchymal stroma interaction; thus an investigation summarizing temporal and local molecular events in the entire human UVA is still missing.
We therefore phenotyped epithelial and mesenchymal characteristics in sagittal sections from 24 female fetuses and two female newborns by immunostaining with cytokeratins (CKs) 8, 13, 14, 17, p63, bcl-2, bmp4, CD31, VEGF, SMA and vimentin.

Epithelial differentiation followed a caudal-to-cranial direction in the UVA. Due to the cytokeratin profile of CK 8, 13, 14, the characteristics of the different epithelial zones in the UVA could already be recognized in middle-age fetuses. Vaginal epithelium was derived from the urogential sinus (UGS) in the lower portion and induced the transformation of vimentin-positive Müllerian epithelium in the upper vaginal portion. During prenatal development the squamo-columnar junction (SCJ) was clearly detectable from week 24 onwards and was always found in the endocervix. Early blc-2 positivity within the surrounding mesenchyme of all the vagina including the portio region pointed to an organ-specific mesenchymal influence.
Prenatal findings in human specimens clearly show that fornix epithelium up to the SCJ is of vaginal Müllerian origin, the (endo)cervical epithelium cranial to the SCJ is of uterine Müllerian origin and includes cells with plasticity to transform into squamous epithelium.

Kategorie: Poster

Poster 48

Rubrik: Entwicklungsbiologie

Titel: Promiscuous epithelial/mesenchymal localization of prominin-1(cd133) versus exclusively epithelial confinement of prominin-2 in developing mice

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Abstract:
The initial description of murine Prominin-1/CD133 highlighted a confinement of this molecule to certain embryonic and adult epithelial cell populations and its propensity for being localized in plasmalemmal protrusions. Although expression of this molecule in human hematopoietic stem/progenitor cells was early on recognized, its non-epithelial expression in the body, under physiological conditions, was not described.

Murine embryonic tissues composed of epithelial and non-epithelial (i.e. mesenchymal) cells were analyzed by mapping the spatial distribution of Prominin-1/CD133 transcripts - in comparison to its sister molecule (paralogue) Prominin-2 - by non-radioactive in situ hybridization combined with immunohistochemical detection of PCNA. A markedly distinct localization of the two Prominin-paralogues was uncovered. At embryonic day 11.5, besides embryonic epithelia both mesoderm– and neural crest–derived mesenchyme have shown significant Prominin-1/CD133 expression. In the developing limb, for instance, the mesenchymal cells giving rise to muscles and the primordial skeleton were strongly labeled for Prominin-1/CD133. The apical ectodermal ridge (AER) of the overlaying surface ectoderm was, however, devoid of this transcript. By contrast, expression of Prominin-2 was exclusively epithelial, marking also the AER of the developing limb. Other regions of the body were also characterized by significant mesenchymal expression of Prominin-1/CD133, while expression of Prominin-2 was kept restricted to the epithelium. Thus, proliferating ectomesenchymal cells of the developing face marked by PCNA, contained Prominin-1/CD133 transcripts that were negative for Prominin-2. Both genes were, however, simultaneously detectable in the nasal epithelium.

These data collectively show a promiscuous epithelial/mesenchymal localization of Prominin-1/CD133 over an exclusively epithelial confinement of Prominin-2.

Kategorie: Poster

Poster 49

Rubrik: Entwicklungsbiologie

Titel: Myocilin modulates programmed cell death during retinal development

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Abstract:
Purpose: Myocilin is a secreted glycoprotein of the olfactomedin family that modulates Wnt-signaling. The biological function(s) of myocilin are largely unclear. Mutations in myocilin are causative for some forms of glaucoma, a neurodegenerative disease that is associated with apoptosis of retinal ganglion cells. In previous studies we observed that myocilin-deficient mice have a higher number of optic nerve axons and retinal ganglion cells than their wild-type littermates. In the present study we investigated if this phenotype is caused by differences in programmed cell death during retinal development.
Methods: Myocilin-deficient mice and βB1-Crystallin-Myocilin mice with ocular overexpression of myocilin were investigated. Apoptosis of retinal neurons during development was visualized by TUNEL-labeling and quantified. Western blotting was used to investigate Wnt/β-catenin and PI3K-Akt-signaling.
Results: Myocilin-deficient mice show less apoptosis of retinal neurons in the different retinal layers during developmental programmed cell death. This effect is completely rescued when myocilin-deficient mice are crossed with βB1-Crystallin-Myocilin mice with ocular overexpression of myocilin. Western blot analysis does neither indicate involvement of canonical Wnt/β-catenin nor of PI3K-Akt-signaling.
Conclusions: Myocilin modulates programmed cell death during retinal development, an effect that leads to a higher number of optic nerve axons and retinal ganglion cells in myocilin-deficient mice.

Kategorie: Poster

Poster 50

Rubrik: Reproduktionsbiologie

Titel: Influence of a maternal diabetes mellitus type 1 on epigenetic processes in rabbit preimplantation embryos

Autoren: Knelangen J.(1),Baisch H.(1),Schindler M.(1),Gürke J.(1),Hauke E.(1),Fischer B.(1),Navarrete Santos A.(1),

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Abstract:
Developmental conditions experienced by the embryo in utero programme metabolism and predispose for pathophysiological alterations later in life and may pioneer functional impairment in senescence. The embryo and embryonic cells are sensitive to nutritional and hormonal changes and have an astonishing capacity to adapt within a short time to metabolic changes to secure survival and differentiation (metabolic programming. DNA methylation and histone modifications are two epigenetic mechanisms, which seem to be responsible for metabolic programming effects.
To study effects of maternal diabetes mellitus on early embryo development we induced a type I diabetes through alloxan treatment of female rabbits. In six-day-old blastocysts from diabetic rabbits histone modifications like methylation and acetylation marks of lysine 9 in the histone H3 were measured by specific antibodies. The methylated marks are connected to inactive and the acetylated ones to active chromatin. In the blastocysts of diabetic rabbits the lysine 9 methylation of H3 (H3K9me3) was increased and the lysine acetylation (H3K9ac) decreased.
SUV39H1 is a methyltransferase that methylates H3K9. The acetylation of H3K9 is removed by the histone deacetylase HDAC1. Both enzymes have key functions in heterochromatic gene silencing. SUV39H1 and HDAC1 expression were analysed by qRT-PCR in rabbit blastocysts. With the start of gastrulation we found an increase in SUV39H1 and HDAC1 mRNA. These enzymes will now be studied in diabetic blastocyst.
Maternal diabetes influences histone modifications and the expression of related enzymes in the preimplantation embryo, providing a potential mechanism for early metabolic programming.

Kategorie: Poster

Poster 51

Rubrik: Reproduktionsbiologie

Titel: Foetal chromosomal abnormalities

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Abstract:
Chromosomal abnormalities are characterized by the existence of an excess or a deficiency of genetic material. The most common are trisomies 21, 13, 18. The small number of early neonatal deaths, up to 48 hours after birth (0.11% in boys and 0.22% in girls) among foetuses with abnormalities, is due to intrauterine death. In trisomy 18 (Edwards syndrome) cases an unknown number miscarrying during the first trimester and about 70% of spontaneous miscarriages happen during the second and third trimesters. 30% of those who are born with trisomy 18 die within the first month of life and 90% in the first year of life. Disease incidence is estimated at 1/6000 live newborns, increasing with maternal age. Regarding the foetal abnormalities, it was estimated that on the first place were the foetal heart malformations (1/175 births), Down syndrome (1:800), all these problems "going" as common diseases, whose frequency is less than 1 in 1000 births. Low incidences have the chromosomal syndromes, showed a percentage of 0.61% of the total number of present abnormalities, 11 cases. The incidence of chromosomal abnormalities throughout the study group is 0.60%, of which 0.49% is Down syndrome cases and 0.11% is trisomy 18. The incidence of Down syndrome is 1/800 - 1/700 in newborns, and 70%, 80% of foetuses with Down syndrome have increased nuchal translucency at 10-14 weeks of gestation. Keywords: chromosome, foetus, Down syndrome.

Kategorie: Poster

Poster 52

Rubrik: Reproduktionsbiologie

Titel: Ca2+ clearance mechanisms in prostate cancer cell line du145

Autoren: Wolf A.(1),Szczyrba J.(1),Wandernoth P.(1),Wennemuth G.(1),

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Abstract:
Ca2+ is one of the most important signal molecules involved in many important signal cascades. Deregulation of Ca2+ influx and efflux triggers cell differentiation and plays an important role in cancer development. Furthermore, several kinds of cancer are associated with changes in certain Ca2+ pump activity. However, the clearance mechanisms of Ca2+ homeostasis in different cell types are not yet fully understood.

By measuring [Ca2+]i with FURA2-AM we characterized the influence of the four most important Ca2+ pumps/exchangers (PMCA, SERCA, MCU and NCX) in DU145 PCa cells. After inducement of [Ca2+]i peaks by histamine, the decrease in Ca2+ efflux was analyzed under different inhibitory conditions. We discovered that PMCA and SERCA, in contrast to MCU and NCX, are involved in Ca2+ clearance in DU145. The clearance after PMCA or SERCA inhibition at 600nM [Ca2+]i was approx. 25.9nM/s and 16.9nM/s lower than under control conditions, respectively. The highest effect for both pumps was detectable in lower [Ca2+]i ranges. PMCA-blocked cells reached a [Ca2+]i plateau of 356.8nM and did not return to baseline. SERCA-blocked cells with a concentration lower than 160nM displayed a clearance rate of <-0.79nM/s. After combined PMCA/SERCA inhibition no further clearance was detectable. Additionally, SERCA pump activity was reduced 72h after incubation with 100nM androstan.

In summary, we identified PMCA and SERCA as the two most important pumps in the regulation of [Ca2+]i in DU145 and discovered androstan-dependent changes in pump activity. These results may contribute towards the understanding of [Ca2+]i homeostasis and prostate cancer development.

Kategorie: Poster

Poster 53

Rubrik: Reproduktionsbiologie

Titel: Resveratrol and desferoxamine protect oxldl-treated granulosa cell subtypes from degeneration

Autoren: Schube U.(1),Nowicki M.(1),Hmeidan F.(2),Blumenauer V.(2),Bechmann I.(3),Serke H.(1),

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Abstract:
The effects of resveratrol (RES) and desferoxamine (DFO) are currently a topic of numerous animal and human studies. It is reported that both substances exert a wide range of pharmacological effects: anti-inflammatory and antioxidant. The present study was designed to determine whether RES and DFO prevent follicle cell subtypes from the oxLDL-dependent degeneration. We examined effects of the compounds on apoptosis, cell proliferation, and survival.
Follicle cells were obtained from patients undergoinig IVF-therapy. Cultures of cytokeratin-positive/negative (CK+, CK-) granulosa cells and of cumulus cells were treated with 150 µg/ml oxLDL and 30µM/ml RES, DFO or normal LDL (nLDL) under serum-free conditions for up to 36 h. Dead cells were determined by uptake of propidium iodide. The oxLDL-binding receptor such as LOX-1, toll-like receptor 4 (TLR4), and CD36 as well as HO-1, cleaved caspase-3 (an apoptosis marker) and LC3 (an autophagy marker) were examined by Western blots.
The oxLDL-degeneration of the follicle cells were preventing by effects of RES- and DFO-treatment. It was confirmed by: 1) lack of cell death using propidium iodine uptake; 2) the reduced expressions of the oxLDL-binding receptors LOX-1, TLR4 and CD36, and 3) the increased expressions of HO-1 were decreased under oxLDL-RES/oxLDL-DFO conditions. RES and DFO protect oxLDL-dependent degeneration of follicle cell subtypes.

Kategorie: Poster

Poster 54

Rubrik: Reproduktionsbiologie

Titel: Leptin-deficient (ob/ob) mouse ovaries show fatty degeneration, enhanced apoptosis and decreased expression of steroidogenic acute regulatory enzyme

Autoren: Serke H.(1),Nowicki M.(1),Kosacka J.(2),Schröder T.(1),Klöting N.(3),Blüher M.(2),Kallendrusch S.(1),Spanel-Borowski K.(1),

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Abstract:
The majority of follicles disappear by follicular atresia and obese women are often infertile. We suggest that the follicular atresia is driven by oxidative stress caused by obesity. Leptin-deficient (ob/ob) mice are obese and infertile. Dysfunctions of the ovaries are preferentially related to leptin-deficiency. In the current study, we investigated the morphological and functional obesity-dependent changes in ob/ob ovaries. Ovaries were obtained from three-month-old mice either homozygote (ob/ob) and heterozygote (ob/+) or wild-type (C57BL6, WT) for the investigation by light and electron microscopy, Western blot analysis of LOX-1, TLR4, CD36, cleaved caspase-3, LC3, and StAR.Ob/ob ovaries lacked corpora lutea and follicular atresia was at a higher rate compared to controls with corpora lutea. Follicle cells and oocytes accumulated lipid droplets and were characterized through damaged mitochondria and the basement membrane of follicles was thickened. LOX-1 and CD36 expressions were comparable in all three groups. Ob/ob ovaries showed significantly higher levels of TLR4 and cleaved caspase-3 than controls. The high LC3-II/I ratio in the WT and ob/+ ovaries was related to the presence of corpora lutea. The StAR protein was lower in the ob/ob ovaries signifying reduced of steroidogenesis.
Thus, our data supports the view that infertility in obesity is due to direct ovarian dysfunction.

Kategorie: Poster

Poster 55

Rubrik: Reproduktionsbiologie

Titel: Function of cgmp hydrolyzing pdes in epididymis of rat and men

Autoren: Feuerstacke C.(1),Mietens A.(2),Tasch S.(1),Schneider-Huether I.(1),Müller D.(1),Middendorff R.(1),

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Abstract:
During transit through the epididymis, immature and immotile spermatozoa acquire fertilizing capacity and motility. Contractility of epididymal smooth muscle cells therefore contributes to male fertility. Smooth muscle cell function is regulated by the cGMP signalling pathway. By hydrolysing cGMP, phosphodiesterases (PDEs) limit a given cGMP signal and thus impact on epididymal function. However, knowledge of PDEs in the epididymis is limited. In laser-microdissected rat epididymal smooth muscle cells PDE3A, PDE3B and PDE2A could be detected by RT-PCR. Interestingly, PDE3A and PDE3B were absent in human tissue. Function of both, PDE3 and PDE2, was investigated in organ bath studies, using isolated rat epididymal duct segments from the mid-cauda region. A regular spontaneous contraction pattern was observed and the PDE3-specific inhibitor trequinsin dose-dependently reduced contraction frequency. Similarly, the PDE2 inhibitor BAY60-7550 slowed down spontaneous contractions. The cGMP-specific PDE5 was found in epididymal smooth muscle cells of both rat and man by immunohistochemistry and RT-PCR after laser microdissection. Sildenafil, a specific PDE5 inhibitor, reduced the frequency of spontaneous epididymal contractions.

This investigation, localizing PDE2, PDE3 and PDE5 to epididymal smooth muscle cells, shows species-specific differences in PDE expression and demonstrates relevance of PDEs in epididymal contractile function. Our results indicate that the various PDEs dramatically influence cGMP signals. It is not known, however, which of these PDEs is most important in vivo. This warrants further investigation.

Kategorie: Poster

Poster 56

Rubrik: Reproduktionsbiologie

Titel: Correlations regarding the alpha-fetoprotein immunoexpression at placental level and its serology in down syndrome

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Abstract:
The alpha-fetoprotein is a protein of foetal origin synthesized by the foetal liver and at the level of the Yolk sac, the protein crosses into the maternal blood thus being traceable serologically. Recently in obstetrics the test for the presence of the maternal serum alpha-fetoprotein is increasingly used being mandatory in high teratogenic risk pregnancies. Alpha FP per se offers a series of data regarding the future product of conception, by itself or in association with other serum constants, in the so called triple, quadruple and the latest penta screening tests. In all types of tests the identification of AFP is the first and is mandatory in the maternal serum in any type of screening.
At the level of the maternal serum the detection of the AFP is done between the 15th and 20th week of pregnancy and if its level is increased can show the possibility of certain developmental disorders especially defects of the neural tube or of the cephalic pole whereas a low AFP would indicate Down syndrome. The hereby study's goal is to make a retrospective correlation between the maternal serum values and the alpha-fetoprotein immunoexpression at placental level in 26 cases of pregnancies that resulted products of conception with Down syndrome.
Key words: alpha-fetoprotein, Down syndrome, placenta, immunohistochemistry, serology.

Kategorie: Poster

Poster 57

Rubrik: Immunbiologie

Titel: Decreased NK cell functions in obesity can be re-activated by weight loss

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Abstract:
Obesity is an increasing socioeconomic health problem. Obese patients have an increased risk of developing several types of cancer. NK cells are an integral component of the innate immune system, both in the production of cytokines (e.g. IFN-gamma) to stimulate other immune cells and in the direct destruction of cancer cells. Former studies of our group have shown altered Natural Killer (NK) cell activation in rodent and human obesity. The present study investigated a potential normalization of NK cell function by body long-term weight reduction. A three-month standardized activity and nutrition program was conducted with 24 male and female healthy obese (BMI > 30) individuals. Additionally, 15 male and female age- and body weight matched persons served as controls. Results show a significant body weight reduction (-7kg) and an impressive decreased percentage of body fat (from 38% to 33%). Interestingly, the body weight and fat reduction was more pronounced in male subjects. FACS analyses showed no significant effect on numbers of NK cells in the experimental group. However, the decreased expression of TRAIL (TNF-alpha Related Apoptose Inducing Ligand) could be significantly enhanced after body weight loss. Furthermore, levels of intracellular IFN-gamma were significantly increased in male participants. In the control group all investigated parameters remained unchanged. In conclusion the present study shows, for the first time, a reversibility of decreased NK cell functions by body weight reduction in obese individuals. This implicates an important role of weight loss in restoring an effective NK cell defense in tumor surveillance.

Kategorie: Poster

Poster 58

Rubrik: Immunbiologie

Titel: Acute lung infection with pseudomonas aeruginosa in a f344 rat model

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Abstract:
CD26, a type II transmembrane glycoprotein, is highly expressed in the lung. Its main functions are cleavage of some of the inflammatory mediators, interaction with extracellular matrix and co-stimulation of T-cells. Pseudomonas aeruginosa (PA) is one of the most frequent pathogen inducing nosocomial infections. We want to determine the influence of CD26 deficiency to the compartment specific distribution of PA and the degree of the structural damage after an acute bacterial infection.

CD26 positive and CD26 deficient Fischer rats were intratracheally instilled with a fluorescent PA (1x1010 CFU, TB CF 10839) or with NaCl. The distribution of the fluorescent PA in the narcotized rats was evaluated immediately and 6h after instillation using the IVIS (in vivo imaging system). Then the rats were sacrificed, the inflated lungs were fixed by immersion and sampled for light and electron microscopy.

The CD26 positive animals show a tendency to higher CFU values in the lung. Occasionally numerous bacteria and cell detritus were found in the alveoli. However, only few bacteria were found in the alveolar septa. Furthermore, in severely but also in slightly infected areas a more or less pronounced intraalveolar edema, mostly in combination with swelling or fragmentation of the alveolar epithelium, was seen. The surface fraction of intact alveolar epithelium did not differ in both groups. Thus, PA given in a high dosis leads to a significant damage of the blood gas barrier that results in intraalveolar edema formation in both subtypes. Detailed stereological analysis is under way.

Kategorie: Poster

Poster 58a

Rubrik: 7.Immunbiologie

Titel: Disruption of MIF–CD74 signaling abolishes microglial paralysis in malignant gliomas*

Autoren: Savaskan N.(1), Schwarz M.(1),Buchfelder M.(1),Eyüpoglu I.(1),

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Abstract:
Malignant glial brain tumors proliferate and infiltrate brain parenchyma by evading the
inert immune system. Here we searched for paracrine mediators contributing to this immune escape phenomenon and identified macrophage migration inhibitory factor (MIF) as an essential player in glioma-induced microglia paralysis. We performed a survey of all public cancer data bases such as Rembrandt NCBI, BioGPS, and ONCOMINE to evaluate MIF-CD74 in human brain tumors. Further, standard expression and RNAi protocols and syngenic orthotopic brain tumor implantation models were facilitated. Analysis of human samples reveals that MIF is expressed at
high levels in malignant gliomas and is present in cerebrospinal fluids of glioma patients. MIF knock down in vivo leads to alleviated tumor growth and prolonged survival compared to wild-type gliomas. Microglia, the resident inert immune cells of the brain, showed increased infiltration into tumor bulk in MIF deficient gliomas. We investigated MIF signaling in microglia and identified CD74 as the mediator for MIFinduced
microglial paralysis. Silencing CD74 in microglia leads to allayed
downstream ERK1/2 activation and enhanced microglial migration. Furthermore, CD74 comprised microglia attacked glioma cells in vitro and reduces glioma proliferation in a contact-independent manner. Microglial cells with inhibited CD74 infiltrated brain tumors and reduced glioma proliferation, leading to prolonged survival
in vivo. Further analysis revealed that inhibition of CD74 receptor enhances IFN-γ expression in microglia. Moreover, IFN-γ treatment shows tumor growth inhibition activity and alleviates tumor infiltration into brain parenchyma.
We identified glioma-derived MIF secretion as an essential part in bain tumor immune
escape. Interference with glioma induced MIF or microglial CD74 receptor expression
in either way provides the potential for disrupting microglial paralysis in malignant brain tumors.
**This study is supported by the DFG and the ELAN Fonds.*

Kategorie: Poster

Poster 59

Rubrik: Neuroregeneration/Neurodegeneration

Titel: Per-1 deficiency increases susceptibility to brain damage after cerebral ischemia: apoptosis versus autopahgy?

Autoren: Wiebking N.(1),Maronde E.(1),Stehle J.(1),Rami A.(1),

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Abstract:
An increased ischemia incidence during the morning is well documented. It cannot be excluded that neurons exhibit a daytime-dependent variation in the activation of cell death predictive markers in response to ischemia. The clock gene Period1 (Per1) is a central element of circadian oscillators. In the mammalian brains, Per1 is not only expressed in the olfactory bulb, amygdala, cerebellum, but also in the hippocampus. The hippocampus exhibits a high vulnerability to cerebral ischemia. In order to define the possible role of the PER1 in ischemia-induced cell damage, experiments were performed to compare the cell death machinery in the hippocampus of per1–/–mice to those of wildtype mice after forebrain ischemia and reperfusion. Although neuronal death in the hippocampal CA1-subfield was observed in both types of mice, the damage in per1–/–mice was more severe (68% vs. 45%). Because cell death induced by ischemia is both apoptotic and autophagic in nature, an analysis of circadian variation of predictors of cell death was monitored in order (1) to explore the mechanisms underlying the excessive vulnerability of the hippocampus in per1-/- mice and (2) whether hippocampal susceptibility inherits a daytime component. We found higher levels of the proapoptotic factors cytochrome c and Apaf-1 in per1-/- mice. In addition the autophagy marker LC3B was dramatically reduced in per1–/–mice. Our data suggests that basal activities of apoptosis and autophagy are modulated by the clock gene product PER1, and that the autophagic machinery is slowed down when PER1 is absent, possibly related to an accumulation of dysfunctional mitochondria.

Kategorie: Poster

Poster 60

Rubrik: Neuroregeneration/Neurodegeneration

Titel: Beclin-1 deficiency enhanced neuronal vulnerability to apoptosis by slowing the autophagic machinery induced by rapamycin or amino acid starvation

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Abstract:
Basal autophagy is constitutive and needed for normal cell mechanisms to clean up damaged organelles and long-life proteins. Beclin-1 is a critical component in the class III PI3 kinase complex that induces the formation of autophagosomes in mammalian systems. Mice that carry heterozygous disruption of Beclin 1 have a high incidence of spontaneous tumours, and cells with reduced Beclin 1 expression exhibit reduced autophagic activity.
This study examined the potential role of Beclin-1 in an autophagic response in hippocampal HT22 neurons challenged with Rapamycin or amino acid starvation (AAS). Rapamycin or AAS in wild type cultures induced light chain-3 (LC-3)-immunopositive and monodansylcadaverine (MDC) fluorescent dye-labelled autophagosome formation. However in AAS as well as Rapamycin-treated cultures, the autophagic flux was dramatically reduced in Beclin-1 knockdown cells compared to controls. In addition, AAS induced neuronal death without affecting caspase-3-, AIF- or HtrA2-levels. In contrast, in Beclin-1 knockdown HT22 neurons, AAS induced a dramatic upregulation of AIF, a caspase-independent neuronal death, a decrease in the LC3-II/LC3-I ratio, and reduced accumulation of autophagosomes.
Collectively, this study shows that Rapamycin or AAS induced autophagy in cultured hippocampal HT22 neurons. Our data further show that inhibition of autophagy by a knockdown of Beclin-1, (1) enhanced susceptibility to proapoptotic signals, (2) underlines that Beclin-1 may play a role in antiapoptotic signalling and (3) that autophagy is per se a protective than a deleterious mechanism.

This study was supported by a grant of the “Adolf-Messer-Stiftung” to A.R.

Kategorie: Poster

Poster 61

Rubrik: Neuroregeneration/Neurodegeneration

Titel: An autophagic mechanism is involved in hippocampal neurodegeneration upon epileptic seizures in the neonatal rat brain

Autoren: Benz A.(1),Niquet J.(2),Langhagen A.(1),Rami A.(1),

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Abstract:
Hippocampal cell loss the after status epilepticus (SE) remains the most commonly observed lesion. Although apoptosis and necrosis are frequently analyzed, cases of autophagic cell death are relatively few. Autophagy is a highly regulated cellular mechanism for the bulk degradation of cytoplasmic contents which seems to be implicated in a variety of physiological and pathological conditions. Our goal was to examine whether autophagy is involved in mechanisms of cell death after epilepsia. To do so, we used the lithium-pilocarpine rat model of status epilepticus and investigated a variety of autophagy markers in the hippocampus by immunoblotting and immunocytochemistry. We examined the dynamics in the expression of the following proteins: LC3, Beclin-1, Bag3, Hsp70, p62/SQSTM1, p-mTOR/mTOR, Atg3, Atg5, Atg7, Atg12, Atg14, Lamp after SE. Protein levels of autophagic markers were dramatically affected in epileptic rats with, however, altered dynamics compared to controls. Levels of LC3 and Atg5 were dramatically and significantly increased, whereas Beclin-1 levels were unchanged after SE. The dynamics in the expression of Bag3, Hsp70, p62/SQSTM1 and p-mTOR/mTOR were significantly altered and we were also able to characterize the expression of Atg3, Atg7, Atg12, Atg14, Lamp1 following SE. In summary, our results demonstrate for the first time that SE in the immature brain results in significant alterations of autophagy dynamics. Autophagy has an ambiguous function towards cell death and survival. While autophagy promotes cell survival in several situations, it can also lead to cell death in other circumstances. Further studies are necessary to clarify the connections bridging these pathways.

Kategorie: Poster

Poster 62

Rubrik: Neuroregeneration/Neurodegeneration

Titel: The gpr55-ligand l-alpha-lysophosphatidylinositol (lpi) limits neuronal death after excitotoxic damage and initiates microglia migration in vitro

Autoren: Kremzow S.(1),Kallendrusch S.(1),Koch M.(2),Dehghani F.(2),

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Abstract:
Cannabinoids are known to limit neurodegeneration and neuroinflammation through the G-protein coupled receptors (GPR) cannabinoid receptor 1 and 2 (CB1/2). However, CB1/2 knock-out mice indicate the existence of additional targets for cannabinoids such as GPR55, which became of special interest due to activity changes in the presence of various cannabinoids. L-alpha-lysophosphatidylinositol (LPI) reflects the so far best characterized ligand of GPR55. In rat Organotypic Hippocampal Slice Cultures (OHSC) we studied N-methyl-D-aspartate (NMDA; 50 µM) induced excitotoxic neuronal death and microglia activation in the absence and presence of LPI. The effect of LPI on rat primary microglia cultures was examined by use of the Boyden Chamber, Western Blot and FACS analyses. After NMDA-lesion of OHSC, LPI mediated activation of GPR55 was neuroprotective and reduced the accumulation of microglia at sites of neuronal injury, namely the dentate gyrus granule cell layer. LPI mediated neuroprotection was not observed in microglia depleted OHSC, pointing toward a possible microglia dependent mechanism. LPI enhanced microglia migration, but attenuated LPS and ATP induced migration. Comparing LPS and LPI treatment to single LPS treatment no changes in phosphorylation of the MAPK p38 and p42/44 neither in MCP-1 nor TNF-α content were detected. In contrast, iNOS and the cytokine IL-1-alpha were elevated after concomitant LPS and LPI treatment in comparison to LPS alone. These findings show a relevant role of GPR55 in neurodegenerative processes that are most likely mediated by microglial cells.

Kategorie: Poster

Poster 63

Rubrik: Neuroregeneration/Neurodegeneration

Titel: Osteopontin (Opn) expression and associated opn receptor regulation of prevalent integrins and CD44 on the rgc5 cell line

Autoren: Lehmann J.(1),Garreis F.(2),Neumann C.(2),Hemmerlein M.(2),Paulsen F.(2),Scholz M.(2),

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Abstract:
Osteopontin (OPN) is an age-dependent increased aqueous humor factor, significantly associated with degenerative changes of the optic nerve and the retina in DBA2/J mice. By modulating the metabolism of neuronal cells OPN may contribute to wound healing, neovascularization, neuroprotection and remodeling of extracellular matrix in the eye. In this study, we analyzed in vitro the effect of OPN on murine neuronal precursor cells (RGC5) after induction of oxidative stress. Basal expression of OPN as well as the expression of prevalent integrin-subunits and the cell-surface glycoprotein CD44 has been analyzed by RT-PCR. Western Blot analyses and immunofluorescence were performed to confirm expression of all investigated receptors on protein level. Regulation of OPN receptor expression after stress induction with H2O2 (150 µM) was analyzed by Real time RT-PCR. The metabolic cell activity of OPN treated RGC5 cells was investigated after blocking the according receptor pathways using a CellTiter 96 Aqueous MTS Assay. In addition we characterized for the first time the physiological and morphological eye phenotype of the OPN-/- mouse at different ages in comparison to age-matches DBA2/J and C57/Bl6 mice by intraocular pressure detection, ERG measurements and light-/electron microscopy.

Kategorie: Poster

Poster 64

Rubrik: Neuroregeneration/Neurodegeneration

Titel: Influence of neuroprotective drugs on retinae in a glyoxal induced model of diabetic retinopathy and in an alzheimer's disease mouse model

Autoren: Löffler J.(1),Knels C.(1),Wurm A.(1),Karich F.(1),Valtink M.(1),Funk R.(1),Ader M.(2),Knölker H.(3),Schröder C.(4),Knels L.(1),

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Abstract:
Objective: Diabetes mellitus and Alzheimer´s disease are accompanied by retinal neurodegeneration with amyloid plaque deposition via the pathologic amyloid precursor protein (APP) cleavage pathway. Here we tested the potential of neuroactive substances to interfere with formation of advanced glycation end products (AGEs) and/or beta-amyloid plaque deposition.
Methods: Retinal explants from wt C57BL/6 mice were cultured in vitro, treated with glyoxal to induce AGE formation, and incubated with memantine, galantamine, and beta-secretase (BACE) inhibitor N-1590. Retinal explants from adult double transgenic SwAPP/Psen1d9 mice (Alzheimer´s disease model) were cultured and likewise incubated with these neuroactive substances or BACE inhibitor PL166. Samples were then analysed by Western blotting, reverse transcription polymerase chain reaction (RT-PCR) and immunofluorescence staining for AGEs, amyloids, and apoptosis and stress markers (CML, alpha- and beta-secretases, APP cleavage products sAPP-alpha and sAPP-beta, Bax, heme oxygenase-1).
Results: Wt C57BL/6 retinas treated with glyoxal displayed increased pathological processing of APP and amyloid plaques. As shown by immunofluorescence staining and Western blotting, pathogenic amyloid formation was counteracted by memantine, galantamine and N-1590. Formation of AGEs was not altered, but apoptosis and cell stress markers were decreased by neuroactive substances. Memantine or BACE inhibitor PL166 treatment led to increased levels of neuroprotective sAPP-alpha in retinal explants of SwAPP/Psen1d9 mice already after 1 day. After a 3-day, PL166 treatment pathogenic sAPP-beta and amyloid levels were decreased.
Conclusions: Galantamine and memantine are protective against AGE-induced (diabetic-type) retinal degeneration, while BACE inhibition counteracts both AGE-induced and Alzheimer-associated retinal degeneration.

Kategorie: Poster

Poster 65

Rubrik: Neuroregeneration/Neurodegeneration

Titel: CEACAM expression at the ocular surface and in the lacrimal apparatus

Autoren: Garreis F.(1),Hoffmann S.(1),Schröder H.(1),Singer B.(2),Paulsen F.(1),

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Abstract:
The carcinoembryonic-antigen-related cell-adhesion molecules (CEACAMs) are members of the immunoglobulin superfamily and associated with various cellular processes, mediate intercellular adhesion, tumor development and function as receptors for pathogenic microorganisms. All CEACAMs are highly glycosylated, membran-bounded and show a heterogeneous expression pattern in different cell types. In this study, we analyzed the expression profile of epithelial CEACAM1 and -6. For this, we systematically analyzed different human tissues of the lacrimal apparatus and ocular surface from body donors by RT-PCR and immunohistochemistry. In addition, we analyzed ultrathin sections from human eyelid by electron microscopy to identify the subcellular distribution of CEACAM1. The inducibility and regulation of CEACAM1 was studied in cultivated human corneal (HCE) as well as conjunctival epithelial (HCjE) cells after challenge with different pathogen-associated molecular patterns (PAMPs) and proinflammatory cytokines by real-time RT-PCR. Immunohistochemical results revealed expression of CEACAM1 in acinus cells of the lacrimal gland and accessory lacrimal glands of the eye lid. CEACAM1 and -6 expression was also detected in apical cells of the lid wiper, a region of the marginal conjunctiva that wipes the ocular surface during the blink. Furthermore, RT-PCR analysis revealed expression of CEACAM1 and -6 in cultivated HCE and HCjE cells. In HCE cells three different CEACAM1 splice variants, CEACAM1-4L (long cytoplasmatic tail), CEACAM1-3S (short cytoplasmic tail) and CEACAM1-4S, were detected. The data suggest that CEACAM1 and -6 are components of the ocular surface and lacrimal apparatus. Further studies will clarify the (patho)physiological role of CEACAMs expression at the lid margin.

Kategorie: Poster

Poster 66

Rubrik: Neuroregeneration/Neurodegeneration

Titel: Conditional knock out of the type 2 tgf- beta receptor in the mouse retina influences angiogenesis

Autoren: Braunger B.(1),Leimbeck S.(1),Volz C.(2),Jägle H.(2),Tamm E.(1),

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Abstract:
Purpose: Proliferation of capillaries in diabetes causes diabetic retinopathy, a major cause of blindness worldwide. The role of TGF-betas in diabetic retinopathy has been discussed controversially, as both anti- and pro-angiogenic properties have been reported. To learn more about the role of TGF-beta signaling for retinal angiogenesis, we generated mice with a conditional deletion of the TGF-beta receptor II which is essential for TGF-beta signaling.
Methods: Floxed Tgfbr2 mice were crossed with CAG-Cre mice with the coding sequences of Cre recombinase under control of a tamoxifen-responsive promoter. Cre-Reporter mice were used to confirm the induction of Cre recombinase by beta-galactosidase staining. Retinal structure and function were studied by microscopy, fluorescence angiography, real time RT-PCR, and electroretinography.
Results: Newborn Tgfbr2 mice/Cre mice were treated with tamoxifen, which resulted in an intense beta-galactosidase staining throughout the entire retina indicating recombination. Western blot analyses and real time RT-PCR confirmed the conditional deletion of TGF-beta receptor II in mixed Tgfbr2-/-;CAG-Cre animals. At four weeks of age, the mice developed pronounced retinal neo-angiogenesis and multiple microaneurysms, a hallmark of diabetic retinopathy. Newly formed vessels showed a thickened vascular wall and were leaky indicating failure of the blood-retinal barrier. Expression of VEGF-A, FGF-2, angiopoetin 2 and IGF was upregulated significantly.
Conclusion: TGF-betas are potent anti-angiogenic factors in the retina, and lack of TGF-beta signaling promotes retinal angiogenesis. We succeeded in generating an animal model to study the molecular pathogenesis of retinal diseases associated with neo-angiogenesis such as diabetic retinopathy and age-related macular dystrophy.

Kategorie: Poster

Poster 66a

Rubrik: 8.Neuroregeneration/Neurodegeneration

Titel:The normal appearing white matter is anything but normal in a murine model of multiple sclerosis

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Abstract:
The analysis of normal appearing white matter (NAWM) pathology in multiple sclerosis (MS) patients is restricted to MR measurements and the examination of post mortem brain tissue. In contrast to the plethora of studies concerning the NAWM in MS patients, only few groups have dealt with NAWM pathology in animal models of MS. To this end, we studied ultra- and semi-thin sections from EPON-embedded transverse segments of the lumbar spinal cord of myelin oligodendrocyte glycoprotein peptide 35-55-immunized C57BL/6 mice. The NAWM was identified as the region which appeared unaltered using light microscopic analysis but was adjacent to microscopically visible lesion sites. We evaluated distinct features of myelin and axonal pathology: the extent of myelin pathology was assessed by measurements of the g-ratio, features of axonal damage covered axonal loss, axolysis, mitochondrial swelling and a decrease in the nearest neighbor neurofilament distance (NNND). Contrasting the physiological appearance of the NAWM in light microscopy, ultrastructural analysis revealed severe nerve fiber pathologies that were not present in non-immunized control mice, but abundant in white matter lesions. Our data suggest that the NAWM is in an unsteady balance between neurodegeneration and neuroprotection and are in line with results obtained in MS patients. Considering the dimension of the NAWM in contrast to focal lesions, it should be of tremendous value for MS patients to keep this CNS region in balance. Our results clearly encourage the development of targeted neuroprotective therapies, which should be adminsistered in addition to the established anti-inflammatory therapies.

Kategorie: Poster

Poster 67

Rubrik: Peripheres und vegetatives Nervensystem

Titel: Dysregulation of iron metabolism in peripheral diabetic neuropathy (pdn) – an imaginary problem or highway to hell

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Abstract:
Peripheral diabetic neuropathy (PDN) is the most often in the midst of complications observed in diabetes. It leads to impaired peripheral nerve regeneration and is connected with structural and functional changes of nerve fibers, endoneural microvessels and Schwann cells. The growing number of evidence shows a relationship between increased body iron storage and diabetes. Iron is essential but also toxic metal. Imbalance of iron homeostasis is prevalent and possibly involved in the pathogenesis of PDN. The aim of this study was to examine the role of iron in PDN development in diabetic rats.
Male Sprague-Dawley rats were housed in six groups. Each experimental group consisted of 6 animals. In three groups, diabetes was inducted by a single intraperitoneal injection of streptozotocin (STZ). The animals were fed for 3 months a high iron-content, standard and iron-poor diet.
In iron-overloaded STZ-rats, the sciatic nerve motor conduction velocity (CV) was significantly decreased already from the first month of the experiment compared to the other groups. The sciatic nerve sensory CV was affected in the iron-depleted STZ-rats. In all diabetic animals, the extension of the minimal F-wave latency was detected. Interestingly, the spatially resolved elemental analysis of dorsal root ganglia revealed increased iron concentration in neurons and satellite cells of STZ-rats fed with standard diet compared to the non-diabetic ones.
In this preliminary study it has been shown directly for the first time that Diabetes mellitus is connected with iron distribution disorders in nerve cells. Moreover, both iron-overload and iron-depletion accelerate development of PDN.

Kategorie: Poster

Poster 68

Rubrik: Peripheres und vegetatives Nervensystem

Titel: Effects of artemin on trpa1 and trpv1 sensitivity in jugular c-fibers

Autoren: Weske A.(1),Wiegand S.(1),Nandigama R.(1),Kummer W.(1),Nassenstein C.(1),

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Abstract:
Neurotrophic factors have been shown to alter the functional properties of sensory C-fibers in allergic asthma. Artemin is a neurotrophic factor in the Glial Cell Line-Derived Neurotrophic Factor (GDNF) family of ligands, binds to its receptor GFRα3 and initiates intracellular signaling via co-receptor RET. Previously, we were able to demonstrate that a subpopulation of bronchopulmonary vagal C-fibers, namely the jugular cells, co-express GFRα3 and RET (Nassenstein et al., J Physiol 2010). These cells also showed expression of the transient receptor potential channel vanilloid 1 and ankyrin 1 (TRPV1 and TRPA1). Dysfunction of TRPV1 and TRPA1 receptors (which bind many environmental irritants and endogenous inflammatory mediators) has been associated with an enhanced sensory C-fiber excitability. The aim of the study was, therefore, to investigate if artemin is expressed in the airways and to define the role of artemin on TRPV1 and TRPA1 sensitivity in jugular C-fibers. After we were able to demonstrate that artemin was expressed in murine lungs, dissociated vagal sensory neurons were treated with recombinant artemin (50 ng/ml) for 4 h and 24 h and intracellular calcium was analyzed in neural-crest C-fibers in response to increasing doses of cinnamaldehyde (TRPA1 ligand) and capsaicin (TRPV1 ligand). Interestingly, artemin caused a phenotypic switch in jugular C-fibers by increasing the number of TRPA1 responsive cells. Furthermore, artemin increased the capsaicin-induced intracellular calcium influx in jugular C-fibers Taken together, these results indicate that artemin modulates functional properties of neural crest C-fiber neurons.

Kategorie: Poster

Poster 69

Rubrik: Peripheres und vegetatives Nervensystem

Titel: Innervation of murine brown and white adipose tissue: neurochemistry and quantities

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Abstract:
Thermogenesis in brown adipose tissue (BAT) is controlled by sympathetic and sensory nerve fibres, and sympathetic innervation of white adipose tissue (WAT) is considered to control lipolysis and hormone production. Changes in innervation have been linked to altered physiological states but quantitative data on absolute values of BAT and WAT innervation and detailed data on neurochemical coding have not been provided yet. We here addressed these issues in interscapular (BAT) and epididymal fat pad (WAT) of mice by single- and double-labelling immunofluorescence in wild-type and BAC(chrna3)-eGFP transgenic mice (sympathetic axons express eGFP), and innervation by sympathetic and CGRP-positive axons was assessed by stereology. Qualitatively, BAT and WAT arteries are densely innervated by noradrenergic, NPY-positive axons. Adipocytes in BAT are innervated by 1) noradrenergic, NPY-negative sympathetic axons, 2) CGRP/SP axons, 3) CGRP (SP-negative) axons, 4) neurofilament-positive axons, and only exceptionally by VIP- and NOS-positive fibres. Ultrastructurally, nerve fibres located between adipocytes consisted of partially enveloped, single axons. WAT adipocytes are seldomly approached by noradrenergic, NPY-negative sympathetic and CGRP-positive axons. Total sympathetic and CGRP-positive fibre length in interscapular fat was 143 m and 3.7 m (median of N=4), respectively, corresponding to 1,920 m/cm³ and 56.4 m/cm³. In epididymal fat, values were 6.5 m and 0.4 m, respectively, corresponding to 12.5 and 1.25 m/cm³.
In conclusion, BAT innervation is neurochemically more differentiated and much denser than WAT innervation, surpassing even heart ventricular innervation. Innervation of WAT adipocytes, in contrast, is extremely sparse and most WAT adipocytes lie in far distance from autonomic axons.

Kategorie: Poster

Poster 70

Rubrik: Peripheres und vegetatives Nervensystem

Titel: Gdnf decreases sensitivity of trpv1 receptors in nodose c-fibers

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Abstract:
Sensory C-fibers activation contributes to several symptoms in allergic asthma, e.g. cough, dyspnea and bronchoconstriction. Many environmental irritants that cause asthma attacks (including ozone, cold air, and cigarette smoke) are ligands of the transient receptor potential (TRP) channel family members that include TRPV1. Therefore, it has been speculated that dysfunction of TRP channels is responsible for the enhanced excitability of sensory C-fibers. We hypothesized that GDNF (Glial Cell Line-Derived Neurotrophic Factor) may contribute to an enhanced sensitivity of TRPV1 in bronchopulmonary nodose C-fibers in allergic airway inflammation. TRPV1 and GFRα1, the receptor for GDNF were co-expressed in almost all nodose C-fibers. We then examined GDNF expression in the lung. Single-cell RT-PCR in samples collected by laser-assisted microdissection and immunohistochemistry revealed that it is expressed in airway smooth muscle cells and epithelial cells. Interestingly, GDNF mRNA expression was down regulated in the lungs of mice with allergic airway inflammation. We then checked for allergen-dependent regulation of GFRα1 expression in bronchopulmonary C-fibers by single-cell RT-PCR. GFRα1 mRNA expression levels were slightly reduced in mice with airway inflammation. Finally, we investigated the GDNF effect on TRPV1 sensitivity. Vagal sensory neurons were treated with GDNF and intracellular calcium levels in response to increasing capsaicin concentrations were measured. GDNF stimulation caused a significant decrease in the percentage of capsaicin-responsive nodose neurons. In addition, GDNF reduced the amplitude of calcium influx in capsaicin-responsive neurons.
In conclusion, our data show that the enhanced excitability of sensory neurons in asthma might be linked to a loss of GDNF-dependent inhibition of TRPV1 signalling.

Kategorie: Poster

Poster 71

Rubrik: Peripheres und vegetatives Nervensystem

Titel: Chemosensory brush cells in the mouse urethra

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Abstract:
Respiratory and gastrointestinal epithelia contain a slender epithelial cell type with an apical tuft of microvilli (“brush cells”). Respiratory brush cells are cholinergic and sense bitter substances and bacterial quorum sensing molecules, suggesting that they might serve as general door-keepers monitoring entrance sites into the body. Thus, we hypothesized their presence at a previously unrecognized site, i.e. the urethra, and addressed this issue utilizing appropriate eGFP reporter mouse strains, single- and double-labelling immunohistochemistry, and RT-PCR.
Solitary epithelial cholinergic (ChAT-eGFP positive) cells were observed in the male and female urethra but not in the bladder, ureter and renal pelvis. They are immunoreactive for the brush cell marker, villin, and exhibit apical microvilli at ultrastructural investigation. They are distinct from neuroendocrine cells (labeled with chromogranin A-, PGP9.5- and serotonin-antibodies). ChAT-eGFP positive cells are immunoreactive to TRPM5 (a cation channel of taste cells), and a large subpopulation is immunoreactive to phospholipase C(beta2) and alpha-gustducin (components of the taste transduction cascade). An additional population of villin-positive cells is non-cholinergic. RT-PCR revealed expression of taste receptors Tas1r1, Tas1r3 and Tas2r105 in the urethra. Villin-positive cells are approached by cholinoreceptive (Chrna3-eGFP positive) sensory nerve fibres.
This is the first demonstration of brush cells in the urogenital tract. The data strongly suggest that these cells, much like those in the respiratory tract, sense the luminal environment via canonical taste transduction mechanisms and transmit this information to sensory nerve fibres by cholinergic signalling, thereby serving as sentinels for harmful stimuli and bacterial colonization.

Kategorie: Poster

Poster 72

Rubrik: Zentrales Nervensystem/Signaltransduktion und Verschaltung

Titel: Apoer2/vldlr are required for proper migration and correct positioning of mesencephalic dopaminergic neurons

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Abstract:
Migration of mesencephalic dopaminergic (mDA) neurons from the subventricular zone to the substantia nigra compacta (SNc), ventral tegmental area (VTA), and retrorubral field (RRF) are controlled by a plethora of neurotrophic factors, cell adhesion molecules (CAMs) and extracellular matrix molecules (ECM). Reelin and the cytoplasmic adaptor protein disabled 1 (Dab1) have been shown to play important roles for the migration and correct positioning of mDA neurons. Mice lacking Reelin and Dab1, both display phenotypes characterised by migration failure of mDA neurons. ApoER2 and VLDLR are the signalling receptors of Reelin and, thus, involved in signal transduction to intracellular adaptor such as Dab1. Here, we describe the roles of ApoER2 and VLDLR on normal positioning and migration of mDA neurons in mice. Our results demonstrate that VLDLR and ApoER2 mutant mice show a reduction and malpositioning of mDA neurons. This phenotype was more pronounced in VLDLR mutant mice. Moreover, we provide evidence that ApoER2/VLDLR double-knockout mice show a phenotype comparable with the phenotypes observed for Reelin and Dab1 mutant mice. Taken together, our results clearly demonstrate that the Reelin receptors ApoER2 and VLDLR play essential roles in Reelin-mediated migration and positioning of mDA neurons.

Kategorie: Poster

Poster 73

Rubrik: Zentrales Nervensystem/Signaltransduktion und Verschaltung

Titel: The presumed aatypical chemokine receptor cxcr7 affects astrocytes through gi/o proteins

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Abstract:
SDF-1/CXCL12 binds to the chemokine receptors, CXCR4 and CXCR7, and controls cell proliferation and migration during development, tumorigenesis, and inflammatory processes. It is currently assumed that CXCR7 would represent an atypical or scavenger chemokine receptor which modulates the function of CXCR4. Here, we provide evidence that CXCR7 actively mediates SDF-1 signalling in primary astrocytes through pertussis toxin-sensitive Gi/o proteins. We observed that SDF-1-dependent increases in intracellular Ca2+ concentration as well as activation of Erk and Akt signalling persist in primary astrocytes with depleted expression of CXCR4 whereas all responses are abolished in astrocytes with depleted expression of CXCR7. Likewise, we found that the effects of SDF-1 on astrocytic proliferation and migration require CXCR7, but not CXCR4. We further observed that CXCR7-mediated effects of SDF-1 on astrocytic cell signalling and function are all sensitive to pertussis toxin and, hence, depend on Gi/o proteins. Moreover, consistent with a ligand-biased function of CXCR7 in astrocytes, the alternate CXCR7 ligand, I-TAC, stimulated Erk and Akt through beta-arrestin. The demonstration that SDF-1-bound CXCR7 signals through Gi/o proteins in astrocytes could help to explain some of the discrepancies previously observed for the function of CXCR4 and CXCR7 in other cell types.

Kategorie: Poster

Poster 74

Rubrik: Neuroimmunologie

Titel: Involvement of proinflammatory cytokine interleukin 6 and tumor-necrosis-factor-receptor 1 in innate immune response after bacterial meningitis

Autoren: Albrecht L.(1),Merres J.(1),Tauber S.(2),Pufe T.(1),Brandenburg L.(1),

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Abstract:
The most frequent pathogen that causes bacterial meningitis is the Gram-positive bacterium Streptococcus pneumoniae. By entering the brain, host cells will be activated and proinflammatory cytokines like interleukin-6 (IL-6) and tumor necorsis factor-alpha (TNF-alpha) as well as antimicrobial peptides are released. Two different receptors mediate the functions of TNF-alpha. The tumor necrosis factor receptor-1 (TNFR-1) induces the proinflammatory properties of TNF-alpha, while TNFR-2 attenuates these properties. Antimicrobial peptides fight against infiltrated pathogens. Furthermore they have immunomodulatory functions. Our previous results showed a strong induction of cathelin-related antimicrobial peptide (CRAMP) expression after IL-6 and TNF-alpha treatment in glial cells.
The goal of the requested project is to examine the context between cytokine IL-6 as well as TNFR-1 and CRAMP expression and function in vitro and in vivo via an experimental mice model of Streptococcus pneumoniae-induced meningitis. For the experiments IL-6- and TNFR-1-deficient as well as wildtype mice are used.
Our results showed a higher lethality in vivo after bacterial meningitis in TNFR-1-deficient mice and decreased immune response in TNFR-1-deficient astrocytes in comparison to IL-6-deficient and wildtype mice and astrocytes. Furthermore, the increased lethality of TNFR-1-deficient mice correlated with a decreased CRAMP expression and glial cell activation. The results were confirmed in vitro.
Altogether, the results suggest that TNFR-1 plays an important role in the regulation of the viability of the infected mice and CRAMP expression. Furthermore, the cytokines and antimicrobial peptides protect the organism as well as coordinate und control the innate immune response against pathogens.

Kategorie: Poster

Poster 75

Rubrik: Neuroimmunologie

Titel: Lack of antimicrobial peptide cramp resulted in decreased glial cell activation after bacterial stimulation

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Abstract:
Antimicrobial peptides (APs) are an important part of the innate immune system of many organ systems, yet little is known about their expression and function in the brain. We showed the expression and secretion as well as bactericidal properties of a main antimicrobial family, the cathelicidins, in glial cells after bacterial infection. The expression of the antimicrobial peptide cathelicidin CRAMP/LL-37 is up-regulated in bacterial meningitis, but the consequence of cathelicidin expression and function for progression of inflammation and viability are far from clear. Therefore, we used CRAMP deficient and wildtype mice to investigate the role of antimicrobial peptide CRAMP in inflammation and glial cell activation after bacterial stimulation.
Our results showed a decreased viability and proliferation of CRAMP deficient microglial cell after treatment with different bacterial supernatants, whereas treatment of CRAMP deficient astrocytes resulted in higher cytotoxicity and morphological change using cell viability and cytotoxicity as well as immunofluorescence microscopy. The analysis of inflammatory response revealed increased expression of different proinflammatory cytokines by CRAMP-deficient glial cells using realtime RT-PCR after bacterial treatment. Altogether, the results suggest that CRAMP produced by glial cells plays an important part in the innate immune response against pathogens in CNS bacterial infections.

Kategorie: Poster