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Vortrag 1

Rubrik: 4.Zellbiologie
Abstract Nr.:4

Titel:Expression of CD9 in cultured podocytes.

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Abstract:
Podocytes play an important role in supporting the glomerular blood filtration barrier in the kidney. Their function is based on the formation of foot processes and integrin-mediated adhesion to the glomerular basement membrane. Podocytes are sensitive to mechanical stress. Loss of podocytes is associated with proteinuria and several renal diseases. Recently, some tetraspanins were shown to significantly modulate integrin function. Tetraspanins are enriched in microdomains possibly regulating membrane fluidity and signal transduction. Previous gene arrays revealed the upregulation of CD9 in cultured mouse podocytes after mechanical stress.
We investigated CD9 expression in cultured podocyte cell lines (SVI, E11) and in primary podocytes by Western Blot, RT-PCR, immunocytochemistry and transfection experiments. To elucidate whether mechanical stress affects localization of CD9, cells were stretched under defined conditions as described previously. Double labelling experiments were conducted to find out whether CD9 is colocalized to F-actin, actin-associated proteins (e.g. vinculin, alpha-actinin-1, paxillin) and podocyte specific proteins (podocin, nephrin).
CD9 mRNA and CD9 protein were detected in both podocyte cell lines. CD9 was localized to cell membranes, cell-cell contacts and perinuclear vesicles. At sites of cell-cell contact CD9 was colocalized to actin and actin-associated proteins, occasionally. SVI and E11 podocytes showed delicate membrane protrusions positive for CD9. In most of these protrusions no actin-associated proteins were seen. Under mechanical stress CD9 mRNA increased and CD9 protein was clustered in the cytoplasm.
As CD9 is upregulated and located in the cytoplasm after mechanical stress, this indicates an important role in adhesion and membrane stability.

Kategorie: Vortrag

Vortrag 2

Rubrik: 4. Zellbiologie
Abstract Nr.:

Titel: Post-transcriptional regulation of AQP1 in the proximal tubular brush border membrane leading to increased water reabsorption

Autoren: Theilig F. (1), Pohl M. (1), Petsch T. (1), Shan Q. (2), Rickheit G. (3), Bleich M. (2), Jentsch T. (3), Willnow T. (3), Bachmann S. (1)
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Abstract:
The phenomenon known as glomerulo-tubular balance comprises a load dependent fluid reabsorption within the proximal convoluted tubule which varies directly upon spontaneous alterations of the nephron filtrate. AQP1 is localized in the proximal tubule (PT), here in the BBM and the basolateral membrane. Little is known about its regulation. We have analyzed translocation of AQP1 in BBM and its recycling mechanism. Candidate genes for endocytosis and endosomal retrieval were studied with respect to their role in AQP1 recycling.
Megalin specific Cre/lox mice (Meg/Cre+) and ClC5 specific Cre/lox mice (ClC-5/Cre+) with mosaic proximal defects in endocytosis were used for immunohistochemistry, in situ hybridisation and western blot analysis. OK cells were stably transfected with AQP1 and redistribution of AQP1 upon changes in fluid shear stress (FSS), cAMP, cGMP, and AngII were analyzed.
In both, Meg/Cre+ and ClC-5/Cre+ mice, transgenic PT cells exhibited reduced endocytosis capacity which caused higher abundance of AQP1 in BBM (2.6 fold increase) in comparison to intact PT cells ($p<.05$) . The subapical recycling compartment showed reduced AQP1 abundance in the transgenic cells. AQP1 mRNA abundance was not different between transgenic and control PT cells. In OK cells, short-term changes in FSS, cGMP and cAMP increased cell surface AQP1 expression (>35%, $p<.05$) concomitantly with increased water reabsorption (>80%, $p<.05$) . Short-term AngII treatment produced only minor changes.
Mouse models with reduced PT endocytosis capacity reveal impaired recycling of AQP1. The endocytotic apparatus and acidification are important for the endocytosis and trafficking of AQP1. Short-term changes in FSS, cAMP and cGMP may influence AQP1 trafficking. These changes may play a role in glomerulo-tubular balance and therefore in body water homeostasis.

Kategorie: Vortrag

Vortrag 3

Rubrik: 4.Zellbiologie
Abstract Nr.:4

Titel:Zebrafish as a model to study MYH9-associated kidney disease

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Abstract:
Recent publications have shown that polymorphisms of the MYH9 gene, encoding for the heavy chain of non-muscle myosin II A (NMMHC-IIA) increase the risk for focal-segmental glomerulosclerosis (Kopp et al, 2008).
In studying the influence of NMMHC-IIA on kidney function and morphology, MYH9-deficient mice are not feasible for extensive research because of an early mortality. As a powerful alternative, the zebrafish, combined with MYH9-knockdown via morpholino injection, is an ideal model organsim due to its rapid development, its high optical transparency and the similarity between zebrafish and mammalian glomeruli.
In the present study, fertilized eggs of transgenic zebrafish expressing EGFP in podocytes and tubular epithelial cells (WT1b:eGFP) were injected with morpholinos against MYH9. We observed a phenotype in 96% of the larvae three days post-fertilization (3dpf), similar to the effects of blebbistatin incubation at 200 microM for 24h, an inhibitor of non-muscle myosin II. The larvae were processed for an in vivo filtration assay by injecting Alexa647-labelled 10kDa-dextran and FITC-labelled 500kDa-dextran, showing filtration to be slightly but significantly reduced as compared to controls. Immunohistochemical and ultrastructural analyses revealed morphological changes of the glomeruli of MYH9 knockdown larvae, resulting in enlarged but less abundant capillaries, a partial thickening of the glomerular basement membrane and filopodia formation at the surface of primary foot processes in MYH9-morpholino injected larvae as compared to the control-morpholino injected fish. These results strongly suggest an important role of NMMHC-IIA in the proper formation of the glomerular tuft.

Kategorie: Vortrag

Vortrag 4

Rubrik: 4.Zellbiologie
Abstract Nr.:4

Titel:The molecular anatomy of the substrate binding pocket of organic cation transporter rOCT1

Autoren: Volk C.(1),Gorboulev V.(1),Müller T.(2),Koepsell H.(1),

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Abstract:
Polyspecific organic cation transporters of the SLC22 family are involved in the homeostasis of a great variety of organic cations, e.g. monoamine neurotransmitters or cationic drugs in organs as kidney, liver, and brain. The molecular basis of polyspecificity, however, is not sufficiently understood. Our previous data suggest the existance of a complex binding pocket which includes different interaction domains. In this study, we combined single amino acid mutations and computer-based modelling to achieve detailled insights in the structure of the binding pocket. Modelling the interaction of the inhibitor corticosterone with rat organic cation transporter 1 (rOCT1) in the inward- and outward-facing conformation predicted direct binding to five amino acids in transmembrane helix (TMH) 2 (Phe160), TMH 4 (Trp218), TMH 10 (Arg440, Leu447) and TMH 11 (Asp475). Analysis of point mutations in these positions using Xenopus laevis oocytes or stably transfected HEK cells were performed. The affinity to corticosterone was increased from both sides in mutants Phe160Ala and Leu475Phe and decreased in mutant Asp475Glu, while the amount of inhibition was reduced in mutants Trp218Phe and Arg440Lys. Furthermore, in mutants Phe160Ala, Trp218Tyr, Arg440Lys and Leu447Phe the affinities for the substrate MPP+ and in mutant Asp475Glu the affinity for the substrate TEA+ were changed. Our data strongly suggest that these five amino acids are located within an innermost cavity of the binding cleft that is alternatingly exposed to the extracellular or intracellular side during substrate transport.

Kategorie: Vortrag

Vortrag 5

Rubrik: 4.Zellbiologie
Abstract Nr.:4

Titel:Regulatory phosphorylation pathways of kidney distal tubular Na+,Cl- -cotransporter and the activating role of vasopressin

Autoren: Mutig K.(1),Saritas T.(1),Uchida S.(2),Kahl T.(1),Borowski T.(1),Paliege A.(1),Böhlick A.(1),Bachmann S.(1),

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Abstract:
The Na+,Cl- -cotransporter (NCC) is expressed in the early (DCT1) and the late, aldosterone-sensitive (DCT2), portions of distal convoluted tubule. NCC contributes to the fine regulation of urine electrolyte composition. Acute activation of NCC involves their luminal trafficking and phosphorylation by WNK/SPAK kinases. Vasopressin (AVP)-dependent, type 2 receptor (V2R)-mediated activation has been established for the related transporter, NKCC2, but few data testify its effects on NCC. We evaluated the effects of short term treatment with the V2R-agonist dDAVP on the activation of NCC in DCT1 as compared to DCT2. Brattleboro rats with central diabetes insipidus (DI) and suspensions of isolated rat renal cortical tubules were treated with dDAVP or vehicle for 30 min. Immunohistochemistry and Western blot techniques were applied. Immunoelectron microscopy revealed stronger luminal NCC abundance in DI rats upon dDAVP (+23%; $p<0.05$) suggesting increased surface expression of the transporter. SPAK immunoreactivity was co-expressed with NKCC2 and NCC in rat kidney; dDAVP-induced increases in SPAK phosphorylation (S-motif, +50%; $p<0.05$) and in parallel, NCC phosphorylation (conserved N-terminal T53 and T58 or S71, +241%; $p<0.05$), were registered predominantly in DCT1 but barely in DCT2 as distinguished by 11[beta]-HSD2 immunoreactivity (DCT2). In tubular suspensions, NCC phosphorylation was enhanced as well by dDAVP (+72%; $p<0.05$), confirming a direct role of vasopressin in DCT. These data demonstrate acute V2R-mediated activation of NCC which may effectively serve to adjust fine tuning of transepithelial NaCl reabsorption in kidney distal convolutions. Distinct effects of dDAVP in DCT subsegments illustrate differential hormonal regulation at this site.

Kategorie: Vortrag

Vortrag 6

Rubrik: 6.Reproduktionsbiologie
Abstract Nr.:6

Titel:Mechanisms of bicarbonate entry into sperm

Autoren: Mannowetz N.(1),Raubuch M.(1),Wandernoth P.(1),Becker H.(2),Deitmer J.(2),Wennemuth G.(1),

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Abstract:
HCO3- is an essential factor for both enhanced sperm motility and sperm capacitation. There are two possible mechanisms for HCO3-entry. (i) HCO3- enters directly by anion transporters. (ii) Extracellular carbonic anhydrases (CAs) hydrate CO2 to HCO3- which then can be transported into the cell following pathway (i). We focus on the role of both the anion exchanger CFTR and the extracellular CAIV and CAXIV. We previously have shown that sperm respond to HCO3- with an accelerated beat frequency from ~3 Hz to 8-9 Hz. We analyzed sperm' response to HCO3- in the presence of two different CFTR inhibitors (GlyH-101 and CFTRinh-172). Sperm of CAIV-/- and CAXIV-/- mice were investigated with regard to an accelerated beat frequency in the presence of CO2. We also determined the percental CAIV activity related to total CA activity. We show that in the presence of GlyH-101 and CFTRinh-172, sperm respond to HCO3- just as well as untreated sperm. Superfusing sperm with 2% CO2 reveals, that within one minute, CAXIV-/- sperm respond with an accelerated beat frequency of 7-8 Hz, whereas the CAIV-/- sperm' response towards CO2 is delayed and diminished resulting in a beat frequency of ~4 Hz. Furthermore, the assessment of the enzyme activity shows that 30% of the total CA activity derives from CAIV. Our data suggests, that CFTR does not contribute considerably to an accelerated beat frequency in the presence of HCO3-. However, in the presence of CO2, CAIV might play a major role for the acceleration of sperm beat frequency.

Kategorie: Vortrag

Vortrag 7

Rubrik: 4.Zellbiologie
Abstract Nr.:4

Titel:Desmoglein 2-mediated adhesion is required for intestinal epithelial barrier integrity

Autoren: Schlegel N.(1),Meir M.(2),Heupel W.(2),Holthöfer B.(3),Leube R.(4),Waschke J.(2),

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Abstract:
The integrity of intercellular junctions which form the “terminal bar” in intestinal epithelium is crucial for sealing the intestinal barrier. While specific roles of tight and adherens junctions are well known, the contribution of desmosomal adhesion for maintaining the intestinal epithelial barrier has not been specifically addressed. For the present study we generated a desmoglein 2 antibody directed against the extracellular domain (Dsg2 ED) in order to test whether impaired Dsg2-mediated adhesion affects intestinal epithelial barrier functions in vitro. This antibody was capable to specifically block Dsg2 interaction in cell-free atomic force microscopy (AFM) experiments. For in vitro studies of the intestinal barrier we used Caco2 cells following differentiation into a tight enterocyte-like epithelial monolayer. Application of Dsg2 ED to Caco2 monolayers resulted in significantly increased cell dissociation compared to controls in a dispase-based enterocyte dissociation assay. Moreover, under similar conditions Dsg2 antibody significantly decreased transepithelial electrical resistance (TER) indicating that Dsg2 interaction is critically involved in the maintenance of epithelial intestinal barrier functions. As revealed by immunostaining, this was due to antibody-induced rupture of tight junctions. Under these conditions tight junction proteins claudin 1, occludin as well as tight-junction associated protein ZO-1 were partially removed from cell borders. In summary, our data indicate that Dsg2-mediated adhesion affects tight junction integrity and is required to maintain intestinal epithelial barrier properties.

Kategorie: Vortrag

Vortrag 8

Rubrik: 4.Zellbiologie
Abstract Nr.:4

Titel:Serotonin receptor 4 mRNA expression is modulated in patients with diverticular disease

Autoren: Böttner M.(1),Zorenkov D.(1),Bär F.(1),Bruch H.(2),Roblick U.(2),Wedel T.(1),

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Abstract:
Background:
Serotonin is an important regulator of intestinal peristalsis and disturbances in serotonin metabolism have been implicated in various functional GI tract diseases. Patients with diverticular disease show abnormal peristalsis suggesting an underlying intestinal motility disorder. Thus, we screened mRNA expression of the serotonergic system in the tunica muscularis and mucosa by real-time PCR and additionally assessed the localization of the serotonin 4 receptor (5HT4R) by immunocytochemistry.

Material and methods:
Samples of tunica muscularis and mucosa of the sigmoid colon from patients operated for diverticular disease (n=16) were collected and compared to control specimens from patients with diseases unrelated to intestinal motility disorders (n=16). Real-time RT-PCR for serotonin receptors 2A, 3A, 4 as well as for serotonin transporter (SERT) and the synthesizing enzyme tryptophanhydroxylase was performed. Furthermore, localization of 5HT4R was assessed by immunocytochemistry in paraffin-embedded tissue of control patients.

Results:
Our data revealed that mRNA expression for 5HT4R is down-regulated in the tunica muscularis and up-regulated in the mucosa of patients with diverticular disease, whereas mRNA expression of the other candidate genes remained unchanged. Immunocytochemistry revealed strong expression of 5HT4R in smooth muscle layers as well as in enteric ganglia of the human colonic wall.

Conclusions:
Our results suggest that the serotonergic metabolism is compromised in diverticular disease. Altered 5HT4R mRNA expression levels may partly account for the observed clinical and physiological abnormalities in patients with diverticular disease. The data further support the hypothesis that diverticular disease is associated and possibly promoted by intestinal motor disturbances.

Kategorie: Vortrag

Vortrag 9

Rubrik: 3.Neuroanatomie/Neurobiologie
Abstract Nr.:3

Titel:Systematic morphometric analysis reveals an enteric neuropathy in patients with diverticular disease

Autoren: Wedel T.(1),Büsing V.(2),Heinrichs G.(3),Nohroudi K.(4),Bruch H.(5),Roblick U.(5),Böttner M.(1),

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Abstract:
Background & Aims: The pathogenesis of diverticular disease (DD) is attributed to several etiological factors (e.g. age, diet, connective tissue disorders) but also includes distinct intestinal motor abnormalities. Although the enteric nervous system (ENS) is the key-regulator of intestinal motility, data on neuropathological alterations are scarce. The study aimed to investigate the ENS by a systematic morphometric analysis strictly adhering to our recently published international guidelines.
Material & Methods: Full-thickness sigmoid specimens obtained from patients with symptomatic DD (n=27) and controls (n=27) were processed for immunohistochemistry using anti-HuC/D as pan-neuronal marker. Enteric ganglia, nerve and glial cells were quantified separately in the myenteric, external and internal submucosal plexus compartments.
Results: Compared to controls, patients with DD showed significantly (1) reduced neuronal density in all enteric nerve plexus, (2) decrease of mean ganglionic nerve cell content in the myenteric plexus, (3) decreased ganglionic density in the internal submucosal plexus, (4) reduced glial cell density in the myenteric plexus, (5) decrease of mean ganglionic glial cell content in the myenteric plexus and increase in submucosal plexus compartments, (6) increased glia index in all enteric nerve plexus. 44.4% of patients with DD exhibited myenteric ganglia displaying enteric gliosis.
Conclusions: Patients with DD show substantial structural alterations of the ENS mainly characterized by oligo-neuronal hypoganglionosis which may account for intestinal motor abnormalities observed in DD. The morphometric data on the ENS complement established pathogenetic concepts and suggest that DD is associated with an underlying enteric neuropathy contributing to the development of diverticula.

Kategorie: Vortrag

Vortrag 10

Rubrik: 7.Immunbiologie
Abstract Nr.:7

Titel:Insulin-like growth factor-i mrna and peptide are distinctly confined to subtypes of macrophages, antigen-presenting cells, lymphocytes and hev cells in non-neoplastic human lymph node

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Abstract:
IGF-I is a potent hormone that stimulates growth and differentiation and inhibits apoptosis in numerous tissues. Preliminary evidence suggests that IGF-I exerts differentiating, mitogenic and restoring activities also in the immune system. The present study investigates the cellular sites of IGF-I mRNA and peptide on archival human lymph node samples by routine staining, in-situ hybridisation with an IGF-I probe, and immunohistochemistry with antisera specific for human IGF-I and CD3 (T-lymphocytes), CD20 (B-lymphocytes), CD68 (macrophages), CD21 (follicular dendritic cells, DC), S100 (interdigitating DC) and podoplanin (fibroblastic reticular cells) and tumour necrosis factor (TNF)-alpha. Lymph nodes showed no relevant signs of inflammation, in particular neither pronounced follicular hyperplasia nor variegated hyperplasia of the pulp, and no epitheloid cell reaction. Furthermore, only few solitary macrophages revealed TNF-alpha immunoreactivity supporting the resting state of the lymph nodes. Numerous cells within the B- and T-cell compartments showed IGF-I gene and peptide expression the majority of which was identified as macrophages. Solitary follicular DC exhibited IGF-I mRNA and peptide. B-lymphocytes did not contain IGF-I immunoreactive material, while few T-lymphocytes did. Furthermore, IGF-I mRNA and peptide-expressing cells were identified as high endothelial venule (HEV) cells. From this we conclude that the main task of IGF-I in human non-neoplastic lymph node may be autocrine and paracrine regulation of differentiation, stimulation and survival of lymphocytes, antigen-presenting cells and macrophages and differentiation and maintenance of HEV cells, and that the role for IGF-I in formation and sustaining of lymph node structures is more important than thought so far.

Kategorie: Vortrag

Vortrag 11

Rubrik: 7.Immunbiologie
Abstract Nr.:7

Titel:Age-dependent histoarchitectural changes in human lymph nodes: an underestimated process?

Autoren: Hadamitzky C.(1),Spohr H.(1),Debertin A.(2),Guddat S.(3),Tsokos M.(3),Pabst R.(1),

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Abstract:
Experimental evidence shows that human lymph nodes suffer alterations during ageing. This is clinically relevant due to the crucial role of these organs within the immune system and their lymph resorption and drainage capacity. Although some age-related changes in lymph node histoarchitecture already have been described sparsely, traditional depictions of lymph nodes seldom take account of these degenerative processes. Nevertheless, approaches as intranodal vaccination or lymph node transplantation have recently demonstrated the necessity of an accurate knowledge of this phenomenon. In this study, superficial inguinal lymph nodes were obtained from 41 deceased patients between 17 and 98 years old. To minimise immunological influences as chronic diseases only specimens received from forensic pathology autopsies were taken. An immunohistochemical analysis followed, with classification of lymph node degeneration according to a score. The latter was based on numbers of lymphocytes and high endothelial venules, degree of fibrosis and of lipomatosis. We observed an age-dependent tendency towards replacement of areas usually populated with diverse immune cells through connective tissue. Paradoxally, these changes could also be found in some cases in younger age groups. Of note is that lymph nodes can display regressive changes and often diverge from the common description found in text books. These alterations must be taken into account when dealing with lymph nodes in daily clinical practice.

Kategorie: Vortrag

Vortrag 12

Rubrik: 7.Immunbiologie
Abstract Nr.:7

Titel:Migration kinetics of intestinal intraepithelial lymphocytes as visualized by intravital staining and 2-photon microscopy

Autoren: Gebert A.(1),Klinger A.(1),Orzekowsky-Schroeder R.(2),Schueth A.(1),Huettmann G.(2),

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Abstract:
The epithelium of the gut contains large numbers of lymphocytes which are typically located in basal intercellular spaces. In previous studies, we learned that intraepithelial lymphocytes (IEL) are in a steady motion and randomly scan the intestinal epithelial cells (IEC). The IEL move at a speed of about 8 µm/min and need only 13 min to contact 99 % of all IEC. To study in detail how IEL move within the epithelial layer, we combined 2-photon microscopy in anaesthetized mice with intravital staining of lymphocyte nuclei. Intraperitoneal injection of the dye Hoechst 33258 resulted in a strong staining of individual IEL, while the IEC remained almost unstained. This enabled us to three-dimensionally render the shape of IEL during their walk within the epithelium. Computer-based analysis revealed that the IEL periodically switch from a low-speed (< 5 µm/min) to a high-speed state (> 10 µm/min). It took 2–3 min for an IEL to complete one cycle of this stop-and-go behaviour. In parallel, the IEL formed lateral processes in the low-speed state and had a smooth elongated shape in the high-speed state. In addition, some IEL entered and left the epithelium via pores of the basement membrane which were about 3 µm in diameter. Interestingly, this passage did not reduce the IEL speed. Our data show that IEL contained in the gut mucosa vigorously scan the epithelium in an amoeboid-like fashion and thus perform a highly effective immunological surveillance of the mucosal epithelium.

Kategorie: Vortrag

Vortrag 13

Rubrik: 2.Klinische Anatomie/Makroskopie
Abstract Nr.:2

Titel:The anatomy of the iliolumbar ligament for computer-assisted investigation

Autoren: Hammer N.(1),Steinke H.(1),Böhme J.(2),Stadler J.(3),Spanel-Borowski K.(1),

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Abstract:
Though being a widely accepted cause of low back pain and lumbosacral instability, the iliolumbar ligament (IL) is neglected in computer-based biomechanical studies due to the lack of morphometric information. Frozen sections prepared from 29 human subjects were measured and 7-tesla MR images made, distinguishing the anterior (AIL) and the posterior part of the IL (PIL). Cuboids were attributed as geometric figures to both parts of the ligament. This allowed the computer-based calculation of surfaces, volumes and the angle for positional relationship of both ligament parts. Based on 7-tesla MR imaging, virtual reconstruction was conducted for one male pelvis including the IL. While left and right side parameters varied at statistically significant level, no gender-dependencies could be determined. Lengths of 30 and 25 mm were measured for the AIL and PIL, heights of 17 to 19 mm, respectively, and 4 mm in thickness. Correlations between the side-dependent parameters and the AIL and PIL of the same side indicate close functional relationships. Additional dependencies suggest that the IL is capable of compensating age-related as well as bone-attributed alterations in lumbosacral morphology. The IL data and the visualized ligament structure will help to determine the influence of the IL in spinal and sacro-iliac stability by means of computer-assisted biomechanics.

Kategorie: Vortrag

Vortrag 14

Rubrik: 4.Zellbiologie
Abstract Nr.:4

Titel:Pemphigus autoantibody-induced p38MAPK activation is dependent on plakoglobin

Autoren: Heupel W.(1),Efthymiadis A.(1),Drenckhahn D.(1),Waschke J.(1),

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Abstract:
The severe blistering skin disease pemphigus vulgaris (PV) is caused by autoantibodies against desmosomal cadherins. Plakoglobin (PG) appears to be critically involved in pemphigus pathogenesis as indicated by PG-deficient keratinocytes that were shown to be protected against PV-IgG-induced loss of cell adhesion (acantholysis). Besides its well-defined functions as a plaque protein of desmosomal and adherens junctions, PG also has signaling properties which are not fully understood at present. In this study, we examined PG–mediated activation of p38MAPK because this pathway appears to be essentially involved in pemphigus skin blistering. Suppression of PG expression in HaCaT cells via small hairpin RNA resulted in diminished PG levels (PGdim) as compared to control cells. In single molecule tracking experiments using labeled desmoglein 3 (Dsg3), PV-IgG caused a significant decrease in the lateral mobility of Dsg3, which was not observed in PGdim cells. Moreover, adhesion of Dsg3-coated microbeads on the cell surface was largely reduced as revealed by laser tweezer trapping. Consistently, overall intercellular adhesion of keratinocytes was significantly diminished in PGdim cells. However, PV-IgG-induced cellular dissociation was attenuated. In addition, PV-IgG-mediated phosphorylation of p38MAPK was also blunted in PGdim cells but was restored by reconstitution with GFP-PG. Pharmacological activation of p38MAPK increased cell dissociation in PGdim cells only but not in wt cells. In summary, these experiments support the critical role of PG in PV-IgG-induced acantholysis and indicate that PG is part of a signaling mechanism that activates p38MAPK.

Kategorie: Vortrag

Vortrag 15

Rubrik: 5.Entwicklungsbiologie
Abstract Nr.:5

Titel:Simulation of chorioallantoic membrane vascular bifurcation patterns in relation to molecular signals (Ang-2), shear stress, and vessel wall stiffness.

Autoren: Kurz H.(1),Szczerba D.(2),Winnik S.(3),Fehr J.(4),Moser M.(3),Szekely G.(5),

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Abstract:
We used confocal microscopy in the chicken chorio-allantoic membrane (CAM) to demonstrate that, during the ermergence of bifurcating microvessels in the CAM capillary plexus, cytoskeletal filaments in pericytes increased [1]. Orientation was such to predominantly align smooth muscle actin with circumferential and desmin with axial forces. Moreover, adenoviral application of angiopoietin-2 (Ang-2) onto the CAM was shown to transform the capillary plexus into a system of bifurcating microvessels [2]. Ang-2 is known to be regulated by fluid shear stress. We therefore tried to incorporate both molecular signals and vessel wall stiffness into a model of shear stress dependent microvascular remodeling [3]. Our new simulation experiments show that either mechanical maturation or molecular signaling via Ang-2 could be sufficient for intussusceptive microvascular remodeling, control of anastomosis, and emergence of bifurcations from a capillary plexus. In summary, a novel computational approach was used to integrate divergent biological information in a combined model, which has explanatory power and allows predictions about molecular transport and hemodynamics. [1] Kurz H et al. Histochem Cell Biol 2008; [2] Winnik S et al. Cardiovasc Res 2009; [3] Szczerba D et al. LNCS 2006.

Kategorie: Vortrag

Vortrag 16

Rubrik: 5.Entwicklungsbiologie
Abstract Nr.:5

Titel:Beta-catenin, regulator of cell adhesion, differentiation and proliferation in the early neuroepithelium

Autoren: Junghans D.(1),Herzog S.(2),Jumaa H.(2),Kemler R.(3),Frotscher M.(1),

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Abstract:
Forming a complex structure such as the murine brain requires the interplay between different signalling cascades during early embryonic development. beta-Catenin is known to mediate Wnt-signalling and N-Cadherin mediated cell adhesion. To elucidate how cell-adhesion, Wnt-signalling and cytoskeleton regulators orchestrate proliferation, adhesion and differentiation in the early telecenphalon and neuronal stem cells, we used the Cre/loxP system to a) ablate beta-Catenin from restricted embryonic brain regions and b) to induce beta-Catenin mediated Wnt-signalling. Furthermore, we have generated null-mutants of F-Actin regulators to demonstrate how the cytoskeleton is involved in orchestrating the function of beta-Catenin within different signalling cascades. In summary, we demonstrate the pivotal role of beta-Catenin and its regulation in maintaining the integrity of the neuroepithelium and the developing brain in respect to proliferation, cell-adhesion and differentiation.

Kategorie: Vortrag

Vortrag 17

Rubrik: 5.Entwicklungsbiologie
Abstract Nr.:5

Titel:Dynamic expression of the molecular chaperone Mdg1 during embryonic development

Autoren: Pröls F.(1),

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Abstract:
Chaperone proteins are essential for proper folding, secretion, and degradation of proteins. Accordingly they are prevalent in every cell and cell compartiment. The vertebrate specific Mdg1/ERdJ4, which is an endoplasmic reticulum resident chaperone, has been described to play a role during secretion and to control the metastatic potential of tumor cells. In order to gain further insights into the function of Mdg1, we characterized its protein pattern during chick embryonic development. Mdg1/ERdJ4 protein is present in almost every cell and tissue during early development. It exhibits a salt and pepper pattern in mesenchymal cells (as in presomitic and lateral plate mesoderm, somitocoele, sclerotome, or notochord), a polarized protein distribution in pseudostratified or cubic epithelial structures (as in the neural tube, the enteric system, or the epithel of the Wolffian duct), a non-polarized, uniform cellular distribution in flat epithelium (as for example in ectoderm, endoderm, coelomic epithel, or endothelium) and in constitutively secretorily active epithelial cells (as in hepatocytes, enterocytes, ependyme or proximal tubular epithelium). In addition, we observe Mdg1 positive neurons in the cerebrum, which are in the process of assembling to form distinct nuclei and in Purkinje cells of the cerebellum. We conclude that transient elevation of Mdg1 protein levels is necessary for differentiation of epithelial borders or organization of cellular aggregates while constitutive high levels are closely linked to secretory activity.

Kategorie: Vortrag

Vortrag 18

Rubrik: 5.Entwicklungsbiologie
Abstract Nr.:5

Titel:Remodeling of aortic smooth muscle during avian embryonic development

Autoren: Christoph Wiegreffe (1,2,4), Bodo Christ (2),Ruijin Huang (3) and Martin Scaal (1)*

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Abstract:
The dorsal aorta is the earliest formed intraembryonic blood vessel in vertebrates composed of an inner lining of endothelial cells (ECs) and a slightly later forming outer wall consisting of vascular smooth muscle cells (SMCs) and pericytes. We previously identified the sclerotome as the only somitic compartment contributing to aortic SMCs in the trunk of the avian embryo. However, we demonstrated that the first SMCs in the aortic floor are not of somitic origin and must be derived from a different source. Here, we show that the primary SMCs are a transient population of aortic wall cells originating from the splanchnic mesoderm. A model is presented suggesting that wall formation of the early dorsal aorta in chick is a two-step process: The primary, transient SMCs in the aortic floor originate in the splanchnic mesoderm, whereas the secondary, definitive SMCs of the entire aortic wall originate in the sclerotome.

Kategorie: Vortrag

Vortrag 19

Rubrik: 5.Entwicklungsbiologie
Abstract Nr.:5

Titel:The role of Wnt11 in dermis development

Autoren: Morosan-Puopolo G.(1),Yusuf F.(2),Dai F.(3),Rehimi R.(1),Brand-Saberi B.(2),

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Abstract:
The aim of our work was to decipher the role of Wnt11 in dermis development.
For this purpose we constructed a shRNA plasmid coupled with an EGFP sequence. The Wnt11 shRNA-EGFP construct was injected into the somites of stage HH16-17 embryos and subsequently electroporated. After 24 hrs of reincubation, the embryos were analyzed in ovo under fluorescence to detect the transfected region indicated by EGFP. For the possible effects on dermal markers, the transfected embryos were reincubated for a longer period of time. The successfully transfected embryos were submitted for in situ hybridization with specific RNA probes for dermal, myogenic, dermomyotomal and EMT markers. We noticed that the transfected EGFP fluorescent site correlated with the silencing seen in the hybridized samples with Wnt11 probe. In situ hybridisation for c-Dermo1, Shh and myogenic markers like MyoD and Myf5 showed a downregulation following RNAi targeting Wnt11. No change in Paraxis expression, but a strong upregulation of Pax3 was observed. Cdc42, an EMT marker, was remarkably upregulated. Investigated knock out mice for Wnt11 put into evidence a decrease in dermis thickness and reduced number of hair follicles in comparison with the WT littermates.
In conclusion we propose a role of Wnt11 in orientation of the cells which de-epithelialize from the dermomyotome. We could show in our experiments that Wnt11 is involved in dense dermis development of the trunk region.

Kategorie: Vortrag

Vortrag 20

Rubrik: 5.Entwicklungsbiologie
Abstract Nr.:5

Titel: Developmental roles of 17ß-estradiol involve regulation of reelin expression and synaptogenesis in dentate gyrus

Autoren: Roland A. Bender, Lepu Zhou, Jan-Simon Lanowski, Danny Paysen, Almut König, Gabriele M. Rune

Abstract:
Hippocampus-derived 17β-estradiol (E2) has been shown to regulate neuronal function in the mature hippocampus. However, in the immature hippocampus, where substantial activity of aromatase, the final enzyme of estrogen synthesis, is also detectable, the role of the endogenously produced E2 is largely unknown. In this study, we examined potential functions of E2 during development of the hippocampal dentate gyrus (DG). Specifically, we determined whether hippocampal E2 influences synaptogenesis in the molecular layer (ML) of DG early postnatally, when synapse formation in this region is at its peak. For this purpose, immature rats were injected i. p. daily for 7 days (P9-P16) with letrozole, a non-competitive inhibitor of aromatase. This treatment resulted in a significant reduction of spine synapse density in ML, suggesting that inhibition of endogenous E2 production had affected synaptogenesis. A decrease in spine synapse density was also detectable in mature mice that had received letrozole for one week, suggesting that the effect was not limited to the developmental period, but that synapse formation in ML remains under E2 control throughout life. Ovariectomy of mature mice did not affect synaptogenesis in ML, further strengthening the notion that the E2 derives from hippocampal sources. These findings are in line with previously reported findings (Bender et al., Ann. Meet. Anat. Soc., 2008) showing a regulatory effect of hippocampal E2 on reelin expression in Cajal-Retzius cells of the developing DG. Both effects of E2 – on reelin expression and on synaptogenesis – could be mechanistically linked, as will be discussed. Taken together, these findings indicate a significant contribution of hippocampal-derived E2 to neuronal plasticity in DG already early in development. This may play a role for formation of hippocampal connectivity.
This study was supported by: DFG-grant Ru436/4-4

Kategorie: Vortrag

Vortrag 21

Rubrik: 5.Entwicklungsbiologie
Abstract Nr.:5

Titel:Addressing prominin's function in lower vertebrate model organisms

Autoren: Jászai J.(1),Corbeil D.(1),

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Abstract:
Communicated by Richard H.W. Funk, Department of Anatomy, Medical Faculty,TU Dresden**********

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Prominins are evolutionarily conserved integral five transmembrane proteins localized in various plasmalemmal protrusions of disparate epithelial and non-epithelial cell types. A special glycosylated form of Prominin-1, the AC133 antigen, became a widely used cell surface marker for prospective identification of multipotent stem- and progenitor cells from various tissue sources. The function of Prominin-like molecules, however, remains elusive. Vertebrate Prominin-1/CD133 and other family members do not show any obvious sequence homology to other known proteins, nor do their sequences reveal a motif that could provide clues as to their physiological role. Here we show, that antisense depletion of one of the vertebrate members of the Prominin family, zebrafish prominin-like 3, leads to laterality defects as revealed by disturbed molecular asymmetry in the lateral plate mesoderm and concomitant anatomical defects in heart tube looping, i.e. either inversion of the left-right asymmetry (situs inversus) or absence of laterality. Beside the randomization of heart position, the morphants show additional pleiotropic defects with signs of curved body axis, severe pathological pericardial edema at later stages of development. The phenotype of the morphants observed is strikingly reminiscent to vertebrate disease conditions with impaired primary ciliary function. Indeed, the ciliary morphology of the Kupffer’s vesicle is compromised in the morphants. Our study provides evidence, for the first time, for the function of a vertebrate prominin-like gene in vivo.

Kategorie: Vortrag

Vortrag 22

Titel: Increased intracellular Ca^{2+} concentrations inhibit TGF-beta1-mediated Smad2 transcriptional responses via Ca^{2+}/calmodulin-dependent protein kinase II

Autoren: Björn Spittau[1]*, Ming Ming[1,2], Ivan Manzini[3], Kerstin Krieglstein[1]

Adressen: 1) Institute for Anatomy & Cell Biology, Department of Molecular Embryology, Albert-Ludwigs-University Freiburg, Albertstraße 17, 79104 Freiburg, Germany
2) Institute of Health Sciences, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, and Shanghai Jiao Tong University School of Medicine, Shanghai, 200025, PR China
3) Department of Neurophysiology & Cellular Biophysics, University of Göttingen, Humboldtallee 23, 37073 Göttingen, Germany
*Corresponding author

Abstract (Talk):
Transforming growth factor beta (TGF-beta) signalling plays important roles in a variety of tissues and cell types. Impaired TGF-beta signalling contributes to several pathologies, including cancer, fibrosis as well as neurodegenerative diseases. TGF-beta receptor type I-mediated phosphorylation of Smad2, the formation of the Smad2-Smad4 complex and translocation to the nucleus are critical steps of the TGF-beta signalling pathway. Here we demonstrate that thapsigargin-mediated increase of intracellular calcium concentrations inhibited TGF-beta1-induced Smad2 transcriptional activity in the oligodendroglial cell line OLI-neu. We provide evidence that thapsigargin treatment dramatically reduced the phosphorylation of Smad2 without affecting its nuclear translocation after TGF-beta1 treatment. Moreover, using CaMKII inhibitors and a constitutively active CaMKII mutant, we demonstrate that the observed inhibition of TGF-beta signalling in OLI-neu cells was dependent on calcium-mediated CaMKII activation. In summary, we clearly show that TGF-beta1-induced Smad2 phosphorylation is negatively regulated by increased intracellular calcium concentrations resulting in inhibition of Smad2-mediated transcription of TGF-beta target genes. These results underline the importance of intracellular calcium for the regulation of TGF-beta signalling.

Kategorie: Vortrag

Vortrag 23

Rubrik: 3.Neuroanatomie/Neurobiologie
Abstract Nr.:3

Titel:Effect of 17 beta-estradiol on mitochondrial gene expression and function in the mouse spinal cord

Autoren: Johann S.(1),Dahm M.(1),Beyer C.(1),Arnold S.(1),

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Abstract:
Purpose: The regulation of mitochondrial energy metabolism is essential for the function and protection of the CNS. Mitochondrial defects are implicated in the development of many neurodegenerative diseases. Since 17beta-estradiol (E) is well-known to mediate neuroprotection in the brain and spinal cord, we have assessed its role in the regulation of mitochondrial gene expression in the mouse spinal cord.
Methods: The expression of mitochondria-encoded catalytic subunits of the respiratory chain complexes was analyzed by real-time RT-PCR in E treated cell cultures. To investigate E effects in vivo and to assure the feasibility of a sex-specific regulation four weeks old female and male mice were treated with E or vehicle as control by injection into the neck region. The lumbar part of the spinal cord was isolated and analyzed. To evaluate E action on energy production, ATP levels were assessed by using a bioluminescence assay.
Results: The application of E significantly increased the expression of the investigated catalytic subunits in cell culture. The estrogen receptor antagonist ICI 182780 abrogated estrogen effects on cell cultures, suggesting classical genomic signaling. Similar effects of E on mRNA expression were found in in vivo experiments.
Conclusion: Our results show that E increased the expression of mitochondrial-encoded subunits of the respiratory chain in the spinal cord in an in vitro and in vivo model. The potency of 17beta-estradiol-mediated neuroprotection by modulating mitochondrial function in the spinal cord remains to be scrutinized in animal models such as ALS- and other motoneuron degeneration mouse models.

Kategorie: Vortrag

Vortrag 24

Rubrik: 3.Neuroanatomie/Neurobiologie
Abstract Nr.:3

Titel:The opposing roles of ER-alpha and ER-beta in estrogen-regulated spinogenesis

Autoren: Fester L.(1),Zhou L.(1),Hagshenas S.(1),Rune G.(1),

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Abstract:
In previous reports, we showed that hippocampus-derived estradiol regulates spinogenesis. In this study we address the question as to which of the estrogen receptors (ERs), ER-alpha and/or ER-beta, are involved in this process. Hippocampal slice cultures and dispersed cultures were used and treated with specific agonists and antagonists. Application of estradiol to the cultures had no effect on spine synapse number in hippocampal slice cultures. This was also true for the ER-alpha agonist (PPT) and the ER-beta agonist (DPN). Blockade of ERs, using ICI 182 780, which blocks both receptors, was also ineffective. Treatment of hippocampal cultures with letrozole, a potent aromatase inhibitor, decreases estradiol release into the medium, decreases spine synapse number, and upregulates ER-beta but downregulates ER-alpha. In cultures, in which estradiol synthesis was downregulated in response to letrozole, estradiol and PPT rescued spine synapse number and upregulated synaptophysin and spinophilin. This increase was abolished in the presence of specific ER-alpha antagonists. Vice versa, after inhibition of aromatase activity, the ER-beta agonist DPN further promoted letrozole-induced spine synapse loss and downregulated synaptophysin, spinophilin and synaptopodin. This effect, in turn, was not found when the cultures were treated with a specific ER-beta antagonist. Our findings suggest that ER-alpha mediates spine growth, whereas retraction of spines is mediated by ER-beta in estrogen-regulated spinogenesis.

Kategorie: Vortrag

Vortrag 25

Rubrik: 3.Neuroanatomie/Neurobiologie
Abstract Nr.:3

Titel:Neuronal protection by sex steroids in an experimental stroke model

Autoren: Kipp M.(1),Dang J.(1),Beyer(1),

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Abstract:
Purpose: Cerebral stroke is a major cause of death and permanent disability. Typically, the blood supply to the brain is temporarily or permanently obstructed and may result in massive neuronal cell death. The reproductive steroid hormone estrogen has been shown to provide neuroprotection in a variety of experimental insults, but the importance of progesterone as an anti-ischemic treatment has not been explored in detail. Both steroids might cooperate to reduce brain damage. We introduced middle cerebral artery occlusion (MCAO) in adult rats and evaluated histological and functional melioration after hormone application.
Methods: Age-matched male rats underwent 1 h MCAO with the intra-luminal filament technique, followed by 23 hours of reperfusion. Hormones were applied at the beginning of reperfusion by subcutaneous depot injections in the neck. Ipsilateral parietal cortex perfusion was monitored with laser doppler flowmetry throughout ischemia. Infarct volumes were determined with TTC staining. Complex behavioural analysis was additionally performed.
Results: The volume of cortical lesions was significantly reduced in the single hormone- treated groups (estrogen and progesterone) with progesterone being most effective. The combined hormone application displayed similar protective properties as progesterone alone. Stroke-induced behavioural deficits were significantly improved by both hormones either in single or combined doses.
Conclusion: We found that an exogenous steroid therapy ameliorates histological and functional damage after MCAO. Therefore, both steroids might be valuable therapeutic tools in the early stroke therapy.

Kategorie: Vortrag

Vortrag 26

Rubrik: 3.Neuroanatomie/Neurobiologie
Abstract Nr.:3

Autoren: Lepu Zhou, Günter Glassmeier, Ricardo Vierk, Lars Fester, Gabriele M. Rune

Adresse: Institute of Anatomy, University Medical Center Hamburg-Eppendorf, Martinistr. 52, 20246 Hamburg

Abstract:
Recently, inhibition of estrogen synthesis by aromatase inhibitors has become a favoured therapy for breast cancer in postmenopausal women. Estrogen is, however, important for synapse formation in the hippocampus. Inhibition of aromatase induces spine synapse loss in organotypic hippocampal slice cultures. We therefore, studied the effect of systemic treatment with the potent aromatase inhibitor letrozole, for periods of seven days and four weeks, on spine formation and synaptic proteins in the hippocampi of female mice. In cyclic, letrozole-treated females and in ovariectomized, letrozole-treated females, the number of spine synapses was significantly reduced in the hippocampus but not in the prefrontal or cerebellar cortex. Consequently, the expression of NMDA receptor NR1 was significantly downregulated after treatment with letrozole. In cyclic animals the expression of the synaptic proteins synaptophysin and spinophilin was downregulated in response to letrozole. In ovariectomized animals, however, protein expression was downregulated after seven days of treatment, whereas the expression was upregulated after four weeks of treatment. In acute slices of all groups of animals LTP was heavily impaired in the presence of letrozole. Our results indicate that systemic inhibition of aromatase in mice affects synaptic plasticity in the hippocampus. This may be an underlying cause of cognitive deficits in postmenopausal women treated with aromatase inhibitors.

Kategorie: Vortrag

Vortrag 27

Rubrik: 3.Neuroanatomie/Neurobiologie
Abstract Nr.:3

Titel:TSH-beta expression in the mammalian pars tuberalis is essential for induction, but not for suppression of the hypothalamo-hypopysial gonadal axis

Autoren: Yasuo S.(1),Korf H.(1),

Adressen:(1)Goethe-Universität Frankfurt am Main|Dr. Senckenbergische Anatomie, Institut für Anatomie II|D-60590|Deutschland; email:korf@em.uni-frankfurt.de

Abstract:
Seasonal reproduction depends on photoperiod-regulated activation or suppression of the gonadal axis. Recent studies in quail identified long-day induced TSH-beta expression in the pars tuberalis (PT) as rapid trigger of gonadal activation. TSH induces type 2 deiodinase (Dio2) in the ependymal cell layer (EC) of the infundibular recess to stimulate gonadal axis. A similar mechanism is proposed in sheep and mice, but experimental data on the temporal patterns of induction and suppression of TSH-beta and Dio2 expression are missing. In this study, we examined the expression of TSH- and Dio2 in hamsters transferred from short to long day condition for 9 days, and demonstrate induction of TSH-beta and Dio2 on day 8 after transition. Several animals at day 5 to 8 showed the high expression of TSH-beta and low expression of Dio2, suggesting that the response of TSH-beta in hamsters precedes that of Dio2. Temporal expression of TSH-beta and Dio2 in the suppressive pathway was also examined by sc melatonin injection, which mimics the transition from long to short days. Importantly, Dio2 expression is suppressed at day 1 after the onset of injection, whereas TSH-beta expression was not suppressed until day 10. These data indicate that regulated transcription of TSH-beta is involved in the induction of the gonadal axis in mammals, whereas suppression of this axis is mediated by different mechanisms. Notably, recent results from our laboratory point toward endocannabinoids as additional photoperiodic messengers from the hamster pars tuberalis.
Supported by the Alfons und Gertrud Kassel-Stiftung, Frankfurt am Main, Germany.

Kategorie: Vortrag

Vortrag 28

Rubrik: 3.Neuroanatomie/Neurobiologie
Abstract Nr.:3

Titel:Nrf2 is located in the growth cone of neuronal cells and function as sensors for oxidative stress

Autoren: Wruck C.(1),Rosen C.(1),Brandenburg L.(1),Pufe T.(2),Windoffer R.(3),Pufe T.(1),

Adressen:(1)University Hospital Aachen, Medical Faculty RWTH|Institute of Anatomy and Cell Biology|Aachen|Germany; email:cwruck@ukaachen.de; (2)University Hospital Aachen, Medical Faculty RWTH|Institute of Anatomy and Cell Biolog|Aachen|Germany; (3)University Hospital Aachen, Medical Faculty RWTH|MOCA, Institute of Molecular und Cellular Anatomy|Aachen|Germany

Abstract:
In accordance with common hypothesis many neurodegenerative diseases such as Alzheimer's disease (AD), amyotrophic lateral sclerosis (ALS) and Parkinson's disease (PD) can cause by oxidative stress, a lacking effect of neurotrophins like Nerve Growth Factor (NGF) as well as a disturbed axonal transport.
Utilizing ARE-Luciferase assay we give evidence that NGF activates Nrf2 dose dependently in neural PC12 and astroglial C6 cells. Western blot analysis shows that NGF stimulation causes Nrf2 nuclear translocation in PC12 as well as C6 cells and Nrf2 target gene activation in Nrf2-WT but not in Nrf2-knockout mice primary neurons and astrocytes.
With time laps confocal scanning laser microscopy (CSLM) we visualize the vesicular antero- and retrograde transport of Nrf2 in differentiated PC12 cells.
Taken together our study demonstrates that Nrf2 is located in the growth cone of differentiated PC12 cells and function as a sensor for oxidative stress.

Kategorie: Vortrag

Vortrag 29

Rubrik: 4.Zellbiologie
Abstract Nr.:4

Titel:The molecular pathology of the neurodegenerative disease spinal muscular atrophy: role of profilin

Autoren: Nölle A.(1),Grothe C.(1),Niedenthal R.(2),Claus P.(1),

Adressen:(1)Medizinische Hochschule Hannover|Neuroanatomie, OE 4140|Hannover|Deutschland; (2)Medizinische Hochschule Hannover|Physiologische Chemie/Biochemie|Hannover|Deutschland; email:claus.peter@mh-hannover.de

Abstract:
Spinal muscular atrophy is a neurodegenerative disease accompanied by the loss of motoneurons. Either mutations or deletions in the survival of motoneuron (SMN) gene are responsible for this defect. In the nucleus, SMN localizes to nuclear bodies. The number of SMN-positive nuclear bodies is decreased in SMA patients. We have recently shown that the stability of these structures is regulated by nuclear fibroblast growth factor - 2 (FGF-2) (Bruns et al., 2009, PNAS, in press) and that the destabilization is not responsible for disease progression. However, SMN is also found in axons of motoneurons stressing the importance of a non-nuclear disease mechanism.

We previously analyzed the effects of SMN on neuronal differentiation and identified the Rho-Kinase (ROCK) pathway as an important modulator of SMN-dependent actin regulation (van Bergeijk et al., 2007, FASEB J. 21: 1492-1502). How SMN does interact with the ROCK pathway? One putative molecular bridge between SMN and the ROCK pathway is the protein profilin. Here, we analyzed the interaction of profilin with SMN by a new in vivo protein-protein interaction assay (Srivastav and Niedenthal, unpublished). This method employs the SUMO conjugating enzyme Ubc9 for fusion-dependent trans-sumoylation. Site-directed mutagenesis of SMN allowed the determination of the profilin-binding site in SMN. Our data suggest that actin-regulating proteins downstream of ROCK are involved in SMN-dependent neuritogenesis defects and that the SMN-profilin interaction is the putative link between SMN and the ROCK-pathway. Importantly, analyses of this pathway could help to elucidate new molecular targets for a therapy of spinal muscular atrophy.

Kategorie: Vortrag

Vortrag 30

Rubrik: 4.Zellbiologie
Abstract Nr.:4

Titel:Distinct cytoskeleton rearrangement and neuronal maturation of neural stem cells

Autoren: Liebau S.(1),Böckers T.(1),Christian P.(2),

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Abstract:
Neural Stem Cells (NSCs) are self renewing cells that retain the potential to generate cell subtypes, i.e astroglia, oligodendrocytes and neurons in the mammalian central nervous system. NSCs require the ability to adjust their morphology depending on e.g. migration or differentiation. NSCs, which basically reside in the subventricular zone and the hippocampus in the adult brain, may travel a long distance up to the olfactory bulb. Once differentiation into neurons is initiated, generation of neurites and synaptic contacts depends on a specific interplay of proteins and signals. Even subtle changes in the surrounding matrix or neighborhood to communicating cells lead to the accurate outgrowth of membrane structures. This necessitates submembranous microcompartments consisting of proteins responsible for both signaling and influence on the actin fibre rearrangement. The actin cytoskeleton is the most dynamic part of the cytoskeleton and candidate proteins involved in its modification are e.g the Ableson interacting protein (Abi-1), actin related proteins (i.e. ARP2/3) or WASL. Lately, we could show that ion channels of the SK family play an important role in transducing a signal leading to the outgrowth and elongation of filopodia along the cell membrane. The direct protein interaction of the SK channel subtype SK3 with Abi-1 points to the abundancy of such a microcompartment involving a membrane standing channel protein with a protein of the cytoskeleton machinery. As this protein complex is also present in maturating neurons, a possible role in synaptogenesis in differentiating NSCs and primary neurons is likely.

Kategorie: Vortrag

Vortrag 31

Rubrik: 3.Neuroanatomie/Neurobiologie
Abstract Nr.:3

Titel:Upon NMDA-receptor activation, the PSD protein LAPSER1 regulates nuclear localization of beta-Catenin in hippocampal neurons

Autoren: Schmeisser M.(1),Grabrucker A.(2),Bockmann J.(1),Böckers T.(1),

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Abstract:
LAPSER1, a 670 aa long leucine-rich protein, which belongs to the “Fezzin” family of PSD proteins, has been identified as novel interaction partner of ProSAP2/Shank3. As previous work has shown that other “Fezzins” like ProSAPiP1 or PSD-Zip70 bind SPAR family members via their C-terminal Fez1 domain, we have analyzed this binding property in LAPSER1 and were finally able to characterize the Fez1 domain as SPAR family interacting module. Interestingly, beta-Catenin, key molecule of the canonical Wnt pathway, also binds to LAPSER1 in neurons. Upon NMDA-receptor dependent activation, LAPSER1 and beta-Catenin translocate from the synapse to the nucleus leading to the transcription and translation of known beta-Catenin target genes, including Tcfe2a and c-myc. Nuclear export and cytoplasmatic redistribution of beta-Catenin is tightly regulated by LAPSER1 due to its nuclear export signal (NES). This feature is pointing towards a self-limiting function of the LAPSER1/beta-Catenin complex in neuronal cell nuclei after synaptic activation. As activity-dependent changes in gene expression and protein synthesis are said to be the core elements of neuronal circuit formation, LAPSER1 therefore is another important element which further helps to understand the molecular processes of memory formation.

Kategorie: Vortrag

Vortrag 32

Rubrik: 3.Neuroanatomie/Neurobiologie
Abstract Nr.:3

Titel:Postsynaptic neuroligin1 regulates activity-dependent presynaptic maturation

Autoren: Dresbach T.(1),Wittenmayer N.(1),Körber C.(1),Varoqueaux F.(2),Brose N.(2),Kuner T.(1),

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Abstract:
Presynaptic nerve terminals pass through distinct stages of maturation after their initial assembly. Here we show that the postsynaptic cell adhesion molecule Neuroligin1 regulates key steps of presynaptic maturation. Presynaptic terminals from Neuroligin1 knockout mice remain structurally and functionally immature with respect to active zone stability and synaptic vesicle pool size, as analyzed in cultured hippocampal neurons. Conversely, overexpression of Neuroligin1 in immature neurons, that is within the first five days after plating, induced the formation of presynaptic boutons that had hallmarks of mature boutons. In particular, Neuroligin 1 enhanced the size of the pool of recycling synaptic vesicles, the rate of synaptic vesicle exocytosis, and the fraction of boutons responding to depolarization. Moreover, Neuroligin 1 induced the formation of active zones that remained stable in the absence of F-actin, another hallmark of advanced maturation. Acquisition of F-actin independence of the active zone marker Bassoon during culture development or induced via overexpression of Neuroligin1 was activity-dependent. The extracellular domain of Neuroligin1 was sufficient to induce assembly of functional presynaptic terminals, while the intracellular domain was required for terminal maturation. These data show that induction of presynaptic terminal assembly and maturation involve mechanistically distinct actions of Neuroligins, and that Neuroligin1 is essential for presynaptic terminal maturation

Kategorie: Vortrag

Vortrag 33

Rubrik: 3.Neuroanatomie/Neurobiologie
Abstract Nr.:3

Titel:Ankyring is required to maintain axo-dendritic polarity in vivo

Autoren: Sobotzik J.(1),Del Turco D.(2),Bennett V.(3),Deller T.(2),Schultz C.(1),

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Abstract:
Neurons are highly polarized cells that extend a single axon and several dendrites. Studies with cultured neurons indicate that the proximal portion of the axon, denoted as the axon initial segment (AIS), maintains neuronal polarity in vitro. The membrane-adaptor protein ankyrinG (ankG) is an essential component of the AIS. To determine the relevance of ankG for neuronal polarity in vivo, we studied mice with a cerebellum-specific ankG-deficiency. Strikingly, ankG-depleted axons develop protrusions closely resembling dendritic spines. Such axonal spines are enriched with postsynaptic proteins including ProSAP1/Shank2 as well as ionotropic and metabotropic glutamate receptors. Immunofluorescence indicated that axonal spines are contacted by presynaptic glutamatergic boutons. For further analysis, double mutants were obtained by crossbreeding ankG-/- mice with L7/PCP2 mice expressing enhanced green fluorescent protein (EGFP) in Purkinje cells. This approach allowed precise confocal mapping of EGFP-positive spiny axons and their subsequent identification at the electron microscopic level. Axonal spines contained a typical postsynaptic density and established asymmetric excitatory synapses with presynaptic boutons containing synaptic vesicles. In the shaft of spiny axons, typical ultrastructural features of the AIS, including the membrane-associated dense undercoating and cytoplasmic bundles of microtubules, were absent. Moreover, the presence of axonal spines was consistently associated with the absence of an intact myelin sheath. In conclusion, axons of ankG-deficient mice acquire hallmark features of dendrites. Thus, AnkG is important for maintaining appropriate axo-dendritic polarity in vivo. Supported by the DFG (SCHU 1412/2-1).

Kategorie: Vortrag

Vortrag 33a

Rubrik: 3.Neuroanatomie/Neurobiologie
Abstract Nr.: 3

Titel: Dual Modulatory role of $Gaba_B$ receptors in hippocampal parvalbumin-expressing cells

Autoren: Althof D.(1,2), Frotscher M.(1), Vida I.(1,3),Kulik A.(1)

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Abstract:
Metabotropic □-aminobutyric acid receptors ($GABA_BRs$) mediate modulatory influence of the GABAergic system on both excitatory and inhibitory neurotransmission in the hippocampus. We used a combination of ultrastructural and pharmacological approaches to elucidate the pre- and postsynaptic localization and function of $GABA_BRs$ in inhibitory fast-spiking parvalbumin-expressing (PV+) cells.
At the light microscopic level, weak immunostaining for the $GABA_{B1}$ protein was found in somato-dendritic compartments of PV+ interneurons. At the electron microscopic level, the labeling for $GABA_{B1}$ was abundant postsynaptically where immunoparticles were mainly found to be localized to the extrasynaptic membrane of dendritic shafts. Quantitative analysis further revealed an association of $GABA_{B1}$ to asymmetrical putative glutamatergic synapses on dendritic shafts, which was also studied by a computational model. Using transgenic animals, in which either the $GABA_{B1a}$ or $GABA_{B1b}$ isoform of the receptor subunit was knocked-out, we found that the $GABA_{B1b}$ protein was predominant in this compartment. Presynaptically, the immunoreactivity for $GABA_{B1}$ was sparse but immunogold particles for the subunit were consistently present in synaptic and extrasynaptic membranes of axon terminals of PV+ cells. Consistent with results of the immunocytochemistry, puff-application of 100 □M baclofen, a $GABA_BR$ agonist, to the apical dendrites of PV+ cells resulted in a slow inhibitory current in PV+ cells. Furthermore, putative PV+ basket cell-mediated monosynaptic IPSCs in pyramidal cells, elicited by electrical stimulation in the somatic layer in the presence of ionotropic glutamate receptor blockers, were dramatically reduced by 5 □M baclofen applied to the bath.
These results show that functional $GABA_BRs$ are present in the dendrites and axon terminals of hippocampal PV-containing cells. The localization of postsynaptic receptors around asymmetrical synapses suggests that they are involved in the modulation of glutamatergic inputs to the neurons. In contrast, presynaptic $GABA_BRs$ modulate the inhibitory output by regulating GABA release from these interneurons. (Supported by DFG: SFB 780).

Kategorie: Vortrag

Vortrag 34

Rubrik: 3.Neuroanatomie/Neurobiologie
Abstract Nr.:3

Titel:Different patterns of upregulation of small heat shock proteins after various kinds of stresses in cultured rat hippocampal neurons

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Abstract:
Heat shock proteins are upregulated upon various stresses and help the cell to survive under pathophysiological conditions. Eleven small heat shock proteins (sHsps) are known in mammals, some of which have neuroprotective functions or play a role in neurodegenerative diseases. However, sHsps have not been systematically investigated in the brain with respect to expression pattern, response to different kinds of stresses and molecular mechanism of neuroprotection.

We could show that only five sHsps, HspB1, HspB5, HspB6, HspB8 and HspB11, are expressed in rat hippocampus. We applied different stresses to cultured hippocampal neurons and investigated the effect on sHsp expression on mRNA (real-time RT-PCR) and protein level (western blot, immunocytochemistry). Heat shock and hyperosmotic stress lead to an increase only of HspB1 mRNA with highest levels after 0 to 7 hrs and after 24 hrs recovery, respectively. After exposure to oxidative stress the mRNA levels of HspB1, HspB5 and B11 increased and persisted elevated beyond 24 hrs. The induction of sHsps could be verified on protein level with a delayed time course. By immunocytochemistry, HspB1, HspB5 and HspB11 were found to be localized in the cytoplasm of glia and neurons. Interestingly, after stress conditions HspB5 was phosphorylated and recruited to dendritic spines.

In conclusion, the cellular stress response in the hippocampus involves mainly HspB1, HspB5 and HspB11 with a stress specific induction pattern. The interesting finding of stress induced recruitment of phosphorylated HspB5 to dendritic spines hints at a phosphorylation dependent protective function of HspB5 at synapses which needs further investigation.

Kategorie: Vortrag

Vortrag 35

Rubrik: 3.Neuroanatomie/Neurobiologie
Abstract Nr.:3

Titel:L1CAM mutations cause mechanistically distinct neuronal trafficking defects that interfere with l1-mediated axon growth and plasticity

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Abstract:
Mutations of the neural cell adhesion molecule L1 (L1CAM) cause neurological disorders such as X-linked hydrocephalus, spastic paraplegia and mental retardation. Here we investigated pathomechanisms of two missense mutations, R184Q and W1036L, which are associated with severe disease phenotypes. Using magnetofection in dissociated neuronal cultures we show that these clinical mutations cause accumulation of L1 in neuronal cell bodies and dendrites, respectively. As a consequence, both mutations preclude axonal targeting and disrupt L1-mediated axon growth. Single-cell electroporation in organotypic hippocampal slice cultures further demonstrates that these mutations impair L1-mediated axonal plasticity of CA3 pyramidal neurons. Our study provides evidence that different L1CAM mutations cause mechanistically distinct trafficking defects of L1 that interfere with L1-mediated axon growth and plasticity. These findings are important to understand the pathomechanisms of L1-associated neurological disorders.

Kategorie: Vortrag

Vortrag 36

Rubrik: 3.Neuroanatomie/Neurobiologie
Abstract Nr.:3

Titel:Subcellular detection of BDNF in the adult hippocampus

Autoren: Dieni S.(1),Matsumoto T.(2),Rauskolb S.(2),Barde Y.(2),Frotscher M.(1),

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Abstract:
Brain-derived neurotrophic factor (BDNF) is initially synthesized as a precursor protein (pro-BDNF) that is post-translationally processed to mature BDNF. Pulse-chase experiments in hippocampal neurons have shown that this processing occurs rapidly in a hitherto unidentified intracellular compartment (Matsumoto et al., 2008). In an attempt to provide immunohistochemical evidence for BDNF processing, hippocampal sections were treated with antibodies against pro-BDNF and mature BDNF. Confocal analysis revealed co-localization of pro- and mature-BDNF not only in dentate granule cell bodies, but also in mossy fiber terminals. Immunogold-electron microscopy further showed that each peptide is presynaptically localized to large secretory vesicles. In parallel, the pro- and mature-BDNF antibodies were biochemically analysed and shown to detect pro-BDNF, cleaved pro-domain BDNF and mature BDNF in hippocampal extracts at a relative concentration ratio of 1:9:9. Taken together, these immunohistochemical and biochemical findings suggest that the cleaved pro-domain and mature peptides are both transported to the axon terminals; thus, it is conceivable that both peptides may be secreted, possibly from the same vesicles as a protein complex.

Kategorie: Vortrag

Vortrag 37

Rubrik: 3.Neuroanatomie/Neurobiologie
Abstract Nr.:3

Titel: Lamination is critical for information processing in the mouse dentate gyrus

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Abstract:
The dentate gyrus, similarly to other cortical areas, has a laminar structure. Granule cells (GCs), the principal cells of the dentate, show a polarized orientation and have their somata densely packed in the GC layer; this way, they sharply segregate the cell bodies and dendrites of their main hilar targets, the mossy cells (MCs), from the perforant path input. However, the functional relevance of this strict lamination is yet unexplored. To investigate this further, we used the reeler mouse as a model system and performed patch-clamp recordings with recordings of the extracellular population activity in the GC layer. We show for the first time that, in the dentate and hilus, the lack of lamination directly results in aberrant excitatory connections onto MCs. Thus, compared to the homogeneous wild-type recordings, intracellular spikes can occur in a wider time range, e.g. with a higher or lower precision. Consistent with the anatomical heterogeneity, we also observed an increase in cell-to-cell heterogeneity in intracellular currents and firing patterns in both reeler GCs and MCs. Contrary to our initial theory, these received enhanced monosynaptic inhibition. This can be due to the fact that synaptic activation and precision of fast-spiking interneurons is largely unchanged in reeler; whilst excitatory input fibers partially do not reach their target principal neurons, inhibitory fibers might instead fill their place. Thus, changes in the connectivity caused by an altered lamination result in a lower temporal precision of synaptic activation of principal cells, but not of fast-spiking interneurons in the dentate-hilar network.

Kategorie: Vortrag

Vortrag 38

Rubrik: 3.Neuroanatomie/Neurobiologie
Abstract Nr.:3

Titel:Normal dentate gyrus development depends on cooperating reelin and notch signaling

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Abstract:
The hippocampal dentate gyrus (DG) is crucial for higher brain functions such as learning and memory, and malformation and malfunction of the DG are associated with neurological and psychiatric disorders. During DG development several signaling pathways are involved and likely interact with each other. The extracellular matrix molecule Reelin is necessary both for normal development of the dentate gyrus radial glia and neuronal migration. In Reelin-deficient Reeler mice, the hippocampal radial glial scaffold fails to form and granule cells are dispersed throughout the dentate gyrus. Notch1 has recently been implicated in embryonic radial glial differentiation. We investigated the role of Notch1 in Reelin-dependent radial glial formation and granule cell migration in the postnatal hippocampus. Inhibition of Notch signaling in organotypic hippocampal slice cultures induced a phenotype reminiscent of the Reelin-deficient hippocampus, i.e., a reduced density of radial glial fibers and granule cell dispersion. Moreover, a Reelin-dependent rescue of the Reeler phenotype was blocked by inhibition of Notch activation. In the Reeler dentate gyrus we observed reduced Notch1 signaling and found that Disabled1 (Dab1), a component of the Reelin signaling pathway, colocalized with Notch1, thus indicating a direct interaction between the Reelin and Notch1 signaling pathways. Our results suggest that Reelin enhances Notch1 signaling, thereby contributing to the formation of the radial glial scaffold and the normal development of the dentate gyrus.

Kategorie: Vortrag

Vortrag 39

Rubrik: 3.Neuroanatomie/Neurobiologie
Abstract Nr.:3

Titel:Role for reelin in neurotransmitter release

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Abstract:
Reelin, a large extracellular matrix molecule, is known to control neuronal migration during development. Recent evidence suggests that it also plays a role in the development of postsynaptic dendrites and spines as well as in synaptic plasticity. We have analyzed a potential role of Reelin in presynaptic functions. Quantitative electron microscopy of presynaptic boutons in hippocampal region CA1 of wildtype animals and reeler mutants revealed a decrease in the ratio of bouton perimeter/bouton area in reeler. This points to a less convoluted structure of presynaptic terminals in the mutant. In addition we found that the number of vesicles/bouton area was increased in reeler mice. Together, these findings suggest that there is an altered vesicle fusion in the mutant. Vesicle fusion and transmitter release are controlled by the SNARE complex. Therefore we have studied SNARE proteins and other presynaptic proteins in wildtype animals and reeler mutants. While no or minor changes were observed in the SNARE proteins synaptobrevin and syntaxin 1, SNAP25, a third protein of the SNARE complex, was significantly reduced in tissue from reeler mutants. Moreover, addition of recombinant Reelin to reeler tissue could rescue the expression of SNAP25 protein. Finally, paired-pulse facilitation, a presynaptic mechanism associated with transmitter release, was significantly decreased in reeler when compared to wildtype. These novel findings suggest that Reelin signaling is involved in presynaptic release mechanisms.
(Supported by DFG: SFB 592, project A20)

Kategorie: Vortrag

Vortrag 40

Rubrik: 3.Neuroanatomie/Neurobiologie
Abstract Nr.:3

Titel:Rho GTPase-mediated neurite motility of cortical neurons induced by reelin

Autoren: Bock H.(1),Bouché E.(1),Frotscher M.(1),Meyer D.(1),Schwan C.(1),Leemhuis J.(1),

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Abstract:
The neuronal signalling molecule Reelin regulates the positioning and differentiation of postmitotic neurons during neurodevelopment and in the adult nervous system. Binding of Reelin to the lipoprotein receptors ApoER2 and Vldl receptor transmits the signal into responsive cells by inducing Src family kinase-dependent tyrosine phosphorylation of the intracellular adapter protein Disabled-1 and subsequent activation of the phosphatidylinositol 3-kinase (PI3K) pathway. Using time lapse microscopy we found that Reelin increases neurite motiliy of early-stage primary cortical embryonic neurons. This effect was dependent on ApoER2, Dab1 and PI3K but not on Akt or GSK3ß activity, two serine/threonine kinases downstream of PI3K. However, pretreatment of neurons with the clostridial toxin B blocked the Reelin effect on neurite motility, suggesting involvement of GTP-binding proteins of the Rho family. This was confirmed by biochemical pulldown assays, which demonstrated that the Rho GTPase Cdc42 but not Rac was activated by Reelin treatment in primary neurons. Further analysis, including RNA interference-mediated knockdown of Cdc42, fluorescence resonance energy transfer (FRET) detection of Cdc42 activity in living neurons and RhoGDI-dependent inhibition of Cdc42 with the small-molecule pharmacological inhibitor secramine confirmed the association of Reelin-dependent induction of neurite motility and Cdc42 activation. In conclusion, we identify the Rho GTPase Cdc42 as an important Reelin target in responsive neurons, where it mediates reorganization of the actin cytoskeleton during neuronal migration and differentiation.

Kategorie: Vortrag

Vortrag 41

Rubrik: 3.Neuroanatomie/Neurobiologie
Abstract Nr.:3

Titel:Stability matters: new rules for denervation-induced changes in spine density

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Abstract:
Loss of innervation induces dendritic remodelling. This form of neuronal plasticity also involves biphasic changes in dendritic spine density: After axonal denervation spine densities decrease, during the late phase after denervation spine densities recover. At present, it is believed that denervation-induced spine loss and reinnervation-associated spine formation underlie these changes. We have tested this hypothesis, which is based on data from fixed tissues, by using time-lapse confocal microscopy of organotypic entorhino-hippocampal slice cultures. By following single spines over time we demonstrate that changes in spine stability account for the observed changes in spine density. Differently from what has been proposed spine formation rate was not altered and even remained unchanged during the phase when spine densities recovered.
To learn more about the functional consequences of denervation, individual granule cells were patch-clamped and miniature excitatory postsynaptic events (mEPSC) were recorded from the soma of these cells. Interestingly denervated granule cells demonstrated an increase in mEPSC amplitude, suggesting that non-denervated axospinous synapses are strengthened in response to partial loss of innervation. Using a computational approach we were, in fact, able to demonstrate that the observed changes in spine stability can be sufficiently explained by the recorded changes in synaptic strength. These results revise and extent our classical view of denervation induced plasticity and indicate a critical role for changes in excitatory synaptic strength in structural remodelling following denervation.

Kategorie: Vortrag

Vortrag 42

Rubrik: 3.Neuroanatomie/Neurobiologie
Abstract Nr.:3

Titel:Synaptic PRG-1 modulates excitatory transmission via lipid phosphate-mediated signalling

Autoren: Vogt J.(1),Trimbuch T.(1),Beed P.(1),Streu N.(1),Laube G.(2),Strauss U.(1),Aoki J.(3),Ninnemann O.(1),Schmitz D.(4),Nitsch R.(1),

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Abstract:
Excitatory glutamatergic transmission is one of the most important processes in the central nervous system. Here, we describe a new mechanism in which synaptically produced phospholipids interact with presynaptic G-protein coupled receptors, thereby enhancing basal excitatory transmission. Immunofluorescent colocalization studies and fine structural analysis revealed that the G-protein coupled lysophosphatidic acid-receptor-2 (LPA2-R) was located presynaptically and the LPA-synthesizing enzyme autotaxin was localized in the astrocytic processes ensheathing the synaptic clefts. The interaction between phospholipids and LPA-receptors is critically regulated by PRG1 (plasticity-related gene 1), a molecule located at the postsynaptic densities of glutamatergic neurons. Disruption of PRG1 led to higher basal glutamatergic transmission and epileptic seizures. As LPA application in vitro induced hyperexcitability in wildtype but not LPA2-receptor-deficient brain slices, and uptake of phospholipids was reduced in PRG-1-deficient neurons, we generated PRG-1/LPA2-receptor-deficient animals. Electrophysiological analysis revealed that the pathophysiology observed in the PRG-1-deficient mice was fully reverted in PRG-1/LPA2-receptor-deficient animals. Our study shows that PRG-1 is an important player in the modulatory control of hippocampal excitability dependent on presynaptic LPA2-receptor signaling.

This work was supported by the SFB 665 Project B3

Kategorie: Vortrag

Vortrag 43

Rubrik: 3.Neuroanatomie/Neurobiologie
Abstract Nr.:3

Titel:AF9/MLLT3 interferes with tbr1 expression through epigenetic modification of histone H3K79 during development of upper layers in the cerebral cortex

Autoren: Vogel T.(1),

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Abstract:
Mutations of leukaemia associated AF9/MLLT3 are implicated in neurodevelopmental diseases such as epilepsia and ataxia, but little is known about how AF9 influences brain development and functioning. Mouse Af9 is transcribed in various CNS structures including the subventricular zone (SVZ) and the developing cortical plate of the cerebral cortex. Expression of Af9 in the SVZ of the cerebral cortex overlaps with Tbr2, confining its activity to the neurogenic compartment of the SVZ. Analysis of Af9 mouse mutants reveals that Af9 is involved in the maintenance of the pool of Tbr2-positive intermediate progenitor cells (IPCs) in the SVZ and prevents premature exit of IPCs from the cell cycle. Further, in postmitotic neurons of the developing cortical plate, Af9 is implicated in the formation of the 6-layered cerebral cortex by suppressing a Tbr1-positive cell fate mainly in upper layer neurons. We show that the molecular mechanism of Tbr1 suppression is based on the interaction of Af9 with Dot1l, a protein that mediates transcriptional control through methylation of histone H3 at position K79 (H3K79). We show that Af9 associates with the transcriptional start site of Tbr1 and that Af9 suppresses RNAPolII-dependent Tbr1 expression through increased levels of H3K79 dimethylation.

Kategorie: Vortrag

Vortrag 44

Rubrik: 3.Neuroanatomie/Neurobiologie
Abstract Nr.:3

Titel:Cytoarchitectonic mapping of the human frontal pole

Autoren: S. Bludau 1, A. Schleicher 2, K. Amunts 1,3 and K. Zilles 1,2

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Abstract:
Brodmann's area 10 of the frontal pole occupies a larger proportion of the whole brain than in any other species (Semendeferi et al. 2001); it is involved in higher cognitive functions such as planning of future actions and the ability to draw analogies (Cho et al., 2009). However, little is known about its intersubject variability, architecture, connectivity and function. Aim of the present study was to analyse the cytoarchitecture of BA10 in the human brain, to evaluate its extent with respect to gross macroscopic landmarks and neighbouring cortical areas and to create a three-dimensional probability map in standard reference space.
BA10 was mapped in serial histological sections of ten postmortem brains using an observer-independent approach for the definition of areal borders (Schleicher et al., 1999). Multivariate statistical analysis of the cytoarchitecture revealed that BA10 was heterogeneous in its dorsal and ventral cell density measured via the observer-independent approach. BA10 occupied the most rostral parts of the superior and middle frontal gyri including the frontomarginal sulcus. Latero-caudally BA10 bordered to middle frontal BA46, and dorsally to BA9. The anterior end of the olfactory sulcus marked the approximate border to orbito-frontal area BA11, supporting earlier cytoarchitectonic single subject and MRI studies (Semendeferi et al., John et al., 2007). Three-dimensional cytoarchitectonic probability maps were generated, which showed a considerable intersubject variability in space and extent of the areas. This map enables future comparison with in vivo neuroimaging data, e.g., fMRI, in order to provide a better understanding of structural-functional relationships in this part of the brain.

Kategorie: Vortrag

Vortrag 45

Rubrik: 3.Neuroanatomie/Neurobiologie
Abstract Nr.:3

Titel:Impact of a deletion of MEGAP on brain architecture and neuronal plasticity

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Abstract:
Several genes are known to be involved in mental retardation (MR). In 2002, a gene encoding for a protein called MEGAP was discovered, which was disrupted and functionally inactivated in a patient displaying severe mental retardation (MR). Since MEGAP shares homologies with other RhoGAP-proteins, it is thought that MEGAP plays crucial roles in neuronal plasticity and higher brain functions. MEGAP-deficient mice have recently been generated and we have started to analyze them with respect to putative morphological alterations in the brain.
Mutations in oligophrenin1 can induce MR, accompanied by ventricular enlargement. Likewise, MEGAP-deficient mice display enlarged ventricles. This enlargement is not accompanied by reductions in the thickness of fiber tracts or brain areas. Instead, the mentioned areas display increased mean thicknesses; thus, both parameters contribute to an increase in total brain size and weight (brains of MEGAP-deficient mice display an increase in wet-weight of more than 20%).
Consistent features of neurons in patients with MR or in several mouse models of MR are abnormal dendritic structures and/or alterations in dendritic spine morphology. By using computer-based reconstructions of Golgi-stained material, we found that spine densities were not altered in hippocampal CA1 neurons of MEGAP deficient mice, but the individual spine length was increased.
In summary, these data support the notion that MEGAP plays specific roles in the brain. Further investigations will not only provide insight in the roles of MEGAP, but may also be helpful getting insight in fundamental mechanisms involved in neuronal plasticity and the development of mental dysfunctions.

Kategorie: Vortrag

Vortrag 46

Rubrik: 3.Neuroanatomie/Neurobiologie
Abstract Nr.:3

Titel:Enhanced axonal sprouting and functional recovery after spinal cord injury by a peptidic fragment from clostridium botulinum C3 protein

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Abstract:
We have recently identified a region of Clostridium botulinum C3 transferase covering the amino acids 154-182 (C3bot154-182) responsible for enzyme-independent neurotrophic effects on primary hippocampal cultures as well as on organotypical brain slice cultures. Here we report on beneficial effects of this short peptide on the recovery from spinal cord injury in mice. Immediately after contusive spinal cord injury C3bot154-182 was administered to the site of lesion and the restoration of motor function was observed for three weeks. Two behavioural tests, the Basso Mouse Scale for Locomotion and the Rotarod paradigm detected an enhanced recovery in peptide-treated mice. Additionally, the descending corticospinal tract was traced to detect regenerating fibers after lesioning. Treated animals showed a higher number of fibers both proximal and distal to the site of lesion, presumably representing the process of enhanced regenerative sprouting. We also looked for possible effects of treatment with C3 peptide on neuromuscular junctions following spinal cord injury. In the anterior tibial muscle the number of motor endplates was increased by 30% in treated animals. The observed higher number of motor endplates was accompanied by a reduced degree of denervation following the injury.
In conclusion, C3bot154-182 represents a promising tool to foster regeneration following injury to the CNS.

Kategorie: Vortrag

Vortrag 47

Rubrik: 3.Neuroanatomie/Neurobiologie
Abstract Nr.:3

Titel:Neuregulin 1 type 3 induced schwann cell migration along growing scg axons

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Abstract:
Schwann cells (SCs) are the main glial cell type of the peripheral nervous system. Although SCs have been studied intensively for decades, only little is known about how SCs migrate along extending nerves during development. Two factors were suggested to regulate this process. On the one hand side the glial cell line derived neurotrophic factor (GDNF), via non canonical signaling, and on the other hand side Neuregulin (NRG) 1, via ErbB signaling. Overall, analysis of SC migration in mouse is hampered by methodological restrictions. In previous studies different assays, like in vitro scratch assays or in vitro emigration assays from transected nerves were used. All these assays have in common the lack of the physiological environment of the SCs, the axons. In this study we took advantage of a three dimensional superior cervical ganglion (SCG) explant system, in which at first axons elongate under the treatment with NGF, followed by migrating SCs along the axons. In addition to this explant assay, life imaging was used to study migration in near lifetime. By the application of different chemical inhibitors and the use of mutant tissue, derived from GDNF and NRG1 type III KO embryos, this study strongly suggests Neuregulin to be the factor driving SC migration along extending SCG axons.

Kategorie: Vortrag

Vortrag 48

Rubrik: 3.Neuroanatomie/Neurobiologie
Abstract Nr.:3

Titel:Functional and structural integration of transplanted neurons into organotypic cortical slice cultures

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Abstract:
Transplantation of stem cells or neonatal neurons into brain areas may compensate for the cell loss in neurodegenerative disorders. However, it remains unclear to what extent transplanted neurons structurally and functionally integrate into resident neural networks and in which way neurotrophic factors, such as BDNF, play a role in this integration. We analyzed functional and morphological properties of EGFP-expressing cortical neurons derived either from neonatal BDNF-wildtype or BDNF-knockout mice that were transplanted into neonatal cortical slice preparations. After 4 weeks in culture, resident and transplanted neurons were electrophysiologically and morphologically investigated. The morphometric analysis revealed extensive axons with numerous boutons not only for resident cells, but also for transplanted wildtype and BDNF-knockout neurons. The functional integration of transplanted neurons was characterized by the frequency of spontaneous miniature postsynaptic currents (mPSCs) and the spatial distribution of synaptic inputs following focal photolysis of caged-glutamate. The observed spatial distribution of origins for excitatory and inhibitory synaptic inputs indicates that transplanted wildtype cells establish a functional circuit pattern which is comparable to that of resident neurons. However, we found a strong reduction in the frequency of spontaneous, GABAA-receptor mediated mPSCs in transplanted BDNF-knockout neurons. Furthermore, the lack of BDNF in transplanted BDNF-knockout neurons led to a reduced number of origins for inhibitory inputs. Thus, our data indicate that following transplantation neonatal cortical neurons are structurally and functionally integrated into the resident networks of cortical slice cultures. In the course of this integration BDNF might be relevant for establishing proper integration into inhibitory cortical networks.

Kategorie: Vortrag

Vortrag 49

Rubrik: 3.Neuroanatomie/Neurobiologie
Abstract Nr.:3

Titel:The role of mitochondrial morphology and function during neurodegeneration and neuroprotection

Autoren: Arnold S.(1),Singh S.(1),Misiak M.(1),Roemgens A.(1),Beyer C.(1),

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Abstract:
Purpose: The pivotal role of mitochondrial dysfunction in neurodegenerative diseases is gaining increasing acceptance. Our studies focus on acute and chronic changes of morphological and functional mitochondrial properties in in vitro and in vivo models of neurodegeneration and the role of selective protective factors such as steroid hormones therein.
Methods: Expression analyses of mitochondrial proteins in cultured mouse brain astrocytes and neurons using quantitative RT-PCR and Western Blotting were performed and correlated with intracellular ATP and ROS levels and cell viability. Studies of transgenic mice and treatment of animals with toxins and steroids in vivo were designed to verify in vitro data.
Results: An imbalance of mitochondrial fusion/fission proteins and respiratory chain enzymes are apparently involved in the generation and promotion of cell damage in different neurodegenerative animal models, i.e. Huntington's disease, Parkinson's disease, and amyotrophic lateral sclerosis. We could demonstrate a cell type- and brain region-specificity during toxic and protective processes. This also includes gender-specific aspects in the responsiveness towards toxins and hormones.
Conclusions: An impairment of energy metabolism and an increase of oxidative stress by mitochondria are two mechanisms involved in neurodegenerative cell death, whereas interactions of sex steroids with mitochondria may promote cell survival and represent an important mechanism for gender differences in cellular pathology in the CNS.

Kategorie: Vortrag

Vortrag 50

Rubrik: 3.Neuroanatomie/Neurobiologie
Abstract Nr.:3

Titel:Antimicrobial peptide rcramp induce glial cell activation through p2y receptor signalling pathways

Autoren: Brandenburg L.(1),Jansen S.(1),Wruck C.(1),Lucius R.(2),Pufe T.(1),

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Abstract:
Antimicrobial peptides are part of the innate immune system in many organ systems, yet little is known about their expression and function in the brain. An antibacterial cathelicidin, rCRAMP in rat (homologue of the human LL-37), not only exhibits potent bactericidal activities against bacteria, but also functions as a chemoattractant for immune cells. In this study, to further evaluate the role of rCRAMP in innate immunity of the brain cells, we investigated the action of rCRAMP on glial cell activation. We therefore analyzed the activation of rCRAMP-induced signalling by cytokine expression and by Western blotting of different signal transduction pathways and cAMP level measurement in primary rat glial cells (astrocytes and microglia). We demonstrated (i) the induction of proinflammatory cytokine and neurotrophic factors and (ii) the activation of different signal transduction pathways by rCRAMP in glial cells. Moreover, (iii) we could show that rCRAMP-induced IL-6 expression and ERK1/2 phosphorylation in glial cells was attenuated by the antagonists for P2Y receptors. In conclusion, this study provide evidence that rCRAMP may be involved in brain immunity through stimulating cytokine production and glial cell activation and protection of brain cells by induction of neurotrophic factors.

Kategorie: Vortrag

Vortrag 51

Rubrik: 3.Neuroanatomie/Neurobiologie
Abstract Nr.:3

Titel:Homeobox gene Sax2 is required for diet-induced obesity

Autoren: Simon R.(1),Britsch S.(1),Bergemann A.(2),

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Abstract:
Regulation of energy homeostasis is mainly mediated by factors in the hypothalamus and the brainstem. The homeobox gene Sax2 is expressed predominantly in the brainstem, in the vicinity of serotonergic neurons, and in the ventral neural tube starting during early development. Previously we have shown that the deletion of the Sax2 gene in mouse causes growth retardation starting at birth and a high rate of postnatal lethality as well as a dramatic metabolic phenotype (Simon et al, 2007; Simon and Lufkin, 2003). To further define the role of Sax2 in energy homeostasis, age matched adult wild-type, Sax2 heterozygous and null mutant animals were exposed to a high fat diet. Although food uptake among the different groups is comparable, Sax2 null mutants fed a high fat diet exhibit a significantly lower weight gain when compared to control animals. Unlike their counterparts Sax2 null mutants do not develop insulin resistance and exhibit significantly lower leptin levels under both, standard chow and high fat diet conditions. Furthermore NPY, an important regulator of energy homeostasis, is significantly decreased in the forebrain of Sax2 null mutants on a high fat diet. These data strongly suggest a critical role of Sax2 gene expression in diet-induced obesity. Sax2 gene expression may be required to allow the coordinated crosstalk of factors involved in the maintenance of energy homeostasis, possibly regulating the transcription of specific factors involved in energy balance.

Kategorie: Vortrag

Poster 1

Rubrik: 1.Methoden/Unterricht
Abstract Nr.:1

Titel:Intraocular pressure measurement in the adult chicken eye: tonopenxl vs. rebound-tonometer

Autoren: Schrödl F.(1),Strohmeier C.(2),Bogner B.(2),Nischler C.(2),Reitsamer H.(2),

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Abstract:
Introduction: The neuronal control of intraocular pressure (IOP) is not understood. Several non-invasive methods of measurement exist. However, these methods are not validated on the adult chicken eye, a model to study the intrinsic innervation. To compare two methods and to test their reliability compared to absolute IOP values was aim of this study.
Methods: In canulated eyes of anesthetized adult chicken (n=4) a defined IOP (10 to 50 mmHg; steps of 10 mmHg) was set via water column. Subsequentely, 15 measurements were recorded on each IOP set point with two non-invasive instruments: the digital applanation tonometer Tonopen XL® and the rebound tontometer Icare®, human.
Results: A linear IOP relationship was detected when comparing both instruments with the values of the invasive method. The TonopenXL shows an overestimation at lower IOP´s (intersection between 25 to 30 mmHg) and an underestimation at higher IOP´s (actual pressure = 5.2+0.821×deteced pressure; r=0.99, p<=0.05). This relation is more pronounced in the Icare tonometer readings; additionally a clear paraxial shift of the measured curve has been observed (actual pressure = -3,48+0,649× real pressure; r=0.99, p<=0.05). Further, the variability of the single readings in the Icare tonometer are lower compared to the TonoPenXL (95% CI: 0 mmHg – 1,04 mmHg vs. 0,53 mmHg – 3,56 mmHg).
Conclucion: Both devices resulted in valid IOP readings in the pressure range investigated. Compared to the TonopenXL, the Icare tonometer handling is easier and measurements show lower variation.

Kategorie: Poster

Poster 2

Rubrik : 1. Methoden/Unterricht
Abstract Nr.: 1

Titel: Schirmer strip vs. capillary method: A non-invasive way to gain proteins from tear fluid

Autoren: Andreas Posa, Friedrich Paulsen and Lars Bräuer

Adressen: Department of Anatomy and Cell Biology, Martin Luther University of Halle-Wittenberg, Halle/Saale, Germany

Abstract:
Tear fluid is a complex liquid mixture of more than 500 different proteins, fatty acids, sugars and ions produced by serous and seromucous lacrimal glands to lubricate and protect the tissues of the ocular surface. The retrieval of an appropriate amount of tear fluid from the ocular surface is prerequisite for investigations of the composition of tears especially during diverse pathologies of the eye. The most common established technique therefore is using a small glass capillary to draw the tear fluid directly from the conjunctival sac. But, applying this method on patients involves the risk of injuring the eye which in worst case leads to blindness. In order to minimize feasible harm on patients we investigated the content and concentration of proteins within tear fluid collected by performing Schirmer's test, which is usually applied to diagnose a putative dry eye disease. After collecting and extracting the tear fluid from the Schirmer strips, the obtained "Schirmer-tears" were compared with the "capillary-tears" with regard to the total protein concentration measured by means of Bradford protein assay. Both samples showed nearly the same protein content. In addition, using Western blot analysis we could evidence explicit proteins covering molecular weights ranging from 5 kDa to 50 kDa.
Our investigations reveal the potential of using the harmless, non-invasive Schirmer's test not only for diagnostic purposes but also for extracting adequate amounts of protein out of tear fluid. The obtained proteins might then be used in further investigations such as ELISA quantification or Western blot analysis.

Kategorie: Poster

Poster 3

Rubrik: 1.Methoden/Unterricht
Abstract Nr.:1

Titel:Ultrastructural qutitative densitometric assessment of 11-beta hyroxysteroid dehydrogenase enzyme in the hepatic and renal tissues of the rat after microwave fixation

Autoren: Mohamed M., Abusaida H., Abdelaziz M.

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Abstract:
Methods used for Ultrastructural localization of steroid dehydrogenases were not readily available and had proved to be unreliable .Therefore,we attempted to treat 11&beta-hydroxysteroid dehydrogenase histochemistry with the use of microwave irradiation. Different treatments of microwave irradiation and/or glutraldehyde were tested.Ultrafast microwave irradiation was obtained at microseconds duration from a pluse-operated microwave device. This device was used to avoid unpredictable patterns,varation in power levels and inefficient power transfer to the specimens common to microwave ovens.Quantitaive densitometric assessment of different treatment as reference values of 11&beta-hydroxysteroid dehydrogenase for control samples was recorded .The present study showed that microwave irradiation technique has the advantages of stabilization of end product of histochemical reaction and increasing of its contrast in electron microscopic examination.The main conclusion of the present study is the presence of synergistic action between glutraldehyde and microwave irradiation in tissue fixation.

Kategorie:Poster

Poster 4

Rubrik: 1.Methoden/Unterricht
Abstract Nr.:1

Titel:Volumetric measurement of osteonecrotic femoral head using magnetical resonance imaging

Autoren: Sferdian M.(1),Frandes C.(1),Pop A.(1),Goldis D.(1),Damian G.(1),

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Abstract:
Background: Several studies have documented that the size of the osteonecrotic lesion in the femoral head is an essential parameter in determining prognosis and treatment
Methods: Our study tested the accuracy of osteonecrotic lesions measured by a computed magnetic resonance imaging in patients 15 with osteonecrosis of the femoral head (ONFH). The values were compared with direct anatomical measurements of the femoral head after total hip replacement.
Results: Volumetric measurement appeared to be very reliable. The mean absolute deviation between MRI and anatomical measuments was similar to that of two MRI sets.
Conclusions: Quantitative volumetric measurements appear to be the most reliable method to measure the true size of a three-dimensional osteonecrotic lesion of the femoral head. Determination of lesion size must be part of a comprehensive system of staging of this disease.

Kategorie: Poster

Poster 5

Rubrik: 1.Methoden/Unterricht
Abstract Nr.:1

Titel:Anatomo-imaging study about mediastinal lymph nodes in hodgkin's disease

Autoren: Bolintineanu S.(1),Grigorita L.(1),Vaida M.(1),Matu C.(1),Pop E.(1),Sargan I.(1),Sztika D.(1),

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Abstract:
The Hodgkin disease is a cancer of the lymphatic system characterized by the presence of the Reed-Sternberg cells on the background of the lymphocytes, macrophages, fibroblasts and granulocytes.
This study was made by the retrospective examination of a number of 36 patients, all of them suffering of this disease.
As a reference book and woek techinges we used the CT radiological examination and the MRI technique at the Military Hospital from Timisoara.According to these experiments we found out that the radiological examination shows the adenopathy of the lymph node at 70-75 % from the patients.
The CT examination is an election examination showing the changings of the mediastinal ganglion at the patient who were tested and the MRI investigation is superior to CT examination, because it is able to show more clearly the structure changings next to it.
KEY WORDS: Lymph node, Hodgkin disease, CT, MRI.

Kategorie: Poster

Poster 6

Rubrik: 1.Methoden/Unterricht
Abstract Nr.:1

Titel: Modern approaches in the anatomical curriculum at Ulm university

Autoren: Boeckers A.(1),Fassnacht U.(1),Boeckers T.(1),

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Abstract:
Anatomical education demands more than pure teaching of anatomical structures in these days. Centred around the dissection course -the heart piece of preclinical education- anatomy has to define itself as a modern subject again transferring preclinical learning objectives into a clinical context, mediating the clinical relevance of anatomical knowledge and exemplifying medical professionalism as part of the curriculum.
The Institute of Anatomy and Cell Biology of Ulm University presents an anatomical learning concept based on a two year long and clinically orientated learning spiral. Learning contents are repeated but increase in complexity over time. According to the students level of knowledge curricular teaching units are supplemented by extracurricular courses.
Founded by student fees a unique Theatrum anatomicum – in the style of ancient Theatrum anatomicum - was built at Ulm University. In a simulated operating setting students in their preclinical education are allowed to take part in invasive procedures performed on body donors. Clinical colleagues act like mentors and encourage students in developing their role as medical professionals. These procedures plus a surgical scrub course are imbedded in the gross anatomy course. In addition voluntary courses like "Anatomie im Bild (AiB)" and „Dr House revisited“ are based on this preclinical-clinical interlocking and motivate students to learn anatomical knowledge. The presented teaching concept led to an improved teaching climate, excellent student acceptance and better examination results. In November 2008 this teaching concept was gratified with the Landeslehrpreis of Baden-Württemberg.

Kategorie: Poster

Poster 7

Rubrik: 1. Methoden/Unterricht
Abstract Nr.: 1

Titel: A „teach the teacher“ training scession for a seminar on anatomy with clinical context increases the confidence and the background knowledge of physicians and non-medical teachers.

Autoren: Katja Horling[1], Friedrich Paulsen[2], Georg Lüers[3]

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Abstract:
Teaching of anatomy plays a central role in preclinical medical education. Students and physicians consider the anatomical knowledge as the most important fundamental component in medical education and the development of expertise during clinical training. In order to focus on the clinically relevant anatomy, in Germany, the teaching of anatomy in the context of clinical problems is requested by law (*ÄApprO from 2002*). However, since most of the teachers of anatomy are not physicians and thus have never obtained any medical training, the professional teaching of anatomy in a clinical context is a serious challenge.
We have established a seminar on “anatomy with clinical context” and a “teach the teacher” training scession to prepare teachers for the clinical background. For the training, the teachers were paired to tandems of a physician and a non-medical teacher. The teacher tandems recieved a practical training in new didactic methods used in the seminar and were instructed to the required theoretical background. An evaluation of the training revealed that all participants recommended structured training sessions to improve their own competence. The tandem was evaluated as very positive construct that was helpful not only during the actual training but also for preparation of the seminar and the reflections about the new didactic methods and the contents of the seminar. This kind of training might be a valuable instrument to compensate for the increasing lack of medical knowledge among teachers of basic medical sciences and could help to improve the intended teaching of clinically relevant anatomy.

Kategorie: Poster

Poster 8

Rubrik: 1.Methoden/Unterricht
Abstract Nr.:1

Titel:Blended learning in medical education: establishment of the e-learning tool „schoolbook cell biology“ at Hannover medical school

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Abstract:
At Hannover Medical School, cell biology is the major part of the module “Cell Biological and Genetic Principles in Medicine”. It covers cell biology as well as histology and consists of lectures, seminars, and practical courses. In the practical courses histological slides are examined, electron micrographs are discussed and practical experiments are performed. Nevertheless, students have claimed difficulties especially with identifying structures in the light microscope. Therefore, we decided to supplement the curricular teaching starting in November 2008 with an accompanying web-based learning program using the content management system “Schoolbook” (http://www.medicalschoolbook.de/project/).
We started with taking digital images of histological slides used in the course and loading them into the Schoolbook system following the didactic structure of the practical course. At the end of the term students were asked to evaluate the “Schoolbook Cell Biology”. The results showed that more than 90% of the responding participants graded the usability of the software as very good or good. About 85% found the Schoolbook helpful for preparing for the course and about 98% considered it useful for going through the topics again after the curricular lessons. To 95% it was helpful for their preparation for the final exam.
We conclude that the “Schoolbook Cell Biology” is a useful support in teaching cell biology to medical students. Therefore, we plan to expand its contents by adding (1) electron micrographs used in the practical course, (2) further information, e.g. movies, weblinks etc., and (3) a “problems module”, supporting the students in estimating their level of knowledge.

Kategorie: Poster

Poster 9

Rubrik: 2.Klinische Anatomie/Makroskopie
Abstract Nr.:2

Titel:The middle ear of the chinchilla: a microdissectional study

Autoren: Ay H.(1),Ortug G.(1),

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Abstract:
In recent years various animal models have been developed to study the pathophysiology of the different conditions and surgical approach. The chinchilla is helpful for otological research and has been used in basic experimental studies. This study has been undertaken to further clarify the detailed middle ear components of the cavity and temporal bulla. In this study, a total 20 adult chinchilla (C. lanigera) weighing 350 to 450 gm, were used. All the animals under complete ketamine anesthesia were perfused with 2.5 % glutaraldehyde solution in 0.1 M sodium-potassium phosphate buffer (pH 7.2) via carotid artery. The heads of the chinchilla were bisected midsagittally and one half containing the whole temporal bone was completely passed through the microdissectional procedures. Microdissections were made by SMZ 10 Nikon Stereomicroscope and digital photographs were taken. In all animals, anatomically structures in middle ear exhibited similar features.
In conclusion, the present study reveals that there is high similarity between the middle ear morphology of rat, frequently used in experimental studies, and chinchilla which is another member of the rodent family. However, some individual differences are observed. The large septal tympanic bulla is characterized in chinchilla. The incudomalleal joint sellar, and incudostapedial joint are spheroid type. The discus in incudostapedial joint is noticeable. The ossicles are tiny and the facial nerve is seen as very large calibration. The cochlea promontory of the chinchilla and oval window are also very large.

Kategorie: Poster

Poster 10

Rubrik: 2.Klinische Anatomie/Makroskopie
Abstract Nr.:2

Titel: Arterial ramification and initial venous drainage in pig myocardium - a sem study

Autoren: Ruffer S.(1),Schweiger M.(1),Steffl M.(1),Amselgruber W.(1),

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Abstract:
The pig heart is considered to be a well established model in human cardiovascular research and of special interest as a prospective xenotransplant. However, knowledge of cardiac microangioarchitecture is limited. In this study we focused on analyzing myocardial capillary distribution and angiomorphology.
Hearts of 10 pigs at different ages were isolated and prepared for coronary vessel corrosion casting. Various samples of several regions were dissected and prepared for scanning electron microscopy.
We observed different patterns of ramification of coronary arteries as well as the peculiar character of venous drainage in the pig heart. Arteries enter myocardium directly perpendicular after running under the epicardium at their beginning. They split in acute angles until arriving at the size of capillaries, which varies from 3,8 to 5 µm. The capillary bed is typically arranged in an extraordinary dense network, oriented parallel to muscle fibers. Those form multiple layers in slightly alternating angles. Next to arterio-arterial anastomoses, which are quite frequent facing epicardial surface, capillary bed exhibits many intercapillary cross-bridges. Branching of capillaries occurs in right angles, similar to a rope-ladder pattern. On the venous side numerous venules open quickly in larger veins of up to triple size of diameter. Venous valves of different architecture can be noticed even at a very small size of veins, beginning at a diameter of 100 µm.
Our findings clearly show the compact arrangement of capillaries in the pig heart, characterized by arterio-arterial anastomoses, complex layering and a venous drainage, secured by the early emplacement of venous valves.

Kategorie: Poster

Poster 11

Rubrik: 2.Klinische Anatomie/Makroskopie
Abstract Nr.:2

Titel:Is plating of midshaft clavicular fractures with a conventional straight 3.5mm locking compression plate (lcp) possible?

Autoren: Grechenig W.(1),Heidari N.(2),Leitgoeb O.(1),Prager W.(1),Grechenig S.(1),Weinberg A.(3),Tesch N.(4),

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Abstract:
Current literature describes improved clinical outcomes and a minor rate of pseudoarthrosis following operatively treated clavicular fractures
We measured 49 clavicles and investigated the feasibility of using standard 3.5mm AO Locking Compression Plate (LCP) of adequate length for the stabilisation of midshaft fractures.
The mean length was 155mm (SD 12mm) with a mean acromial curvature of 18.1 mm (SD 3.7 mm) and a mean diaphyseal curvature of 12 mm (SD 4 mm).The optimum plate for the clavicle was a 7-hole LCP providing adequate fixation in 48 of the 49 clavicles.In the one case that the 7-hole plate did not fit a 6-hole one did. This turned out to be the shortest clavicle.The 6-hole plate was a good fit in 5 (10.2%) clavicles.In 37 (76%) an 8-hole LCP and in 27 (55%) a 9-hole plate were safely applied.In 17 clavicles (34.7%) the plate stood off the cranial surface of the clavicle medially by a mean of 2.47mm (SD 1.28 mm), in 18 (36.7%) the plate stood off the cranial surface of the clavicle laterally by a mean of 2.72mm (SD 1.13mm) and in 5 (10.2%) clavicles the plate stood off the cranial surface of the bone in the middle by a mean of 2.8 mm (SD 1.3mm) In principle this technique for plate osteosynthesis of the clavicle is feasible. However in longer and more curved clavicles this becomes more complicated with increasing tendency for the plate to stand off the bone. In these circumstances it may be prudent to revert to other metalwork such as the reconstruction plates.

Kategorie: Poster

Poster 12

Rubrik: 2.Klinische Anatomie/Makroskopie
Abstract Nr.:2

Titel:Scapulothoracic unit-anatomical and functional data

Autoren: Petrescu C.(1),Cebzan C.(1),Sisu A.(1),Stana L.(1),Jianu A.(2),Niculescu M.(2),Vartolas L.(3),Tatu F.(3),

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Abstract:
Scapulo-thoracic unit consists of: scapulae box and thoracic cage, fake scapulothoracic joint, setting clavicular baguette, his joints and fixing muscles and parts of mobilizing mobile bones. In almost all shoulder movements, a scapula is moving on the thoracic cage. Thoracic cage is the deep and theoretic fixed frame on that "rest" all the shoulder systems. Its shape is not exactly the one of a semi- trunk open inside, with a large inferior base, because the intercostals arches and intercostal spaces (filled with intercostal muscles) produce an area where the curvature is convex out and inside and from top to down. Reinforced by the thickness and bone relief forces, its anterior face is concave on its whole expanse. Between the two bony plans, we can talk about a scapulothoracic joint. The term of “joint” is meaningless in anatomical terms and relatively true in terms of bio-mechanically and fiziopathological. In order to move efficient the scapula on the thoracic cage, are necessary more mechanical systems: Scapulothoracic “joint” is only a resultant, not an anatomical and mechanical component: there is no ligament, no articular surface that can regulate these movements. It is necessary that plans to run free, but they do not keep the scapula on the place. A muscular couple of major functional importance maintains the scapula plated on the thoracic cage; couple consists of two antagonistic muscles: the middle part of trapezius muscle and serratus anterior muscle. Scapula movements are controlled and directed in part by the sternoclavicular joint.

Kategorie: Poster

Poster 13

Rubrik: 2.Klinische Anatomie/Makroskopie
Abstract Nr.:2

Titel:Active movements in scapulohumeral joint: flexion (antepulsion) and extension (retropulsion)

Autoren: Petrescu C.(1),Sisu A.(1),Cebzan C.(1),Folescu R.(1),Motoc A.(1),Vartolas L.(2),Tatu F.(3),Rusu M.(4),

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Abstract:
Movements antepulsion-retropulsion run in a sagital plane, with the subject in a transverse plane: retropulsion (extension) with an amplitude of 45-50; antepulsion (flexion) with an amplitude of 180 (note that the same position antepulsion of 180 can be defined as a abduction to 180). Still talking about the three times you antepulsion-flexion. The first time (from 0 to 50-60) involves the following motor muscles: the anterior fascicle (clavicular) of deltoid muscle, coracobrachialis muscle, the superior (clavicular) fascicle of the pectoralis major muscle. This antepulsion in scapulo-humeral articulation is limited by two factors: the pressure of coracohumeral ligament and the strength of teres minor muscle, teres major muscle and infraspinatus muscle. The second time (from 60 to 120) involving the game scapular belt: rotation to 60 of scapulae by a movement guided glenoid cavity up and before; axial rotation in sterno-costo-clavicular joint and acromio-clavicular joint, each with 30 attending. Motor muscles are the same as those of abduction: trapezius muscle and serratus anterior muscle. This antepulsion of scapulothoracic joint is limited by the latissimus dorsi muscle strength and the inferior fascicle of pectoralis major muscle. In the third period (from 120 to 180) antepulsion is locked in scapulohumeral joint and scapulothoracic joint.Muscles involved in retropulsion of scapulohumeral joint are: teres major; teres minor, posterior fascicle (spinous) of deltoid muscle, latissimus dorsi. Retropulsia in scapulothoracic joint by scapulae adduction is performed by following muscles: rhomboid, the middle (transversal) fascicle, the trapezius, latissimus dorsi.

Kategorie: Poster

Poster 14

Rubrik: 2.Klinische Anatomie/Makroskopie
Abstract Nr.:2

Titel:Influence of approach in successful intra-articular injection of the radiocarpal joint

Autoren: Tesch N.(1),Heidari N.(2),Grechenig S.(3),Grechenig W.(3),Weiglein A.(4),Clement H.(3),Weinberg A.(5),

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Abstract:
Intra-articular puncture of all joints are performed by a multitude of healthcare professionals for aspiration of effusion to aid diagnosis and inject drugs and radiographic contrast.
42 cadaveric wrists were injected with methylene blue in the radiocarpal joint by a senior registrar in trauma and orthopaedics. 21 were injected dorsally with the entry point 0.5 to 1 cm distal to Lister's tubercle and the other 21 injected laterally from 0.5 to 1 cm distal to the radial styloid through the anatomical snuff box.
3 (14%) injections were peri-articular in the lateral approach group where as only 1 (5%) was peri-articular in the dorsal group. Fisher's exact test gives a two tailed P value of 0.61, showing no significant difference between the two methods.
Intra-articular puncture cannot be guaranteed even in experienced hands. Unintended peri-articular injection can cause complications and a missed intra-articular puncture can frustrate diagnosis. Needle placement may be aided by the use of ultrasound scanning or fluoroscopy.

Kategorie: Poster

Poster 15

Rubrik: 2.Klinische Anatomie/Makroskopie
Abstract Nr.:2

Titel:Frequency of intra-articular puncture of the radiocapitelar joint. a cadaveric study.

Autoren: Weinberg A.(1),Heidari N.(2),Grechenig S.(3),Grechenig W.(3),Weiglein A.(4),Pichler W.(3),Tesch N.(4),

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Abstract:
Intra-articular puncture of all joints are performed by a multitude of healthcare professionals for aspiration of effusion to aid diagnosis and inject drugs and radiographic contrast for therapy and imaging.
76 (38 right and 38 left) cadaveric elbow joints injected with methylene blue. The punctures on the were performed by two specialists in trauma and orthopaedics, one injecting the right and the other injecting the left joint. A lateral approach was used to inject into the radiocapitelar joint. An arthrotomy was then performed to confirm the presence of the dye within the joint.
The injections were peri-articular on 3 (7.9%) occasions on the left and on 2 (5.2%) occasions on the right. No significant difference was observed between the two specialist. The lateral approach is a safe and consistent way to perform intra-articular puncture of the radiocapitelar joint.
Intra-articular puncture cannot be guaranteed even in experienced hands. Unintended peri-articular injection can cause complications and a missed intra-articular puncture can frustrate diagnosis. Needle placement may be aided by the use of ultrasound scanning or fluoroscopy.

Kategorie: Poster

Poster 16

Rubrik: 2.Klinische Anatomie/Makroskopie
Abstract Nr.:2

Titel:Computer aided three dimensional morphometry of the proximal ulna. do anatomically preshaped plates make sense?

Autoren: Puchwein P.(1),Pichler W.(1),Schöffmann S.(1),Heidari N.(2),Windisch G.(3),Grechenig W.(1),Tesch N.(3),

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Abstract:
Anatomically preshaped olecranon plates are increasingly used for stabilization of comminuted olecranon as well as Monteggia fractures. Purpose of this study was to investigate the three dimensional morphology of the proximal ulna using 64-slice CT scans of 30 cadaveric elbows and to discuss the findings focusing on the geometry of different preshaped plates. CT scans were reconstructed and measured using a 3D-software.
Measurements revealed a mean dorsal hook angle of 94.2° (74.66° to 110.77°), with a specific gender differences, a mean distance from the the tip of the olecranon to the insertion of triceps of 25.43mm, a mean varus angulation of 14.26° (6.40° - 22.94°) and a mean anterior deviation of 7.91° (3.04° - 13.58°).
Our results demonstrate that the proximal ulna has a varied morphology. The construction of an anatomically preshaped plate providing a perfect fit in most cases would be a serious challenge. Furthermore the appellation “anatomically preshaped” may misguide the surgeon to reduce the fracture to the plate, inevitably leading to poor functional result in many cases.

Kategorie: Poster

Poster 17

Rubrik: 2.Klinische Anatomie/Makroskopie
Abstract Nr.: 2

Titel:Influence of drug means on the processes of skeleton's growth in an experiment

Autoren: Petrichko S., Krikun E.Belgorod, Kapustin R.

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Abstract:
The investigation has been done with 36 white mongrel rats of immature age, received 1% of solution of morphine hydrochloride in an average experimental dose – 30 mg/kg during 7, 15 and 30 days. The animals of the same age served for the control. In order to study osteometric indexes we have taken out tibia, femur, pelvis bones and third lumbar vertebra. When morphine hydrochloride used we have noticed increasing of longitudinal sizes of bones already at the very beginning. So, by the 15-th day of research the length of tibia has increased by 7,59%, and at the same time the increase in breadth of diaphysis has slowed by 3,43% and fore-back diameter by 4,81% as compared with control indexes. Pelvis bone has increased at length by 9,14%, but it has decreased at width by 8,9%. The length of third lumbar vertebra has increased on average by 10,7%, but breadth has decreased by 7,8% toward the control. By the 30-th day of the experiment the length of tibia bone by 7,93%, pelvis bone by 9,58%, third lumbar vertebra by 9,84% has been greater of control values. Thus, introduction of drug means in the organism of experimental animals influences unfavourably on the processes of skeleton's bones growth that can be seen in breach of the processes of bones' formation.

Kategorie: Poster

Poster 18

Rubrik: 2.Klinische Anatomie/Makroskopie
Abstract Nr.:2

Titel:Can single-slice mr imaging of the thigh predict muscle volume and force?

Autoren: Cotofana S.(1),Hudelmaier M.(1),Wirth W.(1),Himmer M.(2),Ring-Dimitriou S.(3),Sänger A.(4),Eckstein F.(1),

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Abstract:
Measurement of muscle morphology is important in the assessment of muscle volume (MV), length, anatomical cross-sectional area (ACSA) and maximal contraction force in disease, growth or training. Single-slice MR imaging of the ACSA of the femoral extensors has been suggested for estimating extensor muscle volume and maximal contraction forces. The objective was to determine the ideal location in the thigh from which the volume of the extensors, flexors, adductors, and the sartorius can be predicted by single-slice MR imaging.

Transverse MR images of the thigh were acquired in 41 women (age: 50.8±3.2 yrs) with a 1.5 T scanner. Segmentation of the muscles was performed in a region of interest reaching from the femoral neck to the end of the muscular portion of the intermediate vastus. MV was calculated by numerical integration of segmented voxels and ACSA from 3D muscle reconstructions at 10% intervals from proximal to distal.

The extensors occupied 50.1%, the flexors 19.0%, the adductors 27.8%, and the sartorius 3.1% of the total thigh MV. Maximal correlations of ACSA with MV were observed at 40% from proximal in the extensors (r^2=0.73), at 70% in the flexors (r^2=0.72), at 30% in the adductors (r^2=0.82), and at 70% in the sartorius (r^2=0.85). The 50% ACSA displayed correlations r²≥ 0.69 with all MVs.

Single-slice anatomical muscle cross sectional areas permit to estimate thigh muscle volume (and muscle force). Although ideal measurement locations vary, acquisition of a single-slice MR image at the 50% location achieves the best compromise in predicting thigh muscle volumes.

Kategorie: Poster

Poster 19

Rubrik: 2.Klinische Anatomie/Makroskopie
Abstract Nr.:2

Titel:Dorsal nerve of clitoris outside of alcock´s canal overcrossing sacrotuberous ligament

Autoren: Claassen H.(1),Wree A.(2),Schmitt O.(2),

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Abstract:
The gross anatomy of the perineal region has not been fully elucidated, especially in women. In men, entrapment of pudendal nerve in Alcock´s canal can cause refractory unilateral orchialgia and concomitant proctalgia. Here, a variation of Alcock´s canal vessels was recorded during the dissection course of the Institute of Anatomy (University of Rostock) in summer term 2008.
In the left pelvis of a 80-year-old female, a nerve passed the greater sciatic foramen inferior to the piriformis together with the sciatic nerve. It took its origin near to this foramen in form of a fork having the inferior gluteal artery in its centre. After the two roots of the fork have connected, the nerve overcrossed the sacrotuberous ligament and vanished in the fatty tissue of the ischioanal fossa. Following careful preparation, the nerve was identified as the dorsal nerve of clitoris. From the greater sciatic foramen inferior to piriformis to the rim of the superficial transverse perinei it measured approximately 11 cm. However, a part of the pudendal nerve and the internal pudendal artery took their normal way through the Alcock´s canal. Surprisingly, internal pudendal artery has a high origin at the bifurcation of internal iliac artery.
The dorsal nerve of clitoris described here has no protection by obturator internus fascia of Alcock´s canal. Therefore, it can be exposed to repetitive mechanical irritation which can be worsened, for instance, by long-time sitting. Compression of the pudendal nerve in the ischioanal fossa is known from cyclists after long-term bicycle riding.

Kategorie: Poster

Poster 20

Rubrik: 2.Klinische Anatomie/Makroskopie
Abstract Nr.:2

Titel:Patella's role in the knee joint biomechanics

Autoren: Sisu A.(1),Cebzan C.(1),Petrescu C.(1),Samfirescu E.(1),Haivas C.(1),Sargan I.(2),Tatu F.(3),Rusu M.(4),

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Abstract:
Because of its thickness, patella has a certain role in the extension movements to keep distance between the tendon and the femoral trohleea. Moving the quadricipital tendon, towards the axis of rotation of the knee, patella increases the lever arm of quadriceps muscle with 50%. The patellar surface and femoral intercondylar fossa forms a vertical groove, the depth to which patella slides. The quadricipital muscle force forwarded obliquely superior and lateral, is transformed in a strictly vertical one. Because of this, in flexion, patella descends vertically, down the trohleea femoral groove, to the intercondylar fossa. In the flexion movement patella's articular surface has in fact a "circumference movement", because in extension if it is directed back at the end of the journey, so flexible in full, go to the right place of femoral condyles, the upper target. This important move is possible only when the patella is attached to the femur. Conversely, movement of the patella moving extension suprapatellar bursa likely to be caught between the femur and patella, if not drawn up by suprapatellar bursa tensor muscle, which arises from the deep of vast intermediate muscle. Note that the lack of patella after patelectomy not diminishes the strength of extension of calf. Freehafer demonstrated experimentally that the 25 lower limbs cut off for various illnesses, which was measured tensile strength of patellar ligaments before and after patelectomy. The conclusion is that author quoted that patella is only to form a solid bridge between quadricipital tendon and patellar ligament.

Kategorie: Poster

Poster 21

Rubrik: 2.Klinische Anatomie/Makroskopie
Abstract Nr.:2

Titel:Microscopic structure of the knee joint synovial

Autoren: Sisu A.(1),Cebzan C.(1),Petrescu C.(2),Samfirescu E.(2),Haivas C.(1),Vartolas L.(3),Tatu F.(3),Haivas D.(4),

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Abstract:
Synovial is a connective membrane specialized, which covers the internal face of the joint capsule. It is very thin, with variable density and thickness and has two layers: layer deep, connective-elastic, consisting of: cells with globular condroide aspect, adypocites, vessels and nerve threads; superficial layer (internal) comprising: flattened synoviocytes, placed on one or more layers, the synovial free surface (so inwards) is a discontinuous layer of elements of 2 cell types, some like macrophages and others fibroblast-like.These cellular elements are linked by junctional complexes and not reliant on a basal membrane and from this point of view, synovial internal surface is not an epithelium. For the joints are synovial tassels or fringes that have a much supplied connective shaft, covered by synoviocytes. Synoviocytes are large cells with large nuclei and abundant cytoplasm. Morfofunctional describe 3 types: type A, synoviocytes similar to macrophages, with an intense endocytes activity. Synoviocytes types B, fibroblast-like, are fusiform cells with dendritic appearance; present endoreticular departments and have developed Golgi complexes about secretion: hyaluronic acid, glycoproteins, has characteristic secretory granules, which focuses MPZ (PAS positive). Type C cells have characteristics of both types, proliferating in inflammatory diseases, and depending on the stage of development, predominant type A or B. The synovial liquid is a transudate, came from the synovial capillaries, it is secreting hyaluronic acid and glycoproteins. As methods of study using puncture biopsy (HE stain, histochemical, histoenzimological, immunohistochemistry) and synovial fluid cytology (HE stains and Papanicolaou test).

Kategorie: Poster

Poster 22

Rubrik: 2.Klinische Anatomie/Makroskopie
Abstract Nr.:2

Titel:Knee joint ligaments-anatomical data

Autoren: Cebzan C.(1),Sisu A.(1),Petrescu C.(1),Stana L.(1),Jianu A.(1),Vartolas L.(2),Tatu F.(2),Bredicianu R.(2),

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Abstract:
Bone components of the knee joint are maintained in contact by the articular capsule strengthened by a strong ligament device, consisting of six ligaments. Anterior ligament known as “the patellar ligament” is very thick, very resistant, which stretches from the tip of the patella bone to the anterior tuberosity of tibia. Morphological the patellar ligament is considered the ending tendon of quadriceps muscle, located before the knee joint. Posterior ligament stretches the entire posterior face of the knee joint and is composed of three parts: middle part and two lateral parts. Medial collateral ligament is located at the inside of the knee joint. It extends from the medial femoral epicondyle to the tibia. It has 3 types of fibers: vertical, oblique oblique upward and downward. The lateral collateral ligament is located at the outside of the knee joint. It extends from the lateral femoral epicondyle to the head of the fibula. Anterior cruciate ligament extends posterlaterally from the tibia and inserts on the lateral femoral condyle. Posterior cruciate ligament extends anteromedially from the tibia posterior to the medial femoral condyle. Exists two ligaments on the dorsal side of the knee. The oblique popliteal ligament is a radiation of the tendon of the semimembranosus on the medial side, from where it is direct laterally and proximally. The arcuate popliteal ligament originates on the apex of the head of the fibula to stretch proximally, crosses the tendon of the popliteus muscle, and passes into the articular capsule of the knee joint.

Kategorie: Poster

Poster 23

Rubrik: 2.Klinische Anatomie/Makroskopie
Abstract Nr.:2

Titel:Menisci role in the knee joint biomechanics

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Abstract:
There are different theories on the biomechanical role joints menisci, the oldest of his Bouillet and Van Graver. Menisci completed the free space between curve surface of femoral condyles and approximately flat surface of glenoidal surface on the tibial plateau, sitting as a wedge in dihedral angle of the condylo-glenoidale sinus. By their presence the menisci prevent the synovial protrusion and the articular capsule in the cavity during various movements of the knee joint. Menisci are distributing the forces pressure on a larger area. Without menisci, the contact areas between the femoral condyles and glenoid cavities would be much reduced, and uniform pressure (in kg/cm2) would be higher. Menisci reduce friction between the joint surfaces by two mechanisms. First mechanism was described by Hjorstjo, which showed that each of cartilage’s surfaces of a joint depends on type of movements. From this point of view describes 3 types of movements: a) running (“rolling joint"), is similar to a wheel that moves forward on the ground; b) simple friction (“grinding joint ") is a wheel-like movement which skating on the ground; c) increased friction (“accentuated grinding joint), which is a similar movement of a wheel attached to another mobile banging in an opposite direction to that which must follow. The second mechanism by which menisci reduce friction is improving the greasing of articular areas by halving grease (synovial liquid) once between condyle’s cartilage and the top surface of menisci and a second, between the lower surface of menisci and glenoidal cartilage.

Kategorie: Poster

Poster 24

Rubrik: 2.Klinische Anatomie/Makroskopie
Abstract Nr.:2

Titel:Anteromedial or anterolateral arthrocentesis of the ankle, does the approach alter rate of joint puncture?

Autoren: Heidari N.(1),Pichler W.(2),Grechenig S.(2),Grechenig W.(2),Weinberg A.(3),Tesch N.(4),

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Abstract:
Arthrocentesis of the ankle joint may be performed through either an anteromedial or an anterolateral approach for diagnostic or therapeutic reasons. We evaluated the success of an intra-articular puncture in relation to the puncture site in our study. Two orthopaedic surgical trainees injected methylene blue into 38 cadaveric ankles each (76 ankles in total). Eighteen (of 38) were through an anterolateral and 20 through an anteromedial approach. An arthrotomy was then performed to confirm the placement of the dye within the joint. Of the injections through the anteromedial approach 31/40 (77.5%, 95% confidence limits 64.6 - 90.4%) were successful as were 31/36 (86.1%, 95% confidence limits 74.8 - 97.4%) of the anterolateral ones. In total 62/76 (81.6%, 95% confidence limits 72.9 - 90.3%) of the injections were intra-articular with a trend towards greater accuracy with the anterolateral approach. This however was not statistically significant (P = 0.25). In the case of Trainee A, 16/20 (80%) anteromedial injections and 14/18 (78%) of the anterolateral punctures were intra-articular. Trainee B made successful intra-articular punctures in 15/20 (75%) of the anteromedial and 17/18 (95%) of the anterolateral approaches. There was no significant difference between the two trainees, (P = 0.5 for the anteromedial and P = 0.16 for the anterolateral approach). Intra-articular puncture cannot be guaranteed even in experienced hands. Unintended peri-articular injection can cause complications and an unsuccessful arthrocentesis can delay diagnosis. Needle placement may be aided by the use of ultrasound scanning or fluoroscopy.

Kategorie: Poster

Poster 25

Rubrik: 2.Klinische Anatomie/Makroskopie
Abstract Nr.:2

Titel:Intra-articular puncture of the first metatarsophalangeal joint. does experience influence success?

Autoren: Pichler W.(1),Heidari N.(2),Grechenig S.(1),Grechenig W.(1),Weiglein A.(3),Tesch N.(3),Clement H.(1),Weinberg A.(4),

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Abstract:
Intra-articular puncture of all joints are performed by a multitude of healthcare professionals for aspiration of effusion to aid diagnosis and inject drugs and radiographic contrast for therapy and imaging.
76 (38 right and 38 left) cadaveric feet had the first metatasophalangeal joints injected with methylene blue. The puncture on the left side was performed by a specialist in trauma and orthopaedics and on the right by an inexperienced resident. An arthrotomy was then performed to confirm the presence of the dye within the joint.
The specialist missed the joint on 3 (7.9%) occasions whilst the inexperienced resident failed on 5 (13.1%) occasions. A p-value of 0.7 was calculated using Fisher's exact test. We found that experience does not significantly alter intra-articular puncture rate.
Intra-articular puncture cannot be guaranteed even in experienced hands. Unintended peri-articular injection can cause complications and a missed intra-articular puncture can frustrate diagnosis. Needle placement may be aided by the use of ultrasound scanning or fluoroscopy.

Kategorie: Poster

Poster 26

Rubrik: 3.Neuroanatomie/Neurobiologie
Abstract Nr.:3

Titel:Effect of early postnatal amphetamine treatment on the extinction of conditioned fear

Autoren: Walsh I.(1),Hanke J.(2),Schwegler H.(2),Yilmazer-Hanke D.(1),

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Abstract:
Two genetically related inbred mouse substrains selectively bred for a high and low fear-sensitised acoustic startle response (FSS) were studied for the acquisition and extinction of auditory fear-conditioning, and axiety-related behaviour on the elevated plus maze following early postnatal amphetamine treatment.
The two mouse substrains differing in 4 microsatellite loci have been obtained by backcrossing the offspring of C3H/HeJHd mice expressing a high FSS for 8-9 generations onto DBA/2JHd mice with a low FSS, and eventually through inbreeding of high-FSS mice for another 7-9 generations. Finally, two mouse substrains with stable differences in their FSS were generated: Our low-FSS mouse substrain corresponds to the offspring of DBA/2JHd mice, and our high-FSS substrain is homozygous for four C3H/HeJHd microsatellite loci (on chromosomes 13 and 14) on an otherwise completely DBA/2JHd background.
The results of the behavioural experiments showed that high-FSS mice exhibit an enhanced fear conditioning, and extinguish at a lower rate than low-FSS mice. In contrast, no differences are observed in the time spent or number of entries to the open arms, closed arms or centre of the elevated plus maze. Early postnatal amphetamine treatment severely disrupted the extinction of fear in high-FSS mice only. The present findings show that high-FSS mice consistently display a higher fear level than low-FSS mice in the fear-sensitised acoustic startle response and fear-conditioning paradigms but do not differ in the level of their innate anxiety as measured on the elevated plus maze.

Kategorie: Poster

Poster 27

Rubrik: 3.Neuroanatomie/Neurobiologie
Abstract Nr.:3

Titel:The impact of social stress on neuronal morphology in a mouse model for anxiety and depression

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Abstract:
Genetic variations within the 5-HT system, e.g. the length polymorphism in the regulatory region of the serotonin transporter (5-HTT) gene, impact on the development of anxiety- and aggression-related personality traits, which can be aggravated by environmental cues such as stress. 5-HTT knockout mice display an anxious phenotype and exaggerated adrenomedullary stress responses and are a common animal model for stress-related disorders such as depression. Stress exposure causes neuroadaptive mechanisms including structural changes of neuron subgroups. We subjected male wildtype (WT) and 5-HTT deficient mice to a resident-intruder-paradigm, which stresses the animals socially due to a loser experience and subsequently analysed morphological changes of neurons in Golgi-Cox-stained sections of limbic brain areas in stressed and unstressed animals of both genotypes using the computer based microscopy system Neurolucida (Microbrightfield, Inc.). While no differences concerning lengths of apical dendrites, branching patterns and spine density were found in hippocampus and cingulate cortex, significantly shorter apical dendritic tufts and lower spine numbers were detected in neurons of the infralimbic cortex of non-stressed 5-HTT deficient mice compared to non-stressed WT animals. Furthermore, infralimbic pyramidal neurons of stressed WT mice showed an elongation of apical dendrites proximal to the soma compared to non-stressed WT mice. Further morphological analyses are presently being carried out on pyramidal and interneurons in the lateral and basolateral nucleus of the amygdala. In summary, results indicate that, although in this model drastic alterations of neuronal morphology are absent, subtle changes are found in specific brain areas involved in stress- and anxiety-related behaviours.

Kategorie: Poster

Poster 28

Rubrik: 3.Neuroanatomie/Neurobiologie
Abstract Nr.:3

Titel:Glutamate receptor deficit in CPBK mice – a murine model for schizophrenia?

Autoren: Panther P.(1),Nullmeier S.(1),Dobrowolny H.(2),Wolf R.(3),Schwegler H.(1),Schwegler H.(1),

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Abstract:
Glutamate receptors play an important role in the pathophysiology and treatment of schizophrenia. Clinical and experimental evidence has shown that the expression of the NMDA (N-methyl-D-aspartate) receptor subunits, intracellular NMDA receptor interacting proteins and AMPA receptors of the glutamatergic synapse in the hippocampus appears to be reduced in schizophrenia and that the antipsychotic drug clozapine has an affinity to the NMDA receptor.
Here we investigated by comparison to Balb/cJ mice whether the inbred mouse strain CPBK, known for a low density of the hippocampal NMDA and AMPA receptors, might be a model for schizophrenia. Immunhistochemical investigations were carried out by counting neuronal numbers and densities of parvalbumin positive neurons in hippocampus, tyrosinhydroxylase containing neurons in substantia nigra/VTA and serotonin-transporter expressing neurons of the raphe nuclei. Patients suffering from schizophrenia show sensorimotor gating deficit and different social behaviour. Differences between both mice strains were characterized by a complex series of prepulse-inhibition- and social-interaction-tests during three weeks of chlozapine treatment.
We found a significant volume extension in the raphe nuclei in CPBK-mice and a significant reduction in the volume densities of neurons in the caudal linear nucleus raphe and significant higher volume densities of neurons in the dorsal nucleus raphe in CPBK-mice as compared to Balb/cJ-mice. Differences between both mouse strains were not significant in the other brain regions. The results of the prepulse-inhibition- and social-interaction-tests showed a reduction of PPI and social interaction in CPBK mice. These results might reflect several aspects of a murine model for schizophrenia.

Kategorie: Poster

Poster 29

Rubrik: 3.Neuroanatomie/Neurobiologie
Abstract Nr.:3

Titel:The heterozygouse reeler mice – a murine model for schizophrenia?

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Abstract:
It has been shown that schizophrenic patients exhibit a reduced amount of reelin. Here we investigated, whether heterozygous reeler mice with reduced reelin levels are a model for schizophrenia. To characterize a sensorimotor gating deficit, typically for patients, these mice were subjected to a complex series of prepulse-inhibition-tests and were additionally treated with clozapine during three weeks. Immunhistochemical investigations were carried out by counting neuronal numbers and densities of parvalbumin positive neurons in hippocampus, tyrosinhydroxylase containing neurons in substantia nigra/VTA and serotonintransporter expressing neurons of the raphe nuclei. We found no significant differences in acoustic startle response and prepulse-inhibition between naive and clozapine treated reeler rl/-and controls. Histological analysis revealed no differences of cell numbers and densities in hippocampus, substantia nigra/VTA and raphe nuclei. However reeler rl/- had a significant decrease of neuron-diameter in these areas. Further studies are required to elucidate wether these finding is of major significance.

Kategorie: Poster

Poster 30

Rubrik: 3.Neuroanatomie/Neurobiologie
Abstract Nr.:3

Titel:Monoaminergic innervation and serotonin receptor expression of neuropeptide y-producing interneurons in the rat laterobasal amygdala

Autoren: Bonn M.(1),Schmitt A.(2),Asan E.(1),

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Abstract:
The amygdala is a telencephalic nuclear complex playing a central role in emotional stimulus processing, especially for fear- or anxiety-inducing sensory perceptions. Neuropeptide Y (NPY) presumably subserves anxiolytic functions in the amygdala. Pharmacological and behavioural investigations suggested functional interactions between monoaminergic and peptidergic amygdaloid systems in the lateral (La) and basolateral nucleus (BL), which appear relevant for emotional behaviour in experimental animals. In the present study, morphological features of interrelations between serotonin-transporter-immunoreactive(-ir) serotonergic and tyrosine hydroxylase(TH)-ir, presumably dopaminergic afferents and NPY-ir interneurons in the La and BL were analysed in detail on a light microscopic level. In both La and BL, >95% of NPY-ir somata displayed close appositions by serotonergic afferents. Perisomatic appositions of TH-ir axons were observed on ~47% and 62% of NPY-ir neurons in the La and BL, respectively. To assess functional parameters of monoaminergic innervation, a double in situ hybridization method was devised combining high-sensitivity chromogen detection of monoamine receptor mRNA with tyramide-signal-amplified fluorescence detection of peptide mRNA. First studies using this method documented serotonin receptor 1A (5-HT1A) expression in nearly 60% of NPY mRNA-reactive La and BL neurons while 5HT3 mRNA was not detected in NPY mRNA-producing neurons. The results indicate a direct influence of serotonergic afferents on NPY mRNA-producing anxiolytic amygdaloid interneurons. Further analyses using the double in situ hybridization method devised will address expression of additional monoamine receptors and other signal transduction molecules and provide a basis for electrophysiological studies concerning monoaminergic modulation of the activity of these functionally important interneurons.

Kategorie: Poster

Poster 31

Rubrik: 3.Neuroanatomie/Neurobiologie
Abstract Nr.:3

Titel:No morphological and functional alterations in the nigrostriatal system of aged TGF-beta2/GDNF double-heterozygous mice

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Abstract:
The deleterious loss of dopaminergic (DA) neurons in the substantia nigra pars compacta (SNpc) and the resulting decrease in dopamine (DA) levels in the striatum, the main target projection area of SNpc DA neurons, are the hallmarks of Parkinson's disease (PD). Tgf-beta and Gdnf have attracted scientific attention as they are able to increase the numbers of cultured midbrain DA neurons. Moreover, Tgf-beta and Gdnf are able to protect DA neurons in different models of PD, such as 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine and 6-hydroxydopamine. However, the role of Tgf-beta and Gdnf for the maintenance of midbrain DA neurons under normal aging conditions remains elusive. In this study we analyzed the nigrostriatal system of aged Tgf-beta2+/-/Gdnf+/- double heterozygous mice. Using tyrosine hydroxylase immunohistochemistry, we demonstrate that double-heterozygous animals had no morphological changes in the nigrostriatal system as compared with age-matched wild type mice. Moreover, we found no significant differences in the striatal levels of dopamine (DA) and its metabolites dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA). Our results indicate that a combined haploinsufficiency for Tgf-beta2 and Gdnf has no impact on the function and the survival of midbrain DA neurons under normal aging conditions.

Kategorie: Poster

Poster 32

Rubrik: 3.Neuroanatomie/Neurobiologie
Abstract Nr.:3

Titel:Intrastriatal application of botulinum toxin into wistar rats leads to morphologic changes

Autoren: Hawlitschka A.(1),Schmitt O.(1),Antipova V.(2),Benecke R.(3),Wree A.(1),

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Abstract:
Systemic application of anticholinergic drugs ameliorates symptoms of Parkinson's disease (PD), but is hampered by adverse side-effects. Since botulinum toxin (BT) blocks cholinergic transmissions locally we investigated in the 6-hydroxydopamine (6-OHDA) animal model of PD the hypothesis whether intrastriatal application of BT may contribute to an improved therapeutic concept of PD. In a first test series different doses of BT-A (100 pg, 1 ng and 2 ng) were applied stereotactically into the right striatum of young adult Wistar rats and brains were investigated 1 and 3 months post injectionem. In a second test series animals were treated with BT-A 4 weeks after 6-OHDA lesioning of the right medial forebrain bundle (hemiparkinson model) and afterwards brains were examined. Nissl staining and immunohistochemical visualisation of cholinergic neurons via choline acetyle transferase (ChAT) and of dopaminergic neurons via tyrosine hydroxylase (TH) were performed. Application of BT-A to both control and PD rats led to dose-dependent morphologic changes in the injected striatum. Most impressively, vesicle-like presynaptic round structures of different size (2 - 9 µm diameter) appeared, which we tentatively named BT-induced boutons (BiBs). They were immunoreactive for ChAT or TH, but never double-positive for both enzymes. The BiB density, but not the BiB-positive projection area, was increased at higher BT doses of 1 and 2 ng. The number and viability of ChAT-positive neurons was not affected by BT injections. We suppose that BiBs are formed by a retention of presynaptic vesicles thereby inhibiting neurotransmitter release. Parallel performed behavioural studies support this hypothesis.

Kategorie: Poster

Poster 32a

Rubrik: 3. Neuroanatomie/Neurobiologie
Abstract Nr.: 3

Titel: Behavioural improvements of hemiparkinsonian rats after intrastriatal injection of botulinum toxin A

Autoren: Antipova V. (1), Draeger D. (1), Hawlitschka A. (1), Benecke R. (2), Mix E. (2)

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Abstract:
Here we investigated consequences of intrastriatal injection of botulinum toxon A (BT-A) into rats on motor function in an animal model of Parkinson's disease (PD) to analyze the potential applicability of the intrastriatal BT treatment as a therapeutic option of PD. Young adult Wistar rats, which had received 6-hydroxydopamine (6-OHDA) in the right medial forebrain bundle 4 weeks before BT-treatment (Hemiparkinson model) and healthy rats were injected with BT-A at (100 pg, 1 ng, 2ng), respectively, into the right striatum. Behaviour changes were investigated by: apomorphine and amphetamine induced rotations, cylinder test according to Schallert (forepaw preference) and Rotarod test according to Dunham and Miya (forced motor activity). BT application into control rats leads to a slight dose-dependent and transient (<2 months) induction of rotations (2 – 3 per minute) by apomorphine clockwise. In the PD rats deafferentiation of dopaminergic neurons by 6-OHDA causes approximately 8 rotations away from the lesion (anti-clockwise). Ipsilateral injection of BT-A at doses of 1 – 2 ng abrogates these rotations almost completely for at least 3 months. In contrast, amphetamine induces approximately 8 rotations per minute clockwise in PD rats. Here, BT-A injections enhance the rotations, but transiently (1 month) to 12 per minute. No significant alterations in cylinder tests and Rotarod tests were seen during 3 months. In conclusion, BT-A reduces the inhibitory ipsilateral cholinergic inputs in the striatum of PD rats thereby antagonizing pathological apomorphine induced rotations. We suppose that intrastriatal application of BT-A can contribute to an improved treatment of PD.

Kategorie: Poster

Poster 33

Rubrik: 3.Neuroanatomie/Neurobiologie
Abstract Nr.:3

Titel:Further development of cell-based transplantation in a rat model of parkinson´s disease

Autoren: Ratzka A.(1),Nobre A.(1),Kalve I.(1),Wesemann M.(1),Grothe C.(1),

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Abstract:
Parkinson´s disease is a neurodegenerative disorder caused by a progressive loss of dopaminergic (DA) neurons. Exogenous cell replacement, a curative cell therapy, where new DA cells are implanted into the dopamine-denervated striatum, results in functional improvements. Limitations of the method include limited supply of donor cells and the low survival of grafted cells. Further development of our previous work (Timmer et al. Neurobiol. Dis. 2006; Cesnulevicius et al. Stem Cells 2006) focused on higher amounts of DA cells and their genetic modification to over-express neurotrophic factors.
First we produced SV40 large T antigen (SV40Tag) immortalized cell lines derived from the ventral mesencephalon. Analysis of molecular markers for DA differentiation, revealed the expression of early DA markers in primary and also in SV40Tag cells. Although SV40Tag cells express, at least under differentiating conditions (dbcAMP/GDNF), low amounts of late DA marker genes (tyrosine hydroxylase, TH) as determined by RT-PCR, no TH-positive neurons were identified by immunohistochemistry so far.
In a second attempt neural progenitor cells of the ventral midbrain were transfected with a non-viral EGFP (enhanced green fluorescent protein) expression plasmid, which resulted in a high transfection efficiency and a strong EGFP signal for at least 3 weeks in vitro. Furthermore, after grafting those cells into neurotoxin lesioned rat brains, a strong EGFP signal was also detected after 2 weeks in vivo. Current experiments in which we replace EGFP by different neurotrophic factors will allow to study their effects on differentiation and survival of transplanted DA cells.

Kategorie: Poster

Poster 34

Rubrik: 3.Neuroanatomie/Neurobiologie
Abstract Nr.:3

Titel:Impact of melatonin and molecular clockwork components on the expression of thyrotropin beta chain (tshb) and the tsh receptor in the mouse pars tuberalis

Autoren: Unfried C.(1),Ansari N.(2),Yasuo S.(3),von Gall C.(1),Korf H.(3),

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Abstract:
Photoperiodic regulation of reproduction in birds and mammals involves thyrotropin beta chain (TSHb) which is secreted from the pars tuberalis (PT) and controls the expression of deiodinase typ 2 (Dio2) and 3 (Dio3) in the ependymal cell layer of the infundibular recess (EC) via TSH receptors (TSHR). To analyze the impact of melatonin and the molecular clockwork on the expression of Tshb and Tshr we investigated melatonin proficient C3H wildtype (WT), melatonin receptor 1 (MT1) and mPER1-deficient mice. Expression of Tshb and TSHb immunoreactivity in PT were low during day and high during night in WT, high during day and low during night in mPER1-deficient and equally high during day and night in MT1-deficient mice. Melatonin injections into WT acutely suppressed Tshb expression. Transcription assays showed that mPER1 significantly reduced the CLOCK:BMAL1-induced transcription of the Tshb. Tshr levels in PT were low during day and high during night in WT and mPER1-deficient mice and equally low in MT1-deficient mice. Tshr expression in the EC did not show a day/night variation. Melatonin injections into WT acutely induced Tshr expression in PT, but not in EC. TSH stimulation of hypothalamic slice cultures of WT induced pCREB in PT and EC and Dio2 in the EC. Our data suggest that 1) Tshb expression in PT is controlled by melatonin and the molecular clockwork and 2) melatonin activates Tshr expression in PT, but not in EC. They also confirm the functional importance of TSHR in the PT and EC.

Kategorie: Poster

Poster 35

Rubrik: 3.Neuroanatomie/Neurobiologie
Abstract Nr.:3

Titel:Disturbed sleep/wake rhythms and neuronal cell loss in lateral hypothalamus and retina of mice with a spontaneous deletion in the ubiquitin carboxyl-terminal hydrolase l1 gene

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Abstract:
Many neurodegenerative disorders including Parkinson´s disease (PD) and Alzheimer's disease (AD) are associated with sleep disturbances with presumably multifactorial aetiology. In the present study, we investigated locomotor activity rhythms and neuronal integrity of brain regions involved in the circadian control of the sleep wake cycle in gad mice with a spontaneous deletion of the gene coding for the enzyme ubiquitin C-terminal hydrolase L1 (UCH-L1) involved in the pathophysiology of PD and AD. In constant darkness, gad mice showed circadian rhythms in locomotor activity, indicating the integrity of the endogenous circadian rhythm generator. However, gad mice showed an increased activity during the subjective day and a decreased number of orexin-A-immunoreactive neurons in the lateral hypothalamus. In addition, gad mice showed increased locomotor activity in the light period when kept in a standard photoperiod and entrainment to the phase shifts was significantly slower than in WT littermates. This behaviour demonstrates an impairment of circadian light perception in gad mice. Importantly, immunoreaction for the circadian photopigment melanopsin in the retinal ganglion cell layer of gad mice was significantly reduced compared to WT. This could explain impaired non-visual responses to light in gad mice. Studies are underway to examine whether the melanopsin-immunoreactive ganglion cells in the retina of humans are also affected by neurodegenerative diseases.

Kategorie: Poster

Poster 36

Rubrik: 3.Neuroanatomie/Neurobiologie
Abstract Nr.:3

Titel:Autism related mutations of synapse proteins and their morphological impact on rat hippocampal neurons

Autoren: Schön M.(1),Grabrucker A.(2),Bockmann J.(1),Böckers T.(1),

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Abstract:
Recent studies have implicated the role of mutations in several pre- and postsynaptic proteins in autism pathogenesis. These rare mutations were found amongst others in ProSAP2/SHANK3, a major scaffolding protein of excitatory synapses, and neuroligins, a group of membrane adhesion molecules of excitatory and inhibitory synapses. These proteins cross the synaptic cleft and interact with neurexins at the presynaptic site, which are also autism associated.
In our work we investigate the morphological and functional effects of several mutations in postsynaptic proteins on rat hippocampal neurons in culture. This may enlighten the so far poorly understood pathogenesis of autism spectrum disorders.
We used primary hippocampal neurons as a model system. After a defined period of time we transfected the neurons with vector constructs of wildtype and mutated forms of our target genes. By immunostaining we analysed the morphological alterations under a fluorescence microscope.
Our results on postsynaptic proteins implicate that the mutations have an effect on dendritic branching and synapse formation which reinforces the hypothesis of altered synaptic network as a major factor in autism pathogenesis.

Kategorie: Poster

Poster 37

Rubrik: 3.Neuroanatomie/Neurobiologie
Abstract Nr.:3

Titel:Adaptive peripheral immune response increases proliferation of neural precursor cells in the adult hippocampus

Autoren: Wolf S.(1),Steiner B.(2),Wengner A.(3),Lipp M.(3),Kammertoens T.(4),Ullrich O.(1),Kempermann G.(5),

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Abstract:
To understand the link between peripheral immune activation and neuronal precursor biology, we investigated the impact of T cell activation on adult hippocampal neurogenesis in female C57Bl/6 mice. A peripheral adaptive immune response triggered by adjuvant-induced rheumatoid arthritis (2µg/µl mBSA) or staphylococcus enterotoxin B (EC50 0.25 μg/ml per 20g body weight) was associated with a transient increase in hippocampal precursor cell proliferation and neurogenesis as assessed by Immunohistochemistry and confocal microscopy. Both treatments were paralleled by an increase in corticosterone levels in the hippocampus 1-2 fold over the physiological amount measured by quantitative radioimmunoassay. In contrast, i.p. administration of the innate immune response activator lipopolysaccaride (EC50 0.5 μg/ml per 20g body weight) led to a chronic 5-fold increase of hippocampal glucocorticoid levels and a decrease of adult neurogenesis. In vitro exposure of murine neuronal progenitor cells to corticosterone triggered either cell death at high (1.5 nM) or proliferation at low (0.25 nM) concentrations. This effect could be blocked using a viral vector system expressing a transdomain of the glucocorticoid receptor. We suggest an evolutionary relevant communication route for the brain to respond to environmental stressors like inflammation mediated by glucocorticoid levels in the hippocampus.

Kategorie: Poster

Poster 38

Rubrik: 3.Neuroanatomie/Neurobiologie
Abstract Nr.:3

Titel:Trafficking of alpha- and beta-neurexins in primary hippocampal neurons

Autoren: Niesmann K.(1),Brinkhaus L.(1),Missler M.(1),

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Abstract:
Neurexins constitute a family of neuronal cell-adhesion molecules that are essential for neurotransmission and are involved in synapse formation in vitro. Neurexins are encoded by three genes (NRXN1-3) each of which is under the control of two promotors (alpha and beta). alpha- and beta-neurexins differ greatly in their extracellular domains but share the same transmembrane and C-terminal intracellular domains. Our previous data showed that neurexins are trafficked throughout the cell via transport vesicles, but that membrane insertion occurs preferentially in the axonal/synaptic compartment.
We are currently investigating in the mechanisms that control the polarized targeting of neurexin-isoforms to the synapse and ask whether alpha- and beta-neurexins share the same route or whether their putatively different functions also imply different mechanisms of transport. We generated various alpha- and beta-neurexin expression constructs with different fluorophor-tags to study their localisation within hippocampal neurons and to follow their trafficking throughout the cell in live imaging experiments.
Our first analyses demonstrate that for both alpha- and beta-neurexins several populations of vesicles exist that vary in their velocity and the distances they travel during the observation time. Both anterograde and retrograde transport occurs in axons and dendrites, however anterograde transport along the axon appears to be be the predominant route for these molecules. Our experiments shed light onto the intracellular dynamics of neurexin-transport and are the basis for understanding how neurons target these cell-adhesion molecules to synaptic sites.

Kategorie: Poster

Poster 39

Rubrik: 3.Neuroanatomie/Neurobiologie
Abstract Nr.:3

Titel:Genetic analysis of the zinc finger transcription factor bcl11b during adult neurogenesis

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Abstract:
In the adult mammalian brain the dentate gyrus is one of only two locations with continuing postnatal neurogenesis and the primary gateway for inputs into the hippocampus, a cortical structure that is essential for spatial learning and memory. During development of the dentate gyrus, the transition from a proliferating progenitor cell into a postmitotic functional neuron involves the interplay of regulatory transcription factors. Bcl11b encodes a highly conserved C2H2 zinc finger transcription factor that is widely expressed in different regions of the developing and adult brain, such as the hippocampus, neocortex, and striatum. We have previously shown that during development Bcl11b is essential for the differentiation of postmitotic dentate granule cells as well as for proliferation of their precursors. Preliminary results obtained from adult mice with a forebrain specific ablation of Bcl11b demonstrate reduced neurogenesis in the adult dentate gyrus as well. Together, our data indicate a critical role for Bcl11b during postnatal and adult hippocampal neurogenesis. To further determine the specific functions of Bcl11b in the regulation of adult neurogenesis we are establishing a tetracyclin-dependent transactivator system ablating Bcl11b specifically in adult dentate granule cells. This system will allow us to reveal the effect of Bcl11b deficiency on adult neurogenesis in mice which have undergone normal postnatal development.

Kategorie: Poster

Poster 40

Rubrik: 3.Neuroanatomie/Neurobiologie
Abstract Nr.:3

Titel:Neurexins binding to neuroligins, neurexophilins, and dystroglycans

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Abstract:
Neurexins (Nrxns) constitute a family of highly variable molecules predominantly located at the presynaptic cell surface. Nrxns are essential for neurotransmission and link calcium channels to the active zone. Furthermore, Nrxns stimulate the building of new synaptic contacts. Recently, several genomic studies have shown that mutations in Nrxns are associated with autism and schizophrenia.
The extracellular domains of Nrxns bind to neuroligins (Nlgns), neurexophilins (Nxphs) and to dystroglycans (DAGs). To understand the molecular basis of Nrxn functions, it is crucial to discriminate the interplay of all three ligands.
After the binding of Nlgns to beta-Nrxn and the sixth LNS domain of alpha-Nrxn has already been extensively studied, we enlightened the other ligands. Alpha-Nrxns interact with Nxphs, a family of neuropeptide-like glycoproteins. We show, that all Nxphs bind to the second LNS domain of Nrxn and present the binding site. The complex is formed by co-expression within the cell and remains extremely stable. It requires denaturing conditions to release Nxph from Nrxn, which can be explained by hydrophobic residues essentially required for Nrxn/Nxph complex formation.
The third ligand DAG is the center piece of the dystrophin-associated glycoprotein complex (DGC). It is composed of alpha- and beta-DAG. We show, that alpha-Nrxn LNS 2 and 6, both bind to alpha-DAG, but beta-Nrxn does not. Beta-Nrxn contains a specific N-terminal sequence that inhibits DAG binding. In conclusion, while alpha-Nrxns bind to all three ligands, Nlgns are exclusive binding partners for beta-Nrxns.

Kategorie: Poster

Poster 41

Rubrik: 3.Neuroanatomie/Neurobiologie
Abstract Nr.:3

Titel:Ultrastructure of bruchpilot accumulations in larval nerves of a serine arginine rich protein kinase (srpk79d) null mutant in drosophila melanogaster

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Abstract:
Bruchpilot, a Drosophila protein homologous with mammalian presynaptic proteins ELKS/CAST/ERC, is required for structural integrity of T-shaped ribbons at presynaptic active zones (“T-bars”). In Drosophilae bearing a mutation in the CG11489 gene encoding a protein homologous to mammalian kinases which phosphorylate serine arginine rich proteins (SRPK79D), accumulations immunoreactive for an antibody against a C-terminal Bruchpilot epitope (nc82) are found in peripheral nerves. We performed electron microscopic analyses and immunohistochemistry on Srpk79D null mutant larval ventral nerves to further characterize nc82-reactive accumulations.
In mutants, numerous large electron dense complexes of various shapes surrounded by differing numbers of clear and, occasionally, dense-core vesicles were found intraaxonally. Immunoelectron microscopy confirmed labeling for nc82. In wild-type larvae, few very small similar structures of much lower complexity were detected. The electron-dense centers of complexes in mutants resembled multiple, partly fused T-bars. No correlation was observed between size and proximodistal localization of the complexes in nerves. Doublelabeling studies showed that nc82-reactive accumulations lacked immunoreactivity for vesicular glutamate transporter and other synaptic vesicle markers.
Thus, lack of SRPK79D causes misplaced, premature assembly of presynaptic dense projections containing Bruchpilot within peripheral axons. Like T-bars, these structures recruit vesicles which, however, do not possess common synaptic vesicle characteristics. Proximodistal size distribution analysis argues against gradual accumulation of electron dense material during transport. Further analyses will address the precise function of SRPK79D for correct assembly and function of Drosophila presynaptic elements which may also be of interest for mammalian active zone structure.

Kategorie: Poster

Poster 42

Rubrik: 3.Neuroanatomie/Neurobiologie
Abstract Nr.:3

Titel:Altered synaptic transmission in nrxn 2 alpha and beta knockout mice

Autoren: Born G.(1),Langhorst H.(1),Missler M.(1),

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Abstract:
Synaptic transmission depends on a highly complex pre- and postsynaptic molecular machinery that regulates various steps in this process. Neurexins are a component of this machinery and encoded by three genes (NRXN1-3), each is under the control of two promotors, leading to two major isoforms (alpha- and beta-neurexins). Our previous data showed that alpha neurexins are essential synaptic cell-adhesion molecules that link calcium-channel function to vesicle release, because genetic deletion of all alpha Nrxns in mice leads to perinatal death and dramatically impaired calcium-dependent transmitter release.
Here, we ask if a single KO of both, the alpha and beta neurexin 2 can also have an effect on synaptic transmission in the central nervous system. Using whole-cell voltage clamp recordings from cortical layer V pyramidal cells, we compared wild-type and neurexin 2 alpha/beta c-terminus KO mice. Mutant neurons showed decreased IPSCs and EPSCs. Furthermore, paired-pulse stimuli, used to evoke short-term plasticity, failed to initiate any facilitation during recordings of EPSCs in mutant animals. However, both control and KO mice showed paired-pulse depression during recordings of IPSCs.
Our current experiments demonstrate that already the impairment of a single gene of neurexins is sufficient to produce specific defects in synaptic transmission, supporting the view that single-gene mutations in patients may result into cognitive diseases such as autism spectrum disorders (ASD). Our analyses indicate that the common mechanisms in ASDs is related to changes in the excitatory/inhibitory balance and the inability to produce important forms of synaptic plasticity in relevant brain areas.

Kategorie: Poster

Poster 43

Rubrik: 3.Neuroanatomie/Neurobiologie
Abstract Nr.:3

Titel:Alphapix (ARHGEF6) interacts with CALEB/NGC and modulates CALEB/NGC-mediated dendritic spine complexity

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Abstract:
AlphaPIX (ARHGEF6) interacts with CALEB/NGC and modulates CALEB/NGC-mediated dendritic spine complexity

Johannes, S.1, Rosenberger, G.2, Kutsche, K.2, Nitsch, R.1, and Schumacher, S.1
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CALEB/NGC is a neural member of the EGF family which mediates dendritic tree and spine complexity in neurons. However, little is known about signaling molecules that link the transmembrane protein CALEB/NGC to the cytoskeleton. In a yeast two-hybrid screen we found CALEB/NGC to bind to the protein alphaPIX. AlphaPIX (ARHGEF6) belongs to a group of guanine nucleotide exchange factors (GEFs) that mediate activation of members of the Rho GTPase family. Mutations in alphaPIX have been shown to be present in patients with X-linked non-syndromic mental retardation. We confirmed the interaction of CALEB/NGC and alphaPIX with different types of co-immunoprecipitations. In first steps to analyse the function of the CALEB/NGC-alphaPIX interaction we performed two different sets of experiments in primary hippocampal neurons in culture: First we overexpressed alphaPIX in neurons with high dendritic tree and spine complexity due to forced CALEB/NGC expression. Second, we overexpressed CALEB/NGC in neurons with reduced alphaPIX expression due to RNAi-mediated knockdown. Our results point to the view that alphaPIX modulates CALEB/NGC-induced dendritic spine complexity.

Supported by the Gertrud Reemtsma Stiftung.

Kategorie: Poster

Poster 44

Rubrik: 3.Neuroanatomie/Neurobiologie
Abstract Nr.:3

Titel:Neuronal protection by sex steroids in transient mcao

Autoren: Dang J.(1),Beyer C.(1),Kipp M.(1),

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Abstract:
Purpose: Cerebral stroke is a major cause of death and permanent disability. Typically, the blood supply to the brain is temporarily or permanently obstructed and may result in massive neuronal cell death. The reproductive steroid hormone estrogen has been shown to provide neuroprotection in a variety of experimental insults, but the importance of progesterone as an anti-ischemic treatment has not been explored in detail. Both steroids might cooperate to reduce brain damage. We introduced middle cerebral artery occlusion (MCAO) in adult rats and evaluated histological and functional melioration after hormone application.
Methods: Age-matched male rats underwent 1 h MCAO with the intra-luminal filament technique, followed by 23 hours of reperfusion. Hormones were applied at the beginning of reperfusion by subcutaneous depot injections in the neck. Ipsilateral parietal cortex perfusion was monitored with laser doppler flowmetry throughout ischemia. Infarct volumes were determined with TTC staining. Complex behavioural analysis was additionally performed.
Results: The volume of cortical lesions was significantly reduced in the single hormone- treated groups (estrogen and progesterone) with progesterone being most effective. The combined hormone application displayed similar protective properties as progesterone alone. Stroke-induced behavioural deficits were significantly improved by both hormones either in single or combined doses. Expression of several cytokines (MMP9, IL1ß and IL6) was influenced by steroid treatment.
Conclusion: We found that an exogenous steroid therapy ameliorates histological and functional damage after MCAO. Therefore, both steroids might be valuable therapeutic tools in the early stroke therapy.

Kategorie: Poster

Poster 45

Rubrik: 3.Neuroanatomie/Neurobiologie
Abstract Nr.:3

Titel:Immunoreactivity for the astroglial gap junction protein connexin43 in reticular and mediodorsal thalamic nuclei of rat and human brains

Autoren: Gonzalez-Algaba A.(1),Klugmann F.(1),Reuss B.(1),

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Abstract:
Parvocellular neurons of the mammalian mediodorsal thalamic nucleus send their axons to the prefrontal cortex, reciprocally receiving axons of layer VI cortical neurons. Both thalamocortical and corticothalamic fibers send collaterals to the reticular thalamic nucleus, an inhibitory modulator of thalamic activity. Decreased numbers of parvocellular neurons in the mediodorsal thalamic nucleus is a common pathological finding in the schizophrenic brain, etiology of which is not yet understood. A cellular system affecting neuronal survival is astroglial intercellular coupling by gap junctions. Astroglial gap junctions, consisting primarily of Connexin43 (Cx43), provide an intercellular transport route for neuronal nutrients and waste products. Normal astroglial gap junction coupling is therefore thought to act in a neuroprotective manner. In contrast, impaired gap junction coupling probably results in increased neuronal vulnerability and consequently higher rates of cell death. In the present study we analyzed expression of Cx43 in normal mediodorsal and reticular thalamic nuclei of the rat, as well as in postmortem samples of the human thalamus. As revealed by immunohistochemistry, Cx43 immunoreactive gap junction plaques can be indeed detected in reticular and mediodorsal thalamic nuclei of adult rats, as well as in the corresponding structures of the human thalamus. Based on these preliminary findings we will try to further elucidate the role of astroglial gap junctions for survival and functionality of parvocellular neurons in the mediodorsal thalamic nucleus. Finally we would like to thank the medical faculty of our university for the permanent and reliable support of our work.

Kategorie: Poster

Poster 46

Rubrik: 3.Neuroanatomie/Neurobiologie
Abstract Nr.:3

Titel:Betulinic acid changes the amount of Cx43 in human NTera-2/D1 cells and enhances the retinoic acid mediated downregulation of Cx43

Autoren: Klugmann F.(1),Gonzalez Algaba A.(1),Reuss B.(1),

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Abstract:
Betulinic acid (BA), a potential anti cancer drug, induces apoptotic cell death in neuroectodermally derived tumors. It also promotes differentiation of normal cells, however the question for actions on normal neuronal differentiation has not yet been addressed. A subcellular system known to regulate early neuronal differentiation is intercellular communication via gap junctions. In human NTera2/D1 cells gap junctions are downregulated during Retinoic acid (RA) induced neuronal differentiation. We used these cells here to clarify whether BA was able to change the gap junction protein Connexin(Cx)43.
NTera2/D1 cells were treated for 2, 4, and 6 days with up to 10 micromol/l BA, and Cx43 protein was detected using Western blot analysis and immunocytochemistry. A significant downregulation of Cx43 immunoreactivity was detectable at 10 micromol/l BA, whereas lower concentrations showed an upregulation. The scrape loading technique using Lucifer yellow, revealed a significant reduction in functional coupling by 10 micromol/l BA, whereas lower concentrations lead to increased dye spreading. Finally, Western blot analysis for Cx43 immunoreactivity in cells cotreated with 10 micromol/l of each BA and RA revealed an additively increased downregulation of Cx43 by both substances as compared to single treatments.
Together these results demonstrate a concentration dependent effect of BA on amount and function of gap junctions in NTera2/D1 cells, suggesting both a differentiation promoting and a neuroprotective effect, depending on the concentrations used.
We would like to thank the medical faculty of our university for permanent and reliable support of our work.

Kategorie: Poster

Poster 47

Rubrik: 3.Neuroanatomie/Neurobiologie
Abstract Nr.:3

Titel:Chemokine expression during cuprizone-induced demyelination in the mouse brain

Autoren: Buschmann J.(1),Awad H.(1),Beyer C.(1),Kipp M.(1),

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Abstract:
Purpose: Chemokines play an important role for brain development and function. Under pathological conditions, they become critical modulators of leukocyte chemotaxis and tissue homeostasis. We have investigated the regulation of chemokines in a particular CNS pathology, i.e. multiple sclerosis (MS), since their function for MS is largely unknown.
Methods: Acute demyelination was induced in male mice by cuprizone administration over a period of up to five weeks. The complex regulation of chemokines was investigated by an Affymetrix gen chip analysis and followed-up of candidate genes by real-time PCR. Cellular source of chemokines was determined by immunohistochemistry and in-situ hybridization. The functional relevance of chemokines was further investigated in cell culture approaches.
Results: Gene expression analysis disclosed a complex regulation of chemokines. CCL3, CCL6, CCL27a, and CXCL10 expression was induced, whereas CCL19, CCL21a, and CCL22, was decreased. Follow-up analysis indicated a rather dynamic regulation. CCL2 expression followed an undulatory pattern with peaks after week 1 and 5. In contrast, CCL3 mRNA levels peaked at week 1. CCL3 was mainly secreted by activated astrocytes and microglia, whereas oligodendrocytes were the source of CCL2 in the early phase. The contribution of mature oligodendrocytes to chemokine secretion in the injured brain was further demonstrated in cell culture experiments. LPS and TNFα promoted chemokine secretion, whereas glutamate and hydrogen peroxide did not.
Conclusion: These results clearly demonstrate that chemokines are selectively regulated during an acute demyelinating event in an experimental MS animal model. Besides brain resident inflammatory cells, oligodendrocytes are an additional cellular source.

Kategorie: Poster

Poster 48

Rubrik: 3.Neuroanatomie/Neurobiologie
Abstract Nr.:3

Titel:Activation of the subventricular zone in a toxic de- and remyelination animal model

Autoren: Abolfazl A.(1),Beyer C.(1),Kipp M.(1),

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Abstract:
Introduction: In multiple sclerosis (MS), remyelination prevents axonal loss and progressing clinical disability. The subventricular zone (SVZ) of the lateral ventricles is characterized by the presence of multipotential cells with persistent proliferation. It has recently been shown that the SVZ is activated in human MS and might be the source of remyelinating cells. In this study, we used the cuprizone mouse model to investigate the distribution of proliferating cells and early glial progenitors in the SVZ of acute and chronic demyelinated brains.
Methods: Animals received cuprizone (0.2%) for 5 or 13 weeks to induce acute/chronic demyelination. The activation and compositions of the SVZ was analyzed in detail using immunohistochenmical approaches. Cellular composition, cell proliferation, and stem-cell marker expression were analyzed.
Results: Compared to controls, cuprizone SVZ showed a 2 to 3-fold increase in cell density and proliferation in the acute but not chronic phase. Concordantly, proliferating cells can be found more frequently within the SVZ of the acute demyelinated brain. Neuronal stem cells as well as oligodendrocyte precursors are increased in number after 5 weeks. In contrast, the number of precursor cells is similar in both groups in the SVZ.
Discussion: These data indicate that, in agreement with human MS, the activation of gliogenesis in the SVZ occurs in this animal model and suggest the mobilization of SVZ-derived early glial progenitors to periventricular lesions. There, they could give rise to oligodendrocyte precursors. These early glial progenitors could be a potential target for therapeutic strategies designed to promote remyelination in MS.

Kategorie: Poster

Poster 49

Rubrik: 3.Neuroanatomie/Neurobiologie
Abstract Nr.:3

Titel:Methylprednisolone treatment does not interfere with toxic demyelination

Autoren: Schönen U.(1),Clarner T.(1),Beyer C.(1),Kipp M.(1),

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Abstract:
Purpose: The two histopathological hallmarks of multiple sclerosis (MS) are demyelination and inflammation. While in the acute stage, inflammation is driven by inflammatory cells from the periphery, mainly brain-resident immune cells are found during the chronic disease phase. Corticosteroids (CS, methylprednisolone) are widely used for the treatment of acute relapses in MS because of the potent immunosuppressive and anti-inflammatory properties. The effectiveness of CS in the chronic disease stage is barely investigated. The purpose of this study was to analyze whether CS treatment affects T-cell independent demyelination in the brain.
Methods: Acute demyelination without T-cell invasion or blood-brain-barrier breakdown was induced by feeding male mice a diet containing 0.2% cuprizone for up to five weeks. CS were applied (i.p) every other day. The myelination index was analyzed by immunohistochemistry for PLP and APC and gene expression analysis. The cellular composition of infiltrates was additionally investigated. An appropriate CS application was verified by ACTH-ELISA.
Results: ACTH-serum levels were significantly lower in CS-treated animals compared to controls indicating an appropriate application and effectiveness. CS did not affect cuprizone-induced demyelination. Furthermore, no any changes in astrocyte and microglia activation/invasion were seen after CS application.
Conclusion: These results demonstrate that CS treatment does not interfere with “non T-cell driven” demyelination. Furthermore, brain local inflammation was not affected. These data argue against a protective role of CS treatment during the chronic stage of human MS.

Kategorie: Poster

Poster 50

Rubrik: 3.Neuroanatomie/Neurobiologie
Abstract Nr.:3

Titel:Brain region-specific functional effects of 3-nitropropionic acid-induced alterations of cytochrome c oxidase subunit iv isoform expression in astrocytes

Autoren: Misiak M.(1),Singh S.(1),Beyer C.(1),Arnold S.(1),

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Abstract:
Purpose: Cytochrome c oxidase (COX) subunit IV exists in two isoforms (COX IV-1 and COX IV-2) which play a crucial role in regulating enzyme activity, ATP and reactive oxygen species (ROS) production. COX IV-1 is ubiquitously expressed, whereas COX IV-2 is not present in astrocytes under physiological conditions but is up-regulated after treatment with 3-nitropropionic acid (NPA). We investigated the effect of the respective COX isoforms on the viability of NPA-treated primary astrocytes from mouse striatum, cortex, and midbrain.
Methods: A siRNA system was applied to knock-down COX isoform IV-2. Quantitative RT-PCR data were correlated with intracellular ATP content, ROS level, and astrocyte necrosis.
Results: NPA caused an elevation of COX IV-2 transcription in all brain regions but only in striatal astrocytes, COX isoform IV-1 was decreased. Astrocytes from striatum showed the highest up-regulation of ROS production and necrosis, whereas cortical and midbrain cells were apparently protected from necrotic cell death by lower ROS and higher ATP levels in response to NPA. In astrocytes from all three brain regions, knock-down of COX IV-2 led to a reversal of NPA-mediated increase of intracellular ATP and ROS levels and necrosis.
Conclusions: Our data suggest that under toxic conditions, the viability of astrocytes is influenced by COX IV isoform transcription pattern which appears to be regulated in a brain region-dependent fashion. We conclude that astrocytes from striatum compared to cortex and midbrain are mainly affected by NPA. These observations support the concept of a selective vulnerability of the striatum in Huntington's disease.

Kategorie: Poster

Poster 51

Rubrik: 3.Neuroanatomie/Neurobiologie
Abstract Nr.:3

Titel:An unbiased stereological method for efficiently quantifying the myocardial innervation based on total length estimations

Autoren: Mühlfeld C.(1),Papadakis T.(1),Kummer W.(1),

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Abstract:
Quantitative information about myocardial innervation is essential to analyze the physiological and pathological structure-function relationships of the heart. This study presents a new approach for unbiased quantification of the myocardial innervation illustrated on the left ventricle of the mouse heart. The method bases on the following subsequent steps to estimate the total length of axons in a given reference volume: 1) estimation of the reference volume (V(ref)); 2) randomization of location and orientation; 3) counting of nerve fibre profiles (Q) hit by an unbiased counting frame with a defined area (A(CF)) on paraffin sections stained immunohistochemically for PGP 9.5; 4) electron microscopic estimation of the mean number of axon profiles contained in one nerve fibre profile (QQ(axon/nerve)); 5) estimation of the degree of tissue shrinkage of specimens in paraffin (d(shr)); 6) calculation of the total axon length (L(axon,ref)) within the reference volume by L(axon,ref):=2*Q(nerve)/A(CF)*QQ(axon/nerve)*V(ref)*(1-d(shr))^(2/3). In a set of five mouse hearts, the total length of axons ramifying among cardiomyocytes ranged between appr. 50 m and 100 m with a mean of 75.98 m (SD: 23.73 m). Preliminary data from the first application of this method suggest that tumor-associated cardiac atrophy is characterized by a significant decrease in cardiac innervation. Using antibodies specific for different neuron subtypes and immuno-electron microscopy, this method is also suited to estimate the total axon length of neurons expressing different transmitters.

Kategorie: Poster

Poster 52

Rubrik: 3.Neuroanatomie/Neurobiologie
Abstract Nr.:3

Titel:In vivo effects of exogenous polysialic acid on peripheral nerve regeneration across nerve gaps

Autoren: Schaper-Rinkel J.(1),Schmitte R.(2),Rode B.(3),Gerardy-Schahn R.(4),Scheper T.(3),Grothe C.(1),Haastert K.(1),

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Abstract:
We are developing polysialic acid (polySia)-based scaffolds in order to increase peripheral nerve regeneration (PNR) across long gaps. Endogenously polySia bound to the neural cell adhesion molecule promotes neural plasticity associated with learning, memory and regeneration. To test the applicability of exogenous polySia we performed two in vivo studies.
First: silicone tubes filled with Growth Factor Reduced MatrigelTM (Matrigel) (1) alone, (2) plus soluble polySia, (3) plus Schwann cells (SC), (4) plus polySia and SC, were implanted to bridge 10 mm gaps in adult rat sciatic nerves. Eight weeks after surgery, polySia treated animals showed significantly enhanced numbers of regenerated myelinated axons. Also the outcome of motor recovery (electrodiagnostic measurements) was positively influenced. Pre-labeling of transplanted SC and retrograde tracing experiments revealed no negative effects of polySia on SC survival and regenerating motor and sensory neurons. Second: 13 mm nerve gaps were bridged either by tubes similar to (4) Matrigel + polySia + SC, or by autologous nerve grafts (clinical standard). Over 10 weeks functional recovery was monitored by Rotarod-test and sciatic function index (motor), pinch- and withdrawal-test (sensory). While the pinch-test revealed a higher speed of sensory recovery after transplantation of polySia–supplemented nerve grafts, no difference between sample and control group was observed in other tests. Electrodiagnostic measurements, finally, showed reinnervation of the gastrocnemius muscle in 30% of the polySia-graft animals but 100% in the group implanted with autologous grafts.
We demonstrate here biocompatibility of exogenous polySia and show its potential to improve PNR.
Grant: DFG-FOR-548-GR-857/20-3&24-1

Kategorie: Poster

Poster 53

Rubrik: 4.Zellbiologie
Abstract Nr.:4

Titel:In search for synapto-nuclear transport mechanisms

Autoren: Heinrich J.(1),Proepper C.(1),Boeckers T.(1),

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Abstract:
Chemical synapses are unidirectional, functional connections between neurons and other cell types that are responsible for intercellular communication. They are able to undergo fast morphological changes and this plasticity of synapses seems to be essential for learning and memory formation. Abelson interacting protein 1 (Abi-1) has been characterized as a component of the postsynaptic density (PSD) and was found to be important for dendrite branching, spine morphology and synapse formation. Abi-1 is targeted to PSDs via its direct interaction partner ProSAP2/SHANK3 that functions as a master scaffolding protein. Upon NMDA receptor stimulation Abi-1 translocates from the postsynapse to the nucleus. We are interested in the molecular mechanisms that underly these trasnsport processes. Therefore, we performed a yeast two-hybrid screen of a fetal human brain library using a complete cDNA of Abi-1 as bait. Among several candidate genes we found a partial cDNA of a brain specific N-kinesin, namely Kif26b. This is a motor protein of the kinesin superfamily that moves along the microtubular system carrying cargo molecules. First experiments show that Abi-1 specifically interacts with Kif26b also in vivo and that the overexpression of a GFP-Kif26b fusion protein leads to the accumulation of several protein complexes in the nuclear compartment. Further experiments are set up to elucidate the importance of Kif26b in synapto-nuclear transport mechanisms as well as of subsynaptic signal transduction pathways that seem to be initiated by postsynaptic NMDA receptor activation.

Kategorie: Poster

Poster 54

Rubrik: 4.Zellbiologie
Abstract Nr.:4

Titel:Dynactin mutations in ALS pathogenesis

Autoren: Stockmann M.(1),Liebau S.(1),Ludolph A.(2),Meyer-Ohlendorf M.(3),Böckers T.(1),

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Abstract:
Amytrophic lateral sclerosis (ALS), is the most common adult onset disorder of motor neurons. ALS is a progressive and fatal neurodegenerative disease. Persons afflicted by ALS typically develop a combination of upper and lower motor neuron signs, with progressive muscle weakness, usually combined with pathologically brisk reflex. Eventually the limb and bulbar muscles are also affected.
Current understanding of the pathogenic processes of ALS suggests that there may be a complex interplay between multiple mechanisms including genetic factors, oxidative stress, protein aggregation and damage to cellular processes like axonal transport.
Among others ALS may be caused by mutations in the dynein or dynactin gene. Dynactin is a multi-subunit protein complex that is required for most cytoplasmic dynein activity in eukaryotes and contributes to the activity of another microtubule-based motor, kinesin II.
It was our task to characterize selected sequence changes in the dynactin p150 subunit found in ALS patients in in vitro functional studies.
We expressed constructs inheriting the sequence changes of dynactin in cos7 cells and found out, that a typical morphological feature which distinguishes the wild type from the several mutated proteins is the formation of aggregates.
Furthermore we investigate the question if the mutated dynactin protein may show a malfunction towards known interaction partners.
Another aim is to gain a better understanding of the function of mutated dynactin in the retrograde axonal transport and its role in specific cellular signal pathways.

Kategorie: Poster

Poster 55

Rubrik: 4.Zellbiologie
Abstract Nr.:4

Titel:Identification and characterization of direct interaction partners of tight junction-specific protein occludin using the yeast two-hybrid system

Autoren: Burek M.(1),Drenckhahn D.(2),Förster C.(1),

Adressen:(1)Universität Würzburg|Klinik und Poliklinik für Anästhesiologie|Würzburg|Deutschland; email:Burek_M@klinik.uni-wuerzburg.de; (2)Universität Würzburg|Institut für Anatomie und Zellbiologie|Würzburg|Deutschland

Abstract:
Occludin is an integral transmembrane tight junction protein with two extracellular loops and N- and C-termini in the cytoplasm. The role of occludin is still insufficiently investigated. In order to elucidate its physiological role, we looked for new interaction partners of occludin using the yeast two-hybrid system. A cDNA library from brain microvascular endothelial cells (cEND) has been constructed and screened with the N- and C-terminus of murine occludin cDNA as the bait. We identified several pray clones, like protocadherin gamma C3 (pcdhgC3) and ubiquitin. Interaction of occludin with ubiquitin has been recently reported by Murakami T. et. al. (J Biol Chem, 2009, May 28, publication ahead of print). PcdhgC3 is, as other gamma-protocadherins, most notably expressed in the nervous system. Transfection of Pcdh-gamma genes promotes calcium-dependent cell adhesion in HEK 293 cells. In this study, the interaction of occludin with pcdhgC3 was confirmed as specific by growing yeasts on selective medium, as well as in the beta-galactosidase assay. Further, the cDNA of pcdhgC3 was co-transfected with occludin cDNA into mammalian cells and imunoprecipitated with anti-occludin antibody. The deletion mutant of occludin, which does not contain the N-terminus appeared not to interact with pcdhgC3. Similarly, the mutated N-terminus of occludin showed no interaction with pcdhgC3. Thus, the N-terminus of occludin is necessary for the interaction between these two proteins. Since pcdhgC3 is mostly expressed in the brain, such an interaction with occludin might be specific for the blood-brain-barrier endothelium and could contribute to the specialized character of brain endothelial cells.

Kategorie: Poster

Poster 56

Rubrik: 4.Zellbiologie
Abstract Nr.:4

Titel:The Ca2+-binding protein calretinin is localized in a subpopulation of cells of the adult epithelial rests of malassez

Autoren: Korkmaz Y.(1),Raab W.(1),Blauhut T.(1),Ulbrich H.(2),Klinz F.(2),Addicks K.(2),

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Abstract:
The epithelial rests of Malassez (ERM) are the developmental residues of Hertwig's epithelial root sheath and remain in the mature periodontal ligament (PDL). In epithelial cells, the regulation of intracellular Ca2+ during tooth development by different Ca2+-binding proteins has been described. However, the existence of Ca2+-binding protein calretinin in the adult ERM is unknown and its involvement in the Ca2+ homeostasis in adult PDL has still not been elucidated. The perfusion-fixed, decalcified, frozen-sectioned free-floating sections of the adult rat molars with PDL were incubated with antibodies against TrkA (a marker for ERM) and calretinin. Double incubations were performed combining calretinin and TrkA antibodies. The differences in the ERM number stained by antibodies against TrkA and calretinin polyclonal and calretinin monoclonal antibodies were compared by statistical analysis. TrkA and calretinin were detected in the ERM. In double immunofluorescence experiments, TrkA was detected in all epithelial cell clusters, but co-localization with calretinin was found only in a subpopulation of the ERM at the root surface of the acellular cementum. The number of TrkA labeled ERM was significantly greater than the number of calretinin-immunoreactive ERM. The ERM are not equivalent for staining of calretinin and the ERM can be distinguished by the presence or absence of the Ca2+ binding protein calretinin. The localization of calretinin in a subpopulation of cells in the ERM indicates a buffer and/or sensor role/s in the regulation of Ca2+ homeostasis during the physiological remodeling of acellular cementum in the adult PDL.

Kategorie: Poster

Poster 57

Rubrik: 4.Zellbiologie
Abstract Nr.:4

Titel:Inflammation induces downregulation of TrkA in human ERM

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Abstract:
The epithelial rests of Malassez (ERM) are development residues of the Hertwegs epithelial root shearth (HERS). The functions of the ERM are still unknown, but their persistance thoughout the whole life in the periodontal ligament (PDL) suggest that ERM have important pysiological functions in the PDL, such as the maintenance of the periodontal space and periodontal regeneration. Trk A is a member of the tropomyosin-related kinase (trk) family of rezeptor tyrosine kinases (TrkA, TrkB and TrkC) and is activated by nerve growth factor (NGF). TrkA is also expressed in several non-neuronal tissues including ERM in the PDL of rats. Inflammation dependent regulation of TrkA in ERM of the human PDL is unknown. Therefore, to characterise the role of TrkA and related signaling pathways in human ERM, we examined localization of TrkA in decalcified and frozen-sectioned healthy and inflamed human molars using quantitative immunohistochemistry. The results were statistically analyzed by student's t-test. TrkA was detected in the human ERM. The staining intensity of TrkA in healthy ERM was significantly greater than the inflamed ERM. In our study we could show the expression of Trk A in human healty ERM and a significant decrease of TrkA in the PDL of human theeth with inflammatory diseases. These findings suggest that ERM has functional neutrotrophin rezeptors which may be involved in tisusue maintenance via neuronal signaling in health and disease.

Kategorie: Poster

Poster 58

Rubrik: 4.Zellbiologie
Abstract Nr.:4

3-Titel:Antitumor effect of Nigella sativa oil as a prophylactic therapy of carcinogenic potential of the carbamate fungicide mancozeb in mice;an ultrastructure study.

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Abstract:
The skin cancer is most abundant type among all forms of cancer in the human body. So, the present study tested the effect of Nigella sativa oil on the development of skin cancer induced by mancozeb, (fungicide) in mice. Mancozeb is a polymeric complex of ethylene (dithiocarbamate) is reported to possess carcinogenic and cocarcinogenic activity. In the present work 30female mice were equally divided into3 groups.The first group of mice were only administered mancozeb,by topical application of mancozeb (100 mg/kg body wt) three times per week for 18 weeks. The second group of mice were administered Nigella sativa oil by topical application before topical application of mancozeb(100 mg/kg body wt) three times per week for 18 weeks .The third group is control group The results revealed advanced stages of squamous cell papillomas and keratocanthomas. In addition to apoptosis, DNA damage after 18 weeks of mancozeb topical application. Meanwhile the second group that administered topically Nigella sativa oil before topical application of mancozeb, no obvious changes from that of the control group. In conclusion, A Nigella sativa oil is shown to exhibit anticancer activity in mice. So we recommend its prophylactic usage specially in the developing countries.

Kategorie:Poster

Poster 59

Rubrik: 4.Zellbiologie
Abstract Nr.:4

Titel:Isolation and identification of mesenchymal stem cells from the equine periodontal ligament.

Autoren: Mensing N.(1),Gasse H.(1),Hambruch N.(1),Häger J.(1),Pfarrer C.(1),Staszyk C.(1),

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Abstract:
Mesenchymal stem cells (msc) have been used for regenerative therapies in equine tendon injuries. However, repaired tendons showed inferior biomechanical properties compared to healthy tendons. Hitherto, msc used for such therapies are derived from bone marrow or adipose tissue. To find a source of msc which guarantees a high tenogenic differentiation capacity, our studies focused on the periodontal ligament (pdl), since the pdl combines two remarkable characteristics. It withstands biomechanical strains presenting characteristics similar to a tendon, and it possesses high regenerative capacities.
Cells were obtained from three different pdl areas (apical, middle, subgingival) of a one year old horse. For comparison, subcutaneous cells were cultivated. Population doubling times were determined and colony forming unit assays were performed. Cellular differentiation into osteogenic, adipogenic and chondrogenic lineages was induced using specific in vitro protocols. Specific cellular differentiation markers were assessed by histochemical methods and by RT-PCR.
Pdl cells showed significantly higher colony forming unit numbers than subcutaneous cells, whereas the population doubling time of subcutaneous cells was significantly faster than those of pdl cells. Osteogenic and adipogenic differentiation could be induced in all cells. Marker mRNA for chondrogenic differentiation (Aggrecan, Collagen 2, COMP) was highly expressed by pdl cells from the middle and apical areas. In contrast, subgingival pdl cells and subcutaneous cells showed only expression of mRNA of Aggrecan and COMP.
Equine pdl cells from the middle and apical areas might be a promising source for regenerative msc-therapies. Further studies are intended to examine their tenogenic differentiation capacities.

Kategorie: Poster

Poster 60

Rubrik: 4.Zellbiologie
Abstract Nr.:4

Titel:Expression of anaphylatoxins and complement regulatory proteins in tendon

Autoren: Busch C.(1),Kohl B.(1),John T.(1),Stoll C.(1),Ertel W.(1),Schulze-Tanzil G.(1),

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Abstract:
Tendon injury leads to a local inflammatory response, characterized by the induction of pro-inflammatory cytokines. Since the complement system is involved in posttraumatic tissue inflammation in other tissues, the aim of the present study was to analyze the interrelation of cytokines and the expression of complement components in tendon.
Expression of the anaphylatoxin receptors (C3aR and C5aR) and the cytoprotective complement regulatory proteins (CPRs: CD35, CD46, CD55, CD59) was studied in human tendon and cultured primary human tenocytes by RTD-PCR and immunocytochemistry. Further, the regulation of anaphylatoxin receptors and CRPs by recombinant human interleukin (IL)-6, IL-10, tumor necrosis factor (TNF)-alpha or combinations of TNF-alpha with IL-6 and IL-10 (10 ng/mL, 6, 24, 48 h) was assessed by RTD-PCR.
C3aR and C5aR as well as CD46, CD55, CD59 were expressed at the mRNA level in tendon as well as in cultured tenocytes, whereas CD35 expression could only be found in tendon and the expression of all CPRs was significantly higher in tendon compared with that of cultured tenocytes. C5aR and CD55 were localized immunocytochemically on tenocytes. C3aR expression was upregulated by TNF-alpha, or IL-6 and IL-10 combined with TNF-alpha in tenocyte cultures, whereby IL-10 alone had no effect.
These results indicate that the pro-inflammatory cytokine TNF-alpha modulates the expression of distinct complement components in tenocytes. The obvious discrepancy between the basal expression levels of complement components in tendon and cultured tenocytes strongly suggests the analysis of the influence of systemic regulatory factors in tendon.

Kategorie: Poster

Poster 61

Rubrik: 4.Zellbiologie
Abstract Nr.:4

Titel:Reduced inflammation and expression of the surfactant proteins (SP) A and D after LPS-instillation in DPP4-deficient F344 rats

Autoren: Zientara A.(1),von Hörsten S.(2),Stephan M.(1),Schmiedl A.(1),

Adressen:(1)Hannover Medical School|Institute of Functional and Applied Anatomy|Hannover|Germany; (2)Friedrich-Alexander-University of Erlangen-Nürnberg|Section for Experimental Therapy, Franz-Penzoldt-Center|Erlangen|Germany

Abstract:
Alveolar epithelial type II cells (AEII) synthesize immune modulatory SP and highly express DPP4 (Schade et al, 2008, J Histochem Cytochem), which might influence SP production. DPP4-deficient rats show reduced signs of inflammation in a model of a gram-negative lung inflammation (Stephan et al., 2006, Verh Anat Ges 10). Therefore, we hypothesize that lipopolysaccharide (LPS) instillation leads not only to an ameliorated inflammation but also to reduced SP expression and decreased structural damage in DPP4-deficient rats.
DPP4-positive (WT) and -deficient Fischer rats were intratracheally instilled with 250µl LPS (E.coli, Sigma, Germany) and compared nine hours later to home cage controls (HCC), by immunohistochemistry, morphometry, qRt-PCR, and Western Blot analyses.
Compared to HCC, LPS instillation resulted in: 1) significant substrain dependent differences in the localization of inflammatory cells (WT: predominantly in alveoli, DPP4- deficient: predominantly in alveolar septa), 2) a significant increase of the relative surface fraction of SP-A labelled AEII and of the total number of SP-D labelled AEII only in WT, 3) significant substrain specific differences in SP-D labelled AEII numbers, 4) substrain dependent differences in the SP expression, 5) a significant increase of macrophages in WT, which was at a significant lower level in the DPP4-deficient substrain, 6) a significant increase of the septal thickness, which was significantly more pronounced in WT, 7) a significant decrease of septal surface density (loss of gas exchange areas) in WT.
Thus, ameliorated LPS-induced inflammation in DPP4-deficient rats is accompanied with a lower expression of immune modulatory SP and less structural damage.

Kategorie: Poster

Poster 62

Rubrik: 4.Zellbiologie
Abstract Nr.:4

Titel:Tissue response after UV-ns laser induced injury of murine gut mucosa: an intravital real-time study using 2-photon microscopy

Autoren: Klinger A.(1),Orzekowsky-Schroeder R.(2),Schueth A.(1),Freidank S.(2),Huettmann G.(2),Vogel A.(1),Gebert A.(1),

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Abstract:
Epithelial integrity is crucial for maintaining the barrier function in intestinal epithelium. Temporary damage of the epithelial surface may be caused by dietary compounds and inflammatory bowel diseases, such as ulcerative colitis or Crohn's disease. We here present a novel experimental setup for targeted induction of epithelial defects, using a pulsed UV-ns laser with an emission wavelength of 355 nm coupled to a 2-photon microscope. Visualization of vital murine intestinal mucosa was based on tissue autofluorescence. A novel mouse model enabled us to intravitally depict the effects of cell damage caused by linear UV absorption, leading to mechanical disruption of individual epithelial cells. After local UV damage, cells appeared dark, probably due to destruction of mitochondria and loss of NAD(P)H fluorescence. Within 10 min after cell damage, epithelial cells adjacent to the injured area migrated into the wound to cover the denuded area, resulting in extrusion of the damaged cells from the epithelial layer. Using the nuclear acid stain propidium iodide, we could show that UV manipulation induced necrosis rather than apoptosis. After induced cell damage we did not detect migration of immune cells toward the injured area within observation periods of up to 5 hours. This model will be used in further studies to investigate the intrinsic repair system of intestinal epithelium in vivo and study the biological dynamics in real time.

Kategorie: Poster

Poster 63

Rubrik: 4.Zellbiologie
Abstract Nr.:4

Titel:Uptake of nanoparticles by the small intestinal mucosa – an intravital 2-photon microscopy study in mice

Autoren: Schueth A.(1),Krapf L.(2),Orzekowsky-Schroeder R.(2),Huettmann G.(3),Gebert A.(1),

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Abstract:
The intestinal epithelium is impermeable to macromolecules and microorganisms, except in the gut-associated lymphoid tissue which contains antigen transporting M cells. It is still unknown, whether nanoparticles (NP) are capable of entering the body via ordinary enterocytes or whether this is restricted to M cells. 2-photon microscopy applied to living anaesthetized mice was used to investigate the uptake and further processing of 200 nm latex NP in the small intestine under nearly physiological conditions. The method allows us to repeatedly scan tissue volumes at maximum optical resolution over hours. After applying NP onto the intact gut mucosa, groups of NP were found in M cells and likewise in dendritic cells localized within the FAE and in the underlying lymphoid tissue. NP were also detected outside the gut-associated lymphoid tissue in ordinary villi but to a by far lesser extent. The passage of NP from the gut lumen to phagocytes in the lamina propria took less than 30 min, and microscopy could be done for up to 7 hours after NP application. These findings show that the intact villus epithelium of the small intestine is permeable to NP and that our 2 photon microscopy setup provides a new means for studying quantitatively the different pathways along which NP enter the tissue.

Kategorie: Poster

Poster 64

Rubrik: 4.Zellbiologie
Abstract Nr.:4

Titel:hnRNP K- mediated overexpression of abelson interactor 1 (Abi-1) supports cytoskeletal plasticity and metastatic potential in human colonic carcinoma cells

Autoren: Steinestel K.(1),Pröpper C.(1),Märkl B.(2),Liebau S.(1),Böckers T.M.(1),

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Abstract:
Malignant Neoplasms of the colon are within the leading causes of death from cancer in Germany and the USA. Colorectal tumor biology has long been subject to research, leading to knowledge of multiple pathways resulting in malignant tumor formation. Recent papers provided proof of the expression of heterogenous nuclear ribonucleoprotein K (hnRNP K) in colorectal carcinomas, where the protein seems to support tumor growth and aggressiveness in a way that was so far not completely understood. In our work, we want to show that Abelson interactor 1 (Abi-1), known as a regulator of cytoskeletal reorganization and synaptic maturation, is overexpressed in colorectal carcinoma cells and metastasis compared to healthy colonic mucosa. We further show that hnRNP K interacts with the 3'-UTR of Abi-1 mRNA, leading to increased protein expression of Abi-1 in colonic carcinoma cells. Finally, we want to show that knockdown of both Abi-1 and hnRNP K lead to increased filopodial spread-out and change in morphology of colorectal carcinoma cells. Taken together, our data suggest an important role for hnRNP K-mediated overexpression of Abi-1 in cytoskeletal reorganization and thus metastatic potential in human colorectal carcinoma cells.

Kategorie: Poster

Poster 65

Rubrik: 4.Zellbiologie
Abstract Nr.:4

Titel:Plasma Membrane Ca2+ ATPase (PMCA) Expression in Normal and Neoplastic Colorectal Tissue

Autoren: Rüschoff J.H.(1),Brandenburger T.(2),Aumüller G.(1),Strehler E.(3),Wilhelm B.(1),

Adressen:(1)Philipps-University|Department of Anatomy and Cell Biology|Marburg|Germany; email:jrmr@gmx.de; (2)University Hospital Düsseldorf|Department of Anesthesiology|Düsseldorf|Germany; (3)Mayo Clinic College of Medicine|Department of Biochemistry and Molecular Biology|Rochester, Minnesota|USA

Abstract:
The Plasma membrane Ca2+ ATPase (PMCA) is an exporter of Ca2+ for which four major isoforms (PMCA1-4) have been described in humans and rodents. Tissue distribution of isoforms varies with 1 and 4 being ubiquitous whereas 2 and 3 are primarily expressed in normal brain. In neoplastic cells of colorectum expression of PMCA1(b) und 4(b) has been studied with isoform 4(b) being upregulated in more differentiated cell lines (Aung et al. 2007; Ribiczey et al. 2007). However, a systematic study on a differentiation dependent expression of PMCA isoforms in tissue samples of colorectal cancer (CRC) and its precursors is still missing.
The aim of the present research project is to investigate the expression profile of PMCA1 and 4 in colorectal cancer of different stages and grades as well as in its precursor lesions. In order to elucidate their significance in the adenoma carcinoma sequence, expression at RNA and protein level was studied by real- time PCR, in- situ hybridization and immunohistochemistry in a total of 56 different colonic tumor samples.
Our results are in favor of a downregulation of the PMCA4 protein expression in high grade adenoma and carcinoma while having a constant PMCA1 protein level. However, PMCA1- and PMCA4 RNA was constantly detectable at all adenoma and carcinoma stages. These results could suggest that there is a posttranslational regulation of PMCA4 in CRC. The potential impact on downstream signaling, growth regulation and tumor progression in CRC will be discussed.

Kategorie: Poster

Poster 65a

Rubrik: 4.Zellbiologie
Abstract Nr.:4

Titel: Tumor targeting with specific contrast agents and quantitative imaging techniques

Autoren: Heine MH, Peldschus K, Pöselt E, Schumacher U

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Abstract:
Tumor-specific contrast agents for non-invasive detection of early tumors with Magnetic Resonance Imaging (MRI) will improve to choose the appropriate treatment strategy. Tumor specific antibodies conjugated to superparamagnetic iron oxide particles (AB-SPIO) are promising contrast agents for such imaging efforts possibly offering a high tumor specificity. In particular, these probes will allow monitoring in a non-invasive way how the tumor responds towards systemic treatment. To introduce this approach in clinical medicine a pre-clinical testing in xenograft models is necessary. We therefore screened human tumor cell lines from different tumor entities such as breast, lung, colon, prostate cancer and malignant melanoma for the expression of proteins of the carcinoembryonic antigen (CEA) family with the CEA-specific T84.1 antibody. These receptor molecules are not only highly expressed on the surface of many malignancies (e. g. CEACAM5), but also show neo-expression in newly formed tumor blood vessels (CEACAM1). Several candidate cell lines as HT29 (colon), DU145 (prostate), 5061 (pancreas) and Femx-I (melanoma) showed a high expression level of CEA. In a further verification, we analyzed primary tumors of these candidate cell lines for the presence of CEA. Femx-I tumors showed membrane-bound CEA expression and could therefore be well suited for AB-SPIO treatment. Thus, Femx-I cells were incubated with T84.1 coupled SPIOs and showed positive signals in MRI analysis. The signal could be abolished by preincubation of the tumor cells with the native T84.1 antibody, demonstrating the high specificity of the antibody-SPIO conjugate. In oncoming experiments, these conjugates will be further tested in the Femx-I tumor animal model.

Kategorie: Poster

Poster 66

Rubrik: 5.Entwicklungsbiologie
Abstract Nr.:5

Titel:Wnt signaling pathways regulating dermomyotomal EMT

Autoren: Scaal M.(1),Krück S.(2),

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Abstract:
Earlier work in our laboratory established that Wnt11 signaling from the dorsomedial lip of the dermomyotome and Wnt6 signaling from the surface ectoderm interact to regulate epithelio-mesenchymal transition in the mature dermomyotome, leading to deepithelialization in the central dermomyotome and maintenance of the epithelial dermomyotomal lips. To date the downstream signaling pathways involved in these interactions remain unknown. Using various gain- and loss of function constructs of canonical and non-canonical Wnt signaling, we investigate the intracellular regulation of EMT in the DML and VLL, and of Wnt6 expression in the ectoderm. We present preliminary data arguing for a role of canonical Wnt signaling in DML maintenance.

Kategorie: Poster

Poster 67

Rubrik: 5.Entwicklungsbiologie
Abstract Nr.:5

Titel:Localization and expression of uPAR in the rat tooth germ

Autoren: von Germar A.(1),Barth K.(1),Schwab W.(1),

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Abstract:
Localization and expression of uPAR in the rat tooth germ
The urokinase plasminogen activator receptor (uPAR), a gycosyl-phospatidylinositol-linked glycoprotein is a key regulator in pericellular proteolysis and cell signalling. The biological significance of uPAR expression in the dental development is not fully understood. Therefore, the aim of the present study was to monitor the occurence and distribution pattern of uPAR in cells of the tooth germs by means of immunocytochemistry and western blotting analysis.
In slices of newborn rat tooth germs, uPAR immunoreactivity was detected at the cell membranes of ameloblasts, especially at their distal ends and in the contact region to stratum intermedium cells. In addition, differentiating odontoblasts exhibited moderate uPAR immunostaining.
In dental epithelial cells (HAT-7 cells) uPAR expression was stimulated by the protein kinase C activator PMA. Although we could not detected a substantial colocalization between uPAR and caveolin by double immunostaining experiments, a small part of uPAR was localized in the detergent-resistent membranes of HAT-7 cells suggesting a partial localization with lipid rafts. .
The occurrence of uPAR in cells of the enamel organ and in odontoblasts suggests that the urokinase receptor takes part in the regulation of proteolytic enzymes in the rat tooth germ. In addition, the localization of uPAR in the detergent insoluble membrane fraction of HAT-7 cells may point to an involvement of uPAR in signalling pathways in ameloblasts, providing a basis for further investigations.

Kategorie: Poster

Poster 68

Rubrik: 5.Entwicklungsbiologie
Abstract Nr.:5

Titel:Maternal deprivation leads to retarded morphological postnatal lung development in DPP4 wild type and deficient F344 rats

Autoren: Hupa K.(1),von Hörsten S.(2),Pabst R.(1),Stephan M.(1),Schmiedl A.(1),

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Abstract:
We previously reported that “immunological stress”, e.g. of a postnatal LPS inhalation, resulted in DPP4-dependent differences in morphological lung development (Schünke et al. 2008, DOI 10.3337.anatges). Here, we hypothesize that also “mental stress”, in terms of a prolonged separation (maternal deprivation; MD), will lead to a DPP4-dependend retardation of postnatal lung development. Offspring of wild-type and DPP4-deficient primiparous F344 rats were daily separated from their dams for 2 hours until weaning. Lungs of 7-, 10-, 14- and 21-days-old pups that were either left undisturbed or had been deprived, were analyzed using stereological methods. In addition, the proliferation rate was determined by BrdU-incorporation.
In wild-types, MD resulted in a retarded postnatal lung development: Within the alveolarisation phase, an increased thickness of alveolar septa and a decreased gas exchange area of about 50% were found. At the end of the morphological lung maturation period, gas exchange area and volume of the alveolar septa were still decreased of about 20%. The proliferation rate was also decreased to 50% on day 14. In DPP4-deficient rats, MD caused a decreased volume of the air spaces of about 50% and an increased septal thickness at the end of alveolarization. On day 21, we found a decrease in the volume of the air spaces of about 20% and a decreased proliferation rate of about 50% at day 10.
The less pronounced retardation of the postnatal lung development in the DPP4-deficient rats seems to be caused by an altered mental stress response during postnatal development in this substrain.

Kategorie: Poster

Poster 69

Rubrik: 5.Entwicklungsbiologie
Abstract Nr.:5

Titel:Characterization of progenitor cells in pulmonary vasculature.

Autoren: Saboor F.(1),Berndt C.(1),Müller D.(1),Middendorff R.(1),

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Abstract:
Vascular smooth muscle cells (VSMCs) and pericytes (PCs) which are distinguished by the expression of intermediate filament protein nestin, a neuronal stem cell marker, may represent stem cell-like progenitor cells for organ specific cells. We found that nestin-expressing cells in testicular blood vessels are the progenitors of testosterone producing Leydig cells. Nestin positive progenitor cells have also been observed in adult pituitary, ovary and hair follicle.
Nestin positive cells, found in lung vasculature using nestin-GFP transgenic mice, were characterized as VSMCs and pericytes. Lungs of postnatal nestin-GFP mice showed significantly more nestin positive cells than adult with maximum proliferation 7 days after birth.
Hypoxic mouse model is being used to analyze involvement of nestin expressing cells in vascular remodeling. Western blot analysis for nestin expression in lung tissues showed gradual increase in expression with maximum expression after 1 week of exposure to hypoxia. Proliferative ability of nestin expressing cells is investigated using proliferation markers PCNA and Ki67 where maximum no. of dividing cells was observed after 4 days of exposure to hypoxia. PDGF-B is an important growth factor that regulates VSMCs proliferation and differentitation. Angiogenesis, during vascular remodeling, involves PDGF-B dependant VSMCs/PCs progenitor cell migration and proliferation. A gradual increase in PDGFR-ß expression was observed after exposure to hypoxia while expression of (activity indicating) phosphorylated-PDGFR-ß showed peak after 4-7 days of exposure to hypoxia.
This data suggest an involvement of PDGF in proliferation of nestin expressing cells during vascular remodeling in lungs.

Kategorie: Poster

Poster 70

Rubrik: 5.Entwicklungsbiologie
Abstract Nr.:5

Titel:Ultrastructural changes of renal tubular cells and hepatocytes of neonatal rats suckled milk from mothers orally administered cadmium chloride throughout their entire gestation and lactation periods.

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Abstract:
Distribution and accumulation of cadmium residue in the mammalian tissues and its toxic effect depends on the mode of cadmium administration to the experimental animals. Neonatal offsprings of female rats administered cadmium chloride by the oral route (0.5 mg•kg–1•day–1) throughout their entire gestation and lactation periods. The pups were nursed their mothers until they weaned at the age of 28 days .At the end of the experiment ,rats were anaesthetized by ether, then their hepatic and renal tissues were rapidly removed and processed for transmission electron microscopic examination. Quantitaive densitometric assessments of various fine structures were done. The ultrastructural observations were the prominent decline in following structures: the apical microvilli, basal striation and endocytotic –lysosomal apparatus of the proximal convoluted tubules. Meanwhile hepatocytes showed few, sporadic mitochondria in concomitant with ill developed rough and smooth endoplasmic reticula. In addition to apoptosis. In conclusion, cadmium was accumulated in the hepatic and renal tissues of prenatal rats and also transmitted via milk to the rat pups. Cadmium is potentially toxic to proximal convoluted tubular cells and to lesser extent to hepatic tissues with multifactor mechanisms of cadmium toxicity.

Kategorie:Poster

Poster 71

Rubrik: 6.Reproduktionsbiologie
Abstract Nr.:6

Titel:Effects of DEHP on embryonic cell development and metabolism

Autoren: Hart K.(1),Schmidt J.(1),Fischer B.(1),

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Abstract:
The plasticizer diethylhexylphthalate (DEHP) is known to act as an endocrine disruptor, impairing gonadal development and fertility in the male. Effects on female reproductive health and the developing embryo have not been studied intensively so far. Besides being a risk for reproductive health in probably both sexes, DEHP and its metabolite MEHP are also known to play a role in metabolism, mainly by activation of PPARgamma, a key mediator in lipid metabolism and adipogenesis. To further investigate the effects of DEHP on early embryonic cells during different developmental stages, we have exposed P19 murine embryonic carcinoma cells to different concentrations of DEHP (0.05, 5, 100, 500 µg/ml). The concentration range chosen covers “real world” exposure levels. The P19 cells were exposed in the undifferentiated stage for four days and subsequently differentiated to beating cardiomyocytes. At different stages the expression of key genes of the fatty acid (PPARs, aconitase, FATP1, FABP4) and glucose metabolism (GLUT4, aldolase, PFK), and developmental markers (alpha/beta-MHC, Cx43) were analyzed by qRT-PCR. We found that DEHP interferes with (cardiomyocyte) differentiation and disrupts glucose and fatty acid metabolism during cardiomyogenesis.
Based on the results of the cell culture model mouse blastocysts are currently exposed to DEHP in vivo (=in utero) and in vitro. Our experiments will yield a comprehensive view on the various effects of DEHP on development, differentiation and key metabolic pathways in early embryos and embryonic cells.

Kategorie: Poster

Poster 72

Rubrik: 6.Reproduktionsbiologie
Abstract Nr.:6

Titel:Influence of PDC-109 on Ca2+-atpase activity in lipid raft fractions of bovine sperm membranes

Autoren: Post H.(1),Schwarz A.(1),Aumüller G.(1),Wilhelm B.(1),

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Abstract:
Capacitation of sperm is a prerequisite for successful fertilization. During this Ca2+-dependent event the cholesterol concentration of sperm membrane changes and leads to a reorganization of the lipid and protein content of the so called lipid rafts. Lipid raft microdomains are enriched in cholesterol, sphingomyeline, glycosphingolipids, phospholipids and certain proteins. The plasma membrane Ca2+-ATPase (PMCA) restores Ca2+ concentration in sperm and plays a pivotal role in gaining hypermotility of sperm, a condition precedent to be able to pass the oolemma. In this study we investigated the localization of PMCA in lipid membrane fractions of the sperm membrane and the influence of bovine seminal vesicle major protein PDC-109 on Ca2+-ATPase activity in the lipid fractions. Western blotting experiments identified PMCA in both the lipid raft fraction and the non-lipid raft fraction as well. This localization is not influenced by sperm capacitation or treatment of sperm with the cholesterol acceptor Methyl-beta-cyclodextrine. PDC-109 enhanced the Ca2+-ATPase activity in non-lipid raft fractions of cauda sperm membranes more intensely than in the lipid raft fractions. It is still unknown, as to how between PDC-109 and the Ca2+-ATPase occurs. Contrary to the Ca2+-ATPase assay results lipid overlay experiments with sperm membrane lipid fractions showed that PDC-109 clearly binds to the sphingomyosin-containing lipid raft fraction. Further studies are planned to clarify if a presumptive intermediate partner leads to this effect and how it regulates PDC-109 action on Ca2+-ATPase.

Kategorie: Poster

Poster 73

Rubrik: 6. Reproduktionsbiologie
Abstract Nr. 6

Titel: Functional morphology of the tubular lamina propria testis of infertile men

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Abstract:
The influence of the peritubular lamina propria (LP) on spermatogenesis and sperm transport is unknown, although a thickened LP is found in a large number of infertile men. The LP comprises 5-7 cell layers with interposed extracellular matrix (ECM), the inner 3-5 layers containing myofibroblasts and the outermost layers fibroblasts. The present study analyzed whether a thickened LP displays changes in number, localization and molecular characteristics of contractile myofibroblasts.
Our results indicated three types of morphological changes of the thickened LP. In some tubules, increase in ECM in-between the network of myofibroblasts was the only morphological alteration (group 2). Most frequently, layers of myofibroblasts were found to be separated by a thickened ECM into two strata (group 3). Other tubules were lacking, in addition, the inner layer of myofibroblasts (group 4). Stereological and semi-quantitative investigations revealed from group 1 (normal LP) to group 4 a decrease of the epithelium/wall ratio and of the number of tubules showing intact spermatogenesis. In contrast to previous results, there was no evidence for dedifferentiation of myofibroblasts into fibroblasts. To investigate functional integrity of delocalized myofibroblasts, expression of contractile cell-specific proteins, including components of relaxation-mediating cGMP pathways, was analyzed. Regular expression of these proteins indicated that cells were still capable of exerting physiological functions.
We conclude that thickening of the ECM, but not transformation of delocalized myofibroblasts could be responsible for infertility-associated changes of the LP. The occurrence of distinct changing patterns in one individual testis suggests a gradual and temporal development of LP alterations.

Kategorie: Poster

Poster 74

Rubrik: 6.Reproduktionsbiologie
Abstract Nr.:6

Titel:Modification of bull spermatozoa after in vitro fusion with epididymosomes

Autoren: Schwarz A.(1),Wennemuth G.(2),Aumüller G.(1),Wilhelm B.(1),

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Abstract:
The male gamete development is the result of differentiation from non-polar, amotile sperm to polar, motile sperm. The final maturation step occurs in the epididymis. It is postulated that this process includes the exchange of several components between spermatozoa and epididymosomes. Our earlier studies showed that Ca2+-ATPase-activity is significantly increased in bovine cauda sperm. Furthermore, we demonstrated a splice variant switch from plasma membrane Ca2+-ATPase 4b (PMCA4b) in caput sperm to PMCA4a in cauda sperm.
The aim of the present study was to analyze whether or not proteins and lipids are transferred from epididymosomes to sperm during epididymal transit. Therefore, bovine epididymal sperm and epididymosomes (caput, cauda) were analyzed separately and after in vitro fusion.
The fusion of epididymosomes to spermatozoa in general was analyzed by determining octadylrhodamine (R18) self-quenching. The results indicate that the fusion process of epididymosomes with epididymal spermatozoa is dependent on time and pH. In addition, our experiments clearly indicate that the lipid and protein content in spermatozoa and epididymosomes changes during epididymal transit. Several phospholipids (PC, PE, SM) in spermatozoa and the phosphatidylcholin-content in epididymosomes display a significant decrease in transit from the caput to the cauda epididymis. The cholesterol/ phospholipid ratio in spermatozoa decreases during epididymal transit and after in vitro fusion. Furthermore, Ca2+-ATPase-activity in epididymal sperm was increased (~ 150%) after in vitro fusion.
Further studies have to clarify whether the increased Ca2+-ATPase-activity is a result of epididymal PMCA4a transfer or a consequence of the changed lipid environment.

Kategorie: Poster

Poster 75

Rubrik: 6.Reproduktionsbiologie
Abstract Nr.:6

Titel:The role of fibronectin in migration of adult leydig cell precursors from peritubular location to interstitial space in rat

Autoren: Morcinek K.(1),Wesbuer E.(1),Prokopiak M.(1),Berthold G.(1),Haider S. G.(1),

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Abstract:
Fibronectin - as a main component of extracellular matrix - is an important factor for motility of cells. Several studies have reported that the precursors of adult Leydig cells (ALC) in rat testis move from peritubular position to interstitium during their differentiation. The mechanism of this cell migration is almost unknown. The aim of this research was to analyse the participation of fibronectin in movement of the precursors of ALCs. Therefore, rat testes from postnatal day (pnd) 5 to pnd 21 on each alternate day were processed for immune histochemical reaction of fibronectin. To identify the single precursors of ALCs, the functional marker Luteinizing hormone receptor (LHR) was used in immune histochemical reaction additionally. On pnd 15 we detected fibronectin homogeneously in cytoplasm of few Leydig cells, in cytoplasmic extensions of interstitial fibroblasts and in extracellular space. The tunica albuginea and vascular endothelial cells were distinct positive for fibronectin, the lamina propria of seminiferous tubules only exhibited focally weak reaction. In comparison to pnd 15 testis of sexually adult rats show similar results with regard to extracellular matrix, fibroblasts, vascular endothelial cells and lamina propria of seminiferous tubules. Fibronectin in the adult rats on the contrary was accumulated to a distinct border in the periphery of ALCs. Detailed immunohistochemical data of fibronectin and LHR along with the ontogenetic changes will be presented in the poster.

Kategorie: Poster

Poster 76

Rubrik: 6.Reproduktionsbiologie
Abstract Nr.:6

Titel:Influence of a xenoestrogen di(2-ethylhexyl) phthalate (DEHP) on proliferation of ED1-positive testicular macrophages in rat, an immunohistochemical study

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Abstract:
In recent years increased abnormalities of the male reproductive tract in association with exposure to chemicals in the environment have been reported. One of these chemicals is bis(2-ethylhexyl) phthalate (DEHP), a commonly used plasticizer in many toys, consumer products and medical devices. DEHP - a hormone disruptor - is a member of synthetic xenoestrogens and has an inhibitory effect on testicular steroidogenesis. Testicular macrophages and Leydig cells are functionally interdependent. The aim of the present immunohistochemical work was to study the effect of DEHP on macrophages proliferation in testis of pubertal rats. The 20-days old rats (Rattus norvegicus) were gavaged with DEHP (200 mg/kg) or the corn oil vehicle (control) daily from postnatal day (pnd) 20 to 30, 40 and 50 pnd respectively. Using the specific antibody ED1 we have determined the number of macrophages (per mm2 in paraffin sections) after the DEHP treatment. The treatment of animals with DEHP significantly increased the macrophages numbers in testis compared to control. This is true for all three durations of treatment. These changes were observed already after 10-days treatment. The macrophages were quantified according to the location in the testis: 1. peritubular, 2. Leydig cell vicinity, 3. perivascular. The data showed that the highest number of macrophages was of peritubular location.
To conclude, the significant increase of the macrophages number in testis of DEHP-treated rats can be interpreted as an expression of the inflammatory and/or apoptotic processes.

Kategorie: Poster

Poster 77

Rubrik: 6.Reproduktionsbiologie
Abstract Nr.: 6

Titel:The IHC using monoclonal anti-vim antibody of the primary trophoblast at the placental level in different prematurity stages

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Abstract:
Abstract: The hereby study is one of the conclusions of segment of a large study that uncovers a period of over 5 years of research beginning with the year 2003 until september 2008 regarding the imunohistochemistry at placental level from different prematurely stages placentas.
Prematurity is an important segment in the obstetrical pathology and neonatology at the same time, having an important role in perinatal deaths, early mortality and infant mortality. As study base, we used placentary fragments from 216 placentas that came from births with different prematurity stages from which a number of premature newborns without congenital malformations resulted. This study was mainly addressed to the feto-placental binomial trying to correlate the identified morphological modifications with certain imunohistochemical methods and different stages of newborn prematurity, its survival and potential of adaptation to the extrauterine life.
The hereby study makes reference to the use of monoclonal antibody anti VIM identified in different disorganization stages in the placental architecture especially that of the primary trophoblast.
Key words: imunohistochemistry, prematurity, placental architecture, antiVIM.

Kategorie: Poster

Poster 78

Rubrik: 6.Reproduktionsbiologie
Abstract Nr.: 6

Titel:The immunohistochemical expression of alfa-fp placentas from the births' of encephalic pole developmental problems fetuses

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Abstract:
(Abstract)Alphafetoprotein is a fetal origin protein synthetised in the fetus's liver and in the Yolk sac that communicates with the maternal transplacentary blood so that it can be detected in the serum. In the maternal serum level the detection of AFP is performed between the 16th and the 20th week of pregnancy and its level shows, if it's elevated the possibility o certain development disorders especially frailties of the neural tube or the cephalic pole, whereas low levels of AFP can indicate the Down sindrom. Since the addressability of high risk cases is not made in optimal time for appreciation but long after or at all we consider that the hereby retrospective study can bring more information regarding the correlations of AFP presence at placental levels from which major development disorder fetuses derived. In this study we made use of the anti AFP monoclonal antibody, DAKKO Seryes.
Key words: malformations, cephalic pole, citoarchitectonics, placenta, alphafetoproteine, imunohistochemistry .

Kategorie: Poster

Poster 79

Rubrik: 6.Reproduktionsbiologie
Abstract Nr.:6

Titel:Secretory immunity at mucosal surfaces of vagina and cervix of the pig

Autoren: Telgen L.(1),Steffl M.(1),Schweiger M.(1),Amselgruber W.(1),

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Abstract:
Purpose: Secretory component (SC) in association with immunoglobulin A (IgA) forms secretory IgA (sIgA), the major antibody active at mucosal surfaces. In humans and laboratory animals, sIgA is known to be expressed in different female reproductive organs. However in livestock animals, there is a lack of information about the expression of SC and IgA in the female genital tract.
Methods: Three segments of the oviduct (infundibulum, ampulla, isthmus), uterine horn, cranial and caudal cervical portion and vagina propria were each collected from 13 German Landrace pigs slaughtered at proestrus, estrus, metestrus and diestrus. The localization and distribution of SC and IgA was evaluated by immunohistochemical analysis of paraffin-embedded tissue sections.
Results: The results clearly show that SC is exclusively expressed by the cervix and vagina. The staining of SC was intense in the squamous epithelium and restricted to the superficial cell layer which is not cornificated in the pig. Percentage of SC-positive cells was markedly higher in follicular phase than during the luteal phase of the estrus cycle. In a similar manner, immunostaining characteristics for IgA were also present in cervix and vagina. The cytoplasm of plasma cells stained intensely positive for IgA whereas the luminal surface cells stained moderately.
Conclusions: The luminal defense in the lower genital tract of the pig depends on the stage of the cycle and is mainly due to locally synthesized SC and sIgA.

Kategorie: Poster

Poster 80

Rubrik: 7.Immunbiologie
Abstract Nr.:7

Titel:Induction of antimicrobial peptide psoriasin by bacterial components in glial cells

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Abstract:
Antimicrobial peptides are part of the innate immune system in epithelial and non-epithelial surfaces, and may also have important functions in the brain. However, little is known about the expression of antimicrobial peptides in the CNS and whether glial cells can secrete these peptides. We have used cell cultures, real-time RT-PCR, immunohistochemistry, ELISA and an animal model to get more information about the role of antimicrobial peptides in the CNS. In detail, we have investigated the expression of the antimicrobial peptide Psoriasin also known as S100A7, which was first identified as an over-expressed peptide in psoriatic skin, in rat glial cells (astrocytes and microglia) after incubation with bacterial components. Furthermore, we used cerebrospinal fluid (CSF) and serum from patients with bacterial meningitis to detect Psoriasin. Finally, we investigated the occurrence of Psoriasin in an animal model of bacterial meningitis. We here demonstrate (i) the detection and the (not only for the first time the expression but also) secretion of biological active Psoriasin in glial cells, and (ii) the occurrence of antimicrobial peptides in the cerebrospinal fluid of meningitis patients. Moreover, we could show an involvement of Psoriasin in the rat meningitis model pointing to a role of Psoriasin in the pathogenesis of this disease. Our results suggest that Psoriasin is an important part of the innate immunity in the brain against bacterial CNS pathogens.

Kategorie: Poster

Poster 81

Rubrik: 7.Immunbiologie
Abstract Nr.: 7

Titel: Genetic disruption of Nrf2 impairs articular destruction in a mouse model of rheumatoid arthritis

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Abstract:
Oxidative stress has been implicated in a variety of inflammatory diseases including rheumatoid arthritis. Nuclear factor-erythroid 2 (NF-E2)-related factor 2 (Nrf2) is a transcription factor that is known to maintain the cellular defence against oxidative stress via binding to the antioxidant response element (ARE) within the regulatory regions of antioxidative and detoxifying genes. The extent of oxidative stress in the joints was measured via TBARS-assay.
Antibody-induced arthritis (AIA) was induced in Nrf2-KO mice and in Nrf2-WT control mice by an intra venous injection of 4 mg of monoclonal antibody cocktail against murine typ II collagen. The severity of cartilage destruction was evaluated by histopathology. The mRNA and Protein expression of the inflammatory cytokines TNF-α, IL-1, and IL-6 as well as VEGF was evaluated by Luminex-technique. The activation state of Nrf2 during RA was analyzed via immunhistological staining of Nrf2 of synovial explants from healthy donors and patients suffering from RA. In addition, we used a Xenogen imaging system to measured Nrf2-activity in an ARE-luciferase transgenic mouse during AIA.
These data demonstrate that Nrf2 exerts an anti-inflammatory role during chronic inflammation and tissue damage associated with antibody-induced arthritis, possibly by activating Nrf2 and the resulting up-regulated expression of antioxidative proteins via ARE.
Autoren:, Athanassios Fragoulis[1], Agata Gurzynski[3], Deike Varoga[2], Lars-Ove Brandenburg[1], Thomas Pufe[1] Christoph Jan Wruck[1]

Kategorie: Poster

Poster 82

Rubrik: 7.Immunbiologie
Abstract Nr.:7

Titel:Insulin-like growth factor (IGF)-I and estrogen receptor (ER)-alpha expression in channel catfish B- and T-lymphocytes in vitro

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Abstract:
Aquaculture is a strongly growing food-production field leading to fish-rearing at high densities. Thus, the immune system of fishes and its modes of regulation are of increasing interest. Preliminary evidence suggests that insulin-like growth factor (IGF)-I exerts differentiating, mitogenic and restoring activities also in the immune system of fish. However, only few data are available so far. We are investigating the potential effects of hormones on the growth hormone (GH)/IGF-I axis in interaction with the fish immune system. Thus, we have recently established two Channel catfish T and B lymphocyte cell lines as models for the in vitro investigation of hormonal influences on fish lymphocytes. Using real-time PCR, the expression of estrogen receptor (ER)-alpha as indicator for the efficiency of the in vitro treatment with estrogen was measured. Using real-time PCR and immunohistochemistry, we here for the first time show the expression of IGF-I mRNA and peptide in leukocytes of any fish species identified as T and B lymphocytes. In vitro treatment with 17-alpha-ethinylestradiol (EE2), a xeno-estrogen found at high concentrations in rivers and lakes, induced lymphocyte proliferation at low but cell death at high doses.

Supported by the Swiss National Foundation.

Kategorie: Poster