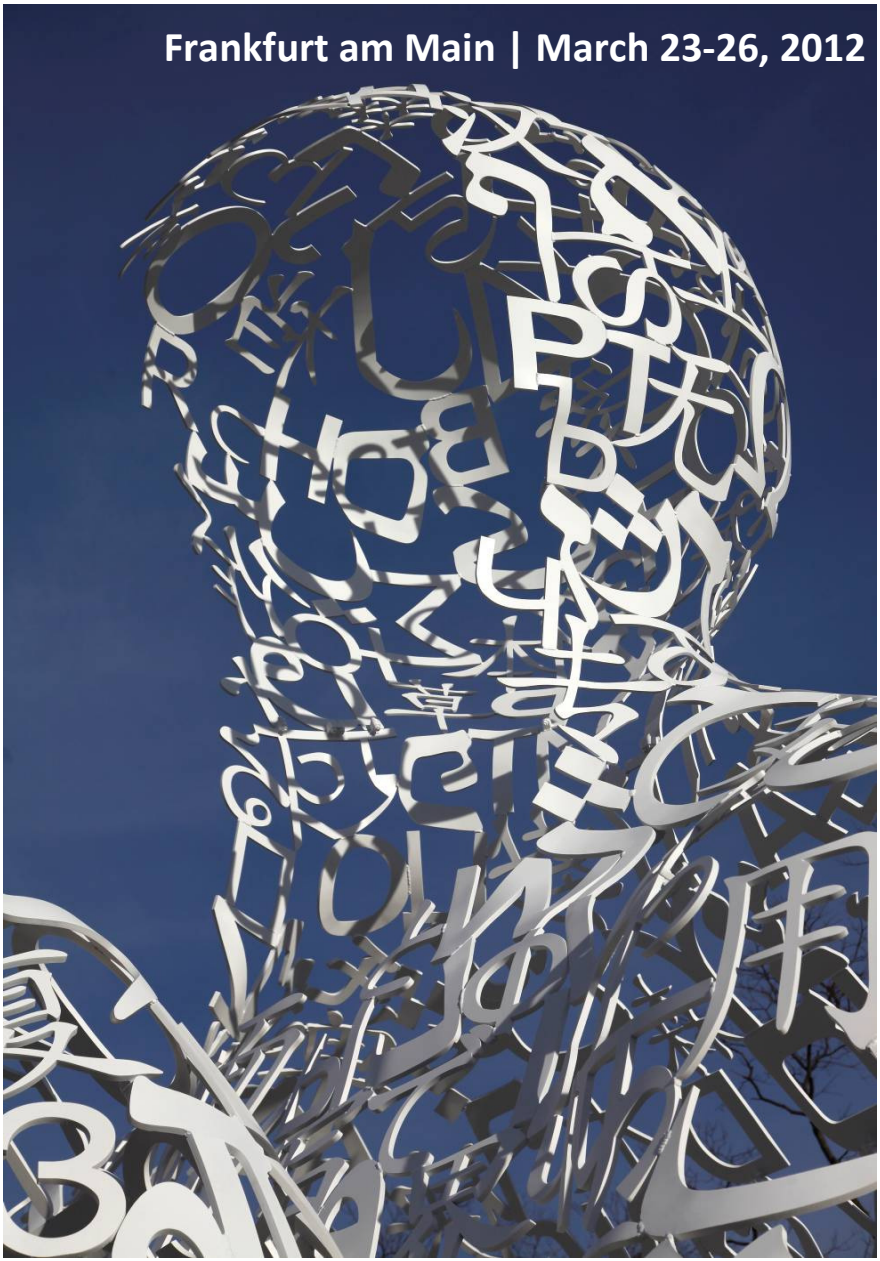
Anatomische Gesellschaft
107th Annual Meeting

Frankfurt am Main | March 23-26, 2012



To find your abstract or an abstract of interest please use the alphabetical list of first authors of lectures and posters starting on next page or use the abstract number which refers to the lecture number given in the meeting program.

Example:
Rubrik: 6.Neuroanatomy/Neurobiology
Abstract Nr.:15

This means lecture 15.

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Abstract Nr.:1

Takahashi J., Dallas (USA)

Clock genes, clock cells and clock networks in the mammalian circadian system

Rubrik: 1.Main Topic I
Abstract Nr.:2

Titel: Sensing light and time using novel photoreceptors

Autoren: Foster R.G.

Adressen: Nuffield Laboratory of Ophthalmology, The John Radcliffe Hospital, University of Oxford, Headley Way, Headington, Oxford OX3 9DU, United Kingdom

Abstract:
Circadian rhythms provide an internal representation of the day and optimise physiology and behaviour to the varying demands of night and day. These clocks require daily adjustment, and the primary time cue used by most vertebrates is the daily change in the amount of environmental light (irradiance) at dawn and dusk, a process termed photoentrainment. Attempts to understand the photoreceptor mechanisms mediating non-image forming responses to light have resulted in the discovery of a remarkable array of different photoreceptors and photopigment families, all of which appear to utilise a basic opsin/vitamin A-based photopigment biochemistry. In non-mammalian vertebrates, specialised photoreceptors are located in many regions of the brain including the pineal and deep brain. Although mammals lack extra-ocular photoreceptors, the eye contains in addition to rod and cone photoreceptors, a third photoreceptor system based upon a subset of melanopsin-expressing photosensitive retinal ganglion cells (pRGCs). In this presentation the photosensory role of two “irradiance detector” photopigments will be considered, the VA opsin photopigments of birds and melanopsin (Opn4) photopigments of mammals: (i) Birds possess photoreceptors located deep within the hypothalamus that regulate seasonal biology. Our new results suggest that VA opsin plays a key role in this critical light detection process; (ii) Mammalian melanopsin (*Opn4*) has two splice variants and appears to transduce light information using an “invertebrate-like” signaling pathway. We have used RNAi knock-down approaches *in vitro* and *in vivo* to provide a functional analysis of this phototransduction pathway and find that different signaling elements regulate different responses to environmental irradiance.

Kategorie: Lecture

Rubrik: 6.Neuroanatomy/Neurobiology
Abstract Nr.:3

Titel:Coordination of daytime-dependent memory processing in mouse hippocampus

Autoren: Rawashdeh O.(1),Jilg A.(1),Stehle J.(1),

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Abstract:
Hippocampal dependent long-term memory processing has been shown to be day-time-dependent. While phenomenologically the circadian system is clearly involved in the temporal gating of hippocampal mnemonic processes and long-term potentiation, the principal mechanisms behind this regulation are poorly understood.
Having demonstrated earlier that clock gene protein abundance cycle rhythmically in mouse hippocampus (Jilg et al., 2010), we here investigated specifically the role of a clock gene that functions as a core element in clockwork plasticity, namely Period1 (Per1). Using a hippocampus-dependent spatial memory task, we detected that the increased performance during daytime learning in wildtype mice was absent in Per1-/--mice. In parallel, the absence of PER1 affected the dynamics of training-induced and long-term memory-dependent signaling cascades. Mechanistically, we dissect that PER1 is essential in regulating PKA/ERK/CREB-dependent signaling, accounts for proper phase alignment of CREB-dependent rhythms in chromatin remodeling, and is responsible for the observed deficiencies in spatial memory performance. As long-term memory is afflicted in both, Per1-/--mice (this study) and Per-mutant flies (Sakai et al., 2004), the memory-associated function of this clock gene is preserved over some 700 million years of evolutionary distance.

Kategorie: Lecture

Rubrik: 1.Main Topic I
Abstract Nr.:4

Titel:Estrogen receptor-mediated hippocampal spine synapse formation is modulated by estrus cyclicity of hippocampal estradiol synthesis

Autoren: Rune G.(1),Zhou L.(1),Noshari S.(1),Behem C.(1),de Vreese X.(1),

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Abstract:
In spite of numerous studies on the role of estrogen receptor (ER) subtypes α and β in estrogen-regulated synaptogenesis in the hippocampus, hippocampal estradiol synthesis has been so far ignored. In this study, we therefore tested whether endogenous levels of estradiol influence ER-mediated signalling regarding synaptogenesis. To this end, we treated hippocampal slice cultures with cholesterol to increase both estradiol synthesis and spine synapse density. To induce opposite effects i.e. inhibit estradiol synthesis and reduce spine synapse density we used letrozole, an aromatase inhibitor. In these slice cultures, we determined the number of spine synapses in response to agonists and antagonists of the ERα and ERβ. In non-treated and cholesterol-treated slice cultures, the agonists of ERα (PPT) and ERβ (DPN) respectively had no effect, while synapse density was clearly reduced in response to the ERα antagonist (MPP) and the ERβ antagonist (PHTTP). Consistently, spine synapse number was reduced in the hippocampus of both the ERα and the ERβ knock-out mouse. However, in slice cultures, in which estradiol synthesis and spine synapse density was reduced by letrozole, PPT induced a significant increase and DPN a significant decrease in spine synapses as compared to the control and to cultures treated with letrozole alone. In contrast, MPP and PHTTP were ineffective in these cultures. Our results reveal that the effects of ER-mediated synapse formation are modulated by neuronal aromatase activity. Neuronal aromatase activity, in turn, varies during the estrus cycle since aromatase activity is regulated by cyclic release of GnRH from the hypothalamus.

Kategorie: Lecture

Rubrik: 1.Main Topic I
Abstract Nr.:5

Titel:Mice deficient for the clock protein bmal1 show impaired adult neurogenesis

Autoren: von Gall C.(1),Prokopiak M.(1),Christ E.(2),Korf H.(2),

Adressen:(1)Institut für Anatomie II|Heinrich Heine University|Düsseldorf|Germany; email:Charlotte.vonGall@med.uni-duesseldorf.de; (2)Institut für Anatomie II|Goethe University|Frankfurt|Germany

Abstract:
Disturbances of the circadian system lead to disintegration of daily body rhythms, reduced life span, cognitive impairment and presenile senescence. A molecular clockwork residing in virtually all cells controls rhythmic cellular processes including gene expression, cell division, und DNA-repair mechanisms. The core molecular clockwork components are transcriptional regulators such as PER1-2, CRY1-2, CLOCK and BMAL1. The BMAL1-deficient (BMAL1-/-) mouse is the model organism of choice for investigating the role of the molecular clockwork in brain function and aging. BMAL1-deficient mice show an altered ROS homeostasis in periphery and brain as well as various signs of premature aging, cognitive deficits and reduced hepatocyte regeneration. Our own observations have shown that the BMAL1-deficiency affects neuronal glutamate signal transduction mechanisms. However, so far little is known about the reasons for cognitive dysfunctions in BMAL1-deficient mice. As adult neurogenesis is implicated in cognitive function, we analyzed the effect of BMAL1-deficiency on neuronal stem cell proliferation in the adult hippocampal subgranular layer. Bromodesoxyuridine (BrdU) was applied intraperitoneally to WT and BMAL1-/- mice on three consecutive days. BrdU as well as neuronal (doublecortin , neuronal nuclei, NeuroD) and glial (glial fibrillary acidic protein) marker were analyzed in the subgranular layer of the dentate gyrus by immunohistochemistry. We found significantly fewer BrdU- and doublecortin- labelled cells in BMAL1-/- mice as compared to WT. This suggests an impaired proliferation of neuronal stem cells which might contribute to impaired cognitive function in BMAL1-/- mice.

Supported by the Dr. Senckenbergische Stiftung

Kategorie: Lecture

Rubrik: 1.Main Topic I
Abstract Nr.:6

Titel:Sensitizing effects of 2-arachidonoylglycerol in hypophysis and hypothalamus of mammals

Autoren: Yasuo S.(1),Unfried C.(2),Bojunga J.(3),Iigo M.(4),Otsuka T.(5),Togo Y.(5),Schreiber Y.(6),Geisslinger G.(6),Korf H.(2),

Adressen:(1)Dr. Senckenbergische Anatomie, Institut für Anatomy II, Dr. Senckenbergisches Chronomedizinisches Institut; Faculty of Agriculture|Goethe University Frankfurt; Kyushu University|Fukuoka|Japan; (2)Dr. Senckenbergische Anatomie, Institut für Anatomy II, Dr. Senckenbergisches Chronomedizinisches Institut|Goethe University Frankfurt|Frankfurt am Main|Germany; (3)Medizinische Klinik I|Goethe University Frankfurt|Frankfurt am Main|Germany; (4)Faculty of Agriculture|Utsunomiya University|Utsunomiya|Japan; (5)Faculty of Agriculture|Kyushu University|Fukuoka|Japan; (6)Institut für Klinische Pharmakologie|Goethe University Frankfurt|Frankfurt am Main|Germany

Abstract:
We recently identified in Syrian hamsters and human an endocannabinoid system in the pars tuberalis (PT), a control center of photoperiodic regulation of reproduction and metabolism. Among endocannabinoids, synthesis of 2-arachidonoylglycerol (2-AG) exhibited photoperiodic fluctuation in the PT of hamsters. Target of PT-derived 2-AG is suggested to be the pars distalis (PD), which contains cannabinoid receptor 1, a G-protein coupled receptor. Using organ cultures of hamster PD we here demonstrate that prolactin secretion was stimulated by 2-AG treatment in combination with forskolin. These effects persisted in the presence of quinpirole, a D2-class dopamine receptor agonist. In the absence of forskolin 2-AG also stimulated prolactin secretion in combination with adenosine treatment, but not with ADP or ATP treatment. To test the hypothesis that 2-AG sensitizes the PD tissue for stimulatory signals, organ cultures were pretreated with 2-AG followed by stimulation with forskolin or adenosine. In agreement with the hypothesis, stimulating effects of forskolin or adenosine were significantly enhanced by the pretreatment of PD with 2-AG. Finally, we demonstrated that 2-AG also sensitized the hypothalamus, another important target of PT-derived 2-AG: pretreatment with 2-AG augmented the forskolin-induced cAMP accumulation in slice culture of mouse hypothalamus. In conclusion, our data suggest that 2-AG sensitizes the PD and hypothalamus to potentiate the effects of endogenous stimulators such as adenosine for the regulation of physiological outputs.

Supported by the Dr. Senckenbergische Stiftung Frankfurt am Main, Kyushu University Interdisciplinary Program in Education and Projects in Research, and Grants-in-Aid for Research Activity Start-up.

Kategorie: Lecture

Rubrik: 1.Main Topic I
Abstract Nr.:7

Titel:Melatonin facilitates reentrainment of the circadian system to light-induced phase advances by acting upon mt2 receptors

Autoren: Pfeffer M.(1),Korf H.(1),von Gall C.(2),

Adressen:(1)Dr. Senckenbergische Anatomie II, Dr. Senckenbergisches Chronomedizinisches Institut|Fachbereich Medizin, Goethe-Universität Frankfurt|Frankfurt|Germany; email:M.Pfeffer@em.uni-frankfurt.de; (2)Institut für Anatomie II|Heinrich Heine Universität Düsseldort|Düsseldorf|Germany

Abstract:
The indolamine melatonin is an important endocrine signal in the circadian system. Exogenous melatonin can entrain circadian rhythms in physiology and behavior, but the role of melatonin and the two melatonin receptor types (MT1 and MT2) in reentrainment of daily rhythms is not understood. The present study analyzed locomotor activity rhythms and clock protein levels in the suprachiasmatic nuclei (SCN) of melatonin-deficient and melatonin-proficient mice as well as in melatonin-proficient mice with targeted deletion of the MT1, the MT2 or both receptors, which were subjected to phase delays or phase advances of the light-dark cycle by 6 h. In all mouse strains and genotypes reentrainment of locomotor activity rhythms was significantly faster after a 6 h phase delay as compared to a 6 h phase advance. Reentrainment after the phase advance was significantly slower in melatonin-deficient animals and in mice lacking functional MT2 receptors as compared to melatonin-proficient animals with intact MT2 receptors. To investigate whether these behavioral differences coincide with differences in reentrainment of clock protein levels in the SCN, mPER1 and mCRY1 immunoreactions were compared between mice kept under the original light-dark cycle and mice subjected to a 6 h phase advance of the light-dark cycle on the third day after phase advance. Again, the deceleration of entrainment was also manifested on clock protein levels within the SCN. Our data suggest that the endogenous melatonin signal facilitates reentrainment of the circadian system to phase advances on the level of the SCN molecular clockwork by acting upon MT2 receptors.

Kategorie: Lecture

Rubrik: 1.Main Topic I
Abstract Nr.:8

Titel:New light on the transcriptional basis of photoreceptor adaptation

Autoren: Kunst S.(1),Wengert A.(1),Grether M.(1),Hölter P.(1),Wolloscheck T.(1),Sticht C.(2),Wolfrum U.(3),Spessert R.(1),

Adressen:(1)University Medical Center of the Johannes Gutenberg University Mainz|Institute of Functional and Clinical Anatomy|Mainz|Germany; email:kunsts@uni-mainz.de; (2)Medical Faculty Mannheim, University of Heidelberg|Medical Research Centre|Mannheim|Germany; (3)Department of Cell and Matrix Biology, Johannes Gutenberg University,|Institute of Zoology|Mainz|Germany

Abstract:
Photoreceptor cells face the challenge of adapting their function to the more than 100-fold daily changes in ambient light intensity. The aim of the present study was to investigate the transcriptional basis of photoreceptor cell adaptation in the rat. For this, genes have been identified that display daily changes in the transcript amount in photoreceptor cells and retina, including those essential for visual processing and pathogenesis of photoreceptor cells. Daily dynamics of the genes were found to be attributable to two patterns: Cyclicity with peak expression during light phase (Zeitgeber time 9-12) directly driven by ambient illumination and cyclicity with peak expression during dark phase (Zeitgeber time 15-24) driven either directly by illumination or by the retinal clock which itself is entrained by light. The results of this study suggest that illumination- and clock-dependent control of gene transcription provides a basis for the adjustment of photoreceptor cell function to the environmental lighting conditions and may also influence the pathogenesis of photoreceptor cells.

Kategorie: Lecture

Rubrik: 1.Main Topic I
Abstract Nr.:9

Titel:The retinal clock drives the expression of kcnv2, a channel essential for visual function and cone survival

Autoren: Hölter P.(1),Kunst S.(1),Wolloscheck T.(1),Kelleher D.(1),Sticht C.(2),Wolfrum U.(3),Spessert R.(1),

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Abstract:
The gene Kcnv2 codes for the voltage-gated potassium channel subunit Kv8.2 which is a silent subunit as a homotetramer. Kv8.2 can co-assemble with Kv2.1 (Shab) subfamily members to constitute functional voltage-gated potassium channels. In the context of the retinal disorder “Cone dystrophy with supernormal rod response (CDSRR)”, mutations in the Kcnv2 gene lead to dystrophy of cones and disturbed visual processing. In this study transcript levels of Kcnv2 and Kv2.1 were found to display daily rhythms, with elevated values during night which were evident in preparations of the whole retina, photoreceptors isolated by laser microdissection and - consistent with the phylogenetic relationship between photoreceptors and pinealocytes – the pineal gland. The daily changes in retinal Kcnv2 and Kv2.1 mRNA levels are retained under constant darkness and therefore are driven by the endogenous retinal clock system which itself is entrained by light. The present data provide evidence that transcriptional regulation of Kcnv2 is a way through which the retinal clock system promotes the daily adaptation of visual function, and, possibly, survival of cones.

Kategorie: Lecture

Rubrik: 5.Experimental Morphology
Abstract Nr.:10

Titel:The clock genes period 2 and cryptochrome 2 differentially balance bone formation

Autoren: Maronde E.(1),

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Abstract:
Clock genes and their protein products regulate circadian rhythms in mammals but have also been implicated in various physiological processes, including bone formation. Osteoblasts build new mineralized bone whereas osteoclasts degrade it thereby balancing bone formation. To evaluate the contribution of clock components in this process, we investigated mice mutant in clock genes for a bone volume phenotype. We found that Per2Brdm1 mutant mice as well as mice lacking Cry2−/− displayed significantly increased bone volume at 12 weeks of age, when bone turnover is high. Per2Brdm1 mutant mice showed alterations in parameters specific for osteoblasts whereas mice lacking Cry2−/− displayed changes in osteoclast specific parameters. Interestingly, inactivation of both Per2 and Cry2 genes leads to normal bone volume as observed in wild type animals. Importantly, osteoclast parameters affected due to the lack of Cry2, remained at the level seen in the Cry2−/− mutants despite the simultaneous inactivation of Per2. This indicates that Cry2 and Per2 affect distinct pathways in the regulation of bone volume with Cry2 influencing mostly the osteoclastic cellular component of bone and Per2 acting on osteoblast Parameters.

Kategorie: Lecture

Rubrik: 9.Cell Biology
Abstract Nr.:11

Titel:Desmoglein 2 and 3 differently regulate p38mapk and keratinocyte cohesion

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

Adressen:(1)Institute of Anatomy and Cell Biology, Department 1|Ludwig-Maximilians University Munich|Munich|Germany; email:jens.waschke@med.uni-muenchen.de

Abstract:
Desmosomes provide intercellular adhesive strength required for integrity of epithelial and some non-epithelial tissues. Within the epidermis, the cadherin-type adhesion molecules desmoglein (Dsg) 1-4 and desmocollin 1-3 build the core of desmosomes. In keratinocytes, several isoforms of these proteins are co-expressed, however the contribution of specific isoforms to overall cell cohesion is unclear. In this study, we investigated the roles of Dsg2 and Dsg3, the latter of which is known to be essential for keratinocyte adhesion based on its autoantibody-induced loss of function in the autoimmune blistering skin disease pemphigus vulgaris (PV). The pathogenic PV antibody AK23 targeting the Dsg3 adhesive domain led to profound loss of cell cohesion. In contrast, a Dsg2 antibody also interfering with Dsg2 adhesion had no effect. SiRNA-mediated knockdown of Dsg3 induced loss of cell cohesion accompanied by activation of p38MAPK. Both effects were not detectable after Dsg2 knockdown. Interestingly, subsequent incubation with AK23 led to drastically enhanced keratinocyte cell dissociation and p38MAPK activation in Dsg2 knockdown cells. These experiments indicate that specific desmosomal cadherins contribute differently to cell adhesion and intracellular signaling in human keratinocytes. Furthermore, an additive mechanism of Dsg2 and Dsg3 for cell adhesion is likely. DFG SFB487

Kategorie: Lecture

Rubrik: 9.Cell Biology
Abstract Nr.:12

Titel:Regulation of connective tissue growth factor expression in human trabecular meshwork cells

Autoren: Fuchshofer R.(1),Kessel S.(1),Junglas B.(1),Tamm E.(1),

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Abstract:
Purpose: There is considerable evidence that CTGF is involved in the pathogenesis of glaucoma. CTGF is a key modulator of the trabecular meshwork (TM) actin cytoskeleton and of ECM synthesis. These alterations lead to an increase in intraocular pressure and to a loss of optic nerve axon in CTGF-overexpression-models. The aqueous humour of glaucoma patients contains higher amounts of CTGF. Molecular mechanisms that lead to a higher expression of CTGF are unknown. In this study, we analyzed various substances and stress conditions related to glaucoma on their capability to stimulate CTGF in TM cells in vitro.

Methods: TM cells were treated with Endothelin-1, Angiotensin-II, IGF or Latrunculin-A. Various stress conditions was simulated by H2O2-treatments and by heat-shock. Changes in the expression of CTGF were examined by real-time-PCR, western blotting and immunohistochemistry.

Results: IGF and Angiotensin-II treatment of HTM cells caused an increase of CTGF within 24h. Endothelin-1 showed a significant increase of CTGF after 3 days. Regarding stress conditions CTGF expression was increased by oxidative stress within 6h after treatment. By heat shock at 42°C CTGF was elevated within 30 min after incubation. Disruption of the actin cytoskeleton by Latrunculin-A led to a decrease of CTGF expression in HTM cells.

Conclusion: CTGF is induced by substances and stress conditions related to glaucoma in HTM cells. Together with the CTGF inducing effect of dexamethasone, TGF-beta1 and 2 these findings led to the assumption that CTGF is a central molecule in the pathogenesis of various forms of glaucoma.

Supported: DFG FOR 1075/TP3

Kategorie: Lecture

Rubrik: 9.Cell Biology
Abstract Nr.:13

Titel:Roles of spak and osr1 for the activation of renal distal na(k)cl-cotransporters by vasopressin

Autoren: Saritas T.(1),Paliege A.(1),McCormick J.(2),Borschewski A.(1),Dathe C.(1),Himmerkus N.(3),Bleich C.(3),Ellison D.(5),Mutig K.(1),Bachmann S.(1),

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Abstract:
The Na+,K+,2Cl- cotransporter (NKCC2) of thick ascending limb (TAL) and Na+,Cl-cotransporter (NCC) of distal convoluted tubule (DCT) are key controller of renal salt handling and are thus essential for the regulation of the arterial blood pressure. The hormone vasopressin (AVP) activates both transporter by phosphorylation. Recent studies suggested that SPAK (SPS-related proline/alanine-rich kinase) and OSR1 (oxidative stress responsive kinase 1) may be involved in the AVP-signaling. To evaluate the impact of these kinases on AVP-induced NKCC2 and NCC activation we stimulated SPAK-deficient (SPAK-/-) mice and AVP-deficient rats with a vasopressin analogue dDAVP and evaluated its effects on the SPAK/OSR1-NKCC2/NCC pathway. Immunohistochemical approach revealed co-localization of SPAK and OSR1 with NKCC2 and NCC. Interactions between the transporters and the kinases were confirmed by means of coimmunoprecipitation. Acute dDAVP administration induced parallel increases of SPAK/OSR1 (pS383/pS325; conserved regulatory domain), NKCC2 (pT96 and pT101), and NCC (pT53, pT58, pS71) phosphorylation levels in WT mice and DI rats indicating that either SPAK or OSR1, or both can be involved in AVP signaling. In SPAK-/- mice, dDAVP induced NKCC2 phosphorylation but failed to increase phospho-NCC level which indicates vital role of SPAK for activation of NCC. We conclude that SPAK is critically involved in the AVP signaling in DCT, whereas activation of NKCC2 in TAL is mediated by OSR1 or alternative kinase pathways.

Kategorie: Lecture

Rubrik: 5.Experimental Morphology
Abstract Nr.:14

Titel:Vasopressin activates soluble epoxide hydrolase in the renal medulla

Autoren: Röschel T.(1),Dathe C.(1),Andree C.(2),Schunck W.(2),Bachmann S.(1),Paliege A.(1),

Adressen:(1)Anatomy|Charité - Universitätsmedizin Berlin|Berlin|Germany; email:tom_roeschel@yahoo.de; (2)Genetics, Nephrology, Hypertension, and Vascular Injury|Max-Delbrück-Center for Molecular Medicine|Berlin|Germany

Abstract:
Epoxyeicosatrienoic acids (EETs) have been shown to exert an inhibitory effect on the type 2 Na-K-2Cl--cotransporter (NKCC2) in the thick ascending limb. Tissue levels of bioactive EETs are determined in part by the activity of soluble epoxide hydrolase 2 (sEH), which metabolizes EETs to their corresponding, less active, diols (DHETs). We here tested the hypothesis that vasopressin (AVP), a major regulator of NKCC2 activity, decreases EET levels within the outer medulla (OM) of AVP-deficient Brattleboro (DI) rats through activation of sEH.
To test this, adult DI rats were treated for 3 days with the V2 AVP receptor agonist desmopressin (dDAVP, 5ng/h; 3d) or its vehicle via osmotic minipump. OM EET- levels were measured by mass spectrometry, total and phosphorylated NKCC2 levels were measured by Western blot and sEH abundance was determined by quantitative real time PCR and Western blot. dDAVP treatment caused significant reductions in OM EET levels as compared to controls (-56±3% for 5,6-EET, -50±3.4% for 11,12-EET and -60±3.7% for 14,15-EET; $p < .05$ each). Concomitantly, OM NKCC2 and sEH protein levels were increased (+116±22%; +105±9.5%; $p < .05$ each). In summary, we have shown that an activation of AVP signaling causes an upregulation of sEH, which is accompanied by a reduction in tissue levels of free EET. Regulation of sEH expression and free EET levels may therefore be an important mechanism of AVP mediated urine concentration in the TAL.

Kategorie: Lecture

Rubrik: 9.Cell Biology
Abstract Nr.:15

Titel:Characterization of the ceacam expression and function in tumor derived microvesicles

Autoren: Singer B.(1),Muturi H.(1),Dreesen J.(1),Jastrow H.(1),Ergün S.(1),

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Abstract:
Normal and malignant cells are capable to release fragments of plasma membrane ranging from 100 nm to 1000 nm in diameter by shedding. These so called microvesicles (MVs) contain numerous proteins and lipids, which reflect the molecular content of the cells from which they originated. Tumor cells release MVs in response to stress, e.g. lack of nutrition or hypoxia. MVs play a role in intercellular communication and can deliver mRNA and proteins. They have been implicated in the process of tumor-mediated immune suppression, metastasis, tumor-stroma interactions and angiogenesis. Interestingly, CEACAMs, which represent a molecule family of cell-cell adhesion receptors, were described to regulate similar cellular processes as MVs. Therefore, it was obvious to speculate that CEACAMs are present on MVs. In this study we identified for the first time by flow cytometry, western blot and sandwich-ELISA that MVs derived from human epithelial tumor cells contain substantial amounts of CEACAM1, CEACAM5 and CEACAM6. Furthermore, MVs derived from human and murine endothelial cells revealed CEACAM1 expression as well. In addition we present data showing that CEACAMs were at least partially responsible for alterated T-cell function induced by MVs. Based on these results, we assume that the presence of CEACAM1 on tumor-derived MVs plays an important role in tumor progression, metastasis and angiogenesis.

Kategorie: Lecture

Rubrik: 9.Cell Biology
Abstract Nr.:16

Titel:Elyra: superresolution microscopy by carl zeiss

Autoren: Paysan J.(1),

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Abstract:
Superresolution microscopy today describes a sub-set of methodologies within the field of fluorescence microscopy, which overcome the resolution limit stipulated by Ernst Abbe for conventional optical microscopes in the 1870s. In simple words \"superresolution\" means \"resolution better than confocal\". There is a broad variety of techniques that can be included into the superresolution family, among these structured illumination microscopy (SIM) and photoactivation localization microscopy (PALM). While substantial improvement of spatial resolution (from around 100 nm down to about 10 nm) can be achieved with the aforementioned techniques, one has to consider also application oriented aspects. The challenge is therefore to provide both, improved resolution and the capability to examine conventional specimens - for example in 3D and without the necessity to radically rethink sample preparation and staining. ELYRA is Carl Zeiss\' superresolution platform based on structured illumination microscopy (SIM) and/or localization microscopy (PALM and dSTORM). In this presentation we review of the underlying technology and introduce the modular concept of the ELYRA system.

Kategorie: Lecture

Rubrik: 12.Reproductive Biology
Abstract Nr.:17

Titel:Reelin synthesis in the reproductive tract

Autoren: Pröls F.(1),Schmalhaus C.(1),Zhou L.(1),Kruse C.(1),Morcinek K.(2),Rune G.(1),

Adressen:(1)Inst. f. Neuroanatomie|Universitätsklinikum Eppendorf|Hamburg|Germany; email:fproels@uke.de; (2)Zentrum Anatomie|Universität Köln|Köln|Germany

Abstract:
Reeler mutant mice and aromatase knockout mice exhibit comparable phenotypes: an impaired hippocampal synaptogenesis and impaired fertility. Increasing data reveal an interdependency of reelin and estrogen synthesis. The impaired fertility in reeler mice could be based on either an impaired hormonal hypothalamus-hypophyses-gonade-axis, due to limited migration of GnRH neurons, or on a direct reelin-dependent defect in sperm or oocyte maturation. Histological investigation of the testis showed a reduced density of dilated tubuli seminiferi in the reeler mice (Cariboni et al., 2005) while no anatomical defect could be detected in epidydimis or ovaries. Prerequisite for a direct reelin-aromatase interaction is the presence of reelin in the gonadal tract. We, therefore, investigated whether reelin protein is synthesized in the gonadal tract of wild type mice. PCR analyses revealed that reelin is synthesized in testis, epididymis, and ovaries. Elevated amounts of reelin transcripts are present in epidydimis when compared to testis or ovary-RNA contents. These RNA data correlated with and could be confirmed by Western blot analyses indicating that gonadal reelin could control fertility. In accordance with this is the finding that reeler mice exhibit an irregular ovarial cycle and a significant reduction in aromatase activity.

Kategorie: Lecture

Rubrik: 12.Reproductive Biology
Abstract Nr.:18

Titel:Can the activation of nrf2 help in preeclampsia?

Autoren: Kweider N.(1),Fragoulis A.(1),Rosen C.(1),Pecks U.(2),Rath W.(2),Pufe T.(1),Wruck C.(1),

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Abstract:
Vascular Endothelial Growth Factor is implicated in placental oxidative stress during preeclampsia. However, little is known how a decrease in VEGF levels augments placental oxidative stress. The principal aim of this work is to investigate the interplay between VEGF and Nuclear factor erythroid 2-related factor-2 (Nrf2), the main regulating factor of the intracellular redox balance. The second objective is to study whether the activation of Nrf2 which, leads to an increase in cellular carbon monoxide, raises VEGF levels.
Material and Methods: This study took place in vitro, the choriocarcinoma cell line BeWo cells were used to study the relationship between VEGF and Nrf2. ELISA, Western blot Assay and the Dual Luciferase Assay were both mainly applied for protein and Nrf2 activity analysis.
Results: our investigations revealed that VEGF165 (10ng/ml) activates Nrf2 in an ERK1/2 dependent manner. Nrf2, in turn, activates the production of antioxidative enzymes thioredoxin, thioredoxin reductase and heme oxygenase-1. The upregulation of HO-1 expression augmented the production of carbon monoxide, which in turn up-regulated the gene expression of VEGF.
In conclusion, VEGF induces the Nrf2 pathway in oder to upregulate the production of antioxidative enzymes. In particular, the Nrf2 driven HO-1 expression elevates the levels of VEGF via carbon monoxide production. Ultimately, the vicious cycle caused by a drop in VEGF levels can be broken by activating Nrf2, in particular, via sulforaphane, a naturally occurring compound derived from broccoli.

Kategorie: Lecture

Rubrik: 12.Reproductive Biology
Abstract Nr.:19

Titel:Lateral cell-cell adhesion of endometrial epithelial cells - establishment of in vitro systems for the examination of hormone-dependent redistribution of junctional complexes and interaction with trophoblast cells

Autoren: Buck V.(1),Flensberg F.(1),Leube R.(1),Hombach-Klonisch S.(2),Gellersen B.(3),Classen-Linke I.(1),

Adressen:(1)Institute of Molecular and Cellular Anatomy|Medical Faculty RWTH Aachen University|Aachen|Germany; email:vbuck@ukaachen.de; (2)Depts. Human Anatomy and Cell Science & Obstetrics, Gynecology & Reproductive Sciences|Faculty of Medicine, University of Manitoba|Manitoba, Winnipeg|Canada; (3)Endokrinologikum|Endokrinologikum Hamburg|Hamburg|Germany

Abstract:
Luminal and glandular endometrial epithelial cells (EECs) form the first barrier for the invading trophoblast in early human implantation. During the 'implantation window' of the menstrual cycle a redistribution of lateral junctional complexes (desmosomes and adherens junctions) was observed in glandular epithelial cells of endometrial tissue samples. This change may have an impact on trophoblast adhesion.
The aims of this study were to establish a 3D in vitro model for the examination of junctional rearrangement in polarised endometrial cells in response to cycle-dependent steroid hormones and to use an endometrial cell-trophoblast coculture system for the analysis of their interaction in vitro.
The 3 D culture system was successfully established by growing the human endometrial epithelial cell line hTERT-EEC in a 3D Matrigel matrix. This resulted in formation of spheroids with internal lumina. Apicobasal polarisation of the surrounding cells could be demonstrated by subapical localisation of the tight junction protein ZO-1 using immunofluorescence and confocal microscopy. These 3D spheroids can now be used for studying steroid hormone regulated effects on EEC junction dynamics and distribution.
Coculture experiments were performed with the human endometrial cell line hTERT-EEC and the human hybridoma cell line AC-1M88, which is derived from primary extravillous trophoblast cells fused with JEG-3 cells. Formation of desmosomes could be observed between epithelial and trophoblast cells by desmoplakin and desmoglein 2 immunofluorescence staining.
In conclusion, the newly developed tools will help to examine hormone- and trophoblast-dependent changes in EEC differentiation at the molecular level.

Kategorie: Lecture

Rubrik: 12.Reproductive Biology
Abstract Nr.:20

Titel:Regulation of permeability of the epididymal epithelium by tgf-beta

Autoren: Stammler A.(1),Konrad L.(2),Wittlif L.(1),Thong A.(1),Middendorff R.(1),

Adressen:(1)Institute of Anatomy and Cell Biology|Justus-Liebig-University|Giessen|Germany; (2)Department of Obstetrics and Gynecology|Justus-Liebig-University|Giessen|Germany

Abstract:
The epididymis mediates transport, maturation and protection of sperm in the luminal liquid. Junctional proteins in the epididymal epithelium form a barrier, the blood epididymis barrier (BEB), which protects sperm during storage and maturation. On the other hand, selective permeability of the epithelium is essential for controling the milieu in the luminal liquid. Interruption of the BEB by e.g. inflammation can disturb the epididymal milieu, which may result in infertility.
We analyzed the localization of junctional proteins in the epididymal epithelium. Further, we developed an in vitro model of epididymal epithelium and analyzed the permeability with transepithelial electrical resistance and tracer diffusion. Lipopolysaccharides (LPS) located in the outer membrane of Gram-negative bacteria were used to simulate bacterial infections. For comparison, possible effects of C-type natriuretic peptide (CNP), which is found in extremely high concentrations in the luminal liquid of the epididymis, were analyzed.
In vitro, the BEB barrier was strongly disturbed after addition of TGFbetas, thus demonstrating the involvement in regulating the permeability of epididymal epithelium. Additionally, dramatic changes in the distribution of junctional proteins were found. These junctional proteins are also present in the epididymal epithelium of mouse and man. Application of LPS modestly increased the permeability of the BEB. In contrast, preliminary data suggest that CNP, whose receptor, the cGMP-generating guanylyl cyclase B, was found in epithelial cells, may contribute to sealing of the epididymal epithelium.
Our data suggest that TGFbetas are important factors for regulation of BEB permeability, thereby possibly mediating bacterial effects.

Kategorie: Lecture

Rubrik: 8.Neuroregeneration/Neurodegeneration
Abstract Nr.:21

Titel:Role of nrf2 in the aging brain

Autoren: Fragoulis A.(1),Henschenmacher B.(2),Rosen C.(1),Siegl S.(3),Zhou L.(4),Rune G.(4),Pufe T.(1),Wruck C.(1),

Adressen:(1)Department of Anatomy and Cell Biology|Medical Faculty, RWTH Aachen University|Aachen|Germany; email:afragoulis@ukaachen.de; (2)Department of Anatomy and Cell Biologym|Medical Faculty, RWTH Aachen University|Aachen|Germany; (3)Department of Pharmacology and Toxicology|Medical Faculty, RWTH Aachen University|Aachen|Germany; (4)Department of Neuroanatomy|University Medical Center Hamburg-Eppendorf|Hamburg|Germany

Abstract:
Background: Oxidative stress has been implicated to provoke a decline of neuronal vitality in the aging brain. 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 genes coding for antioxidative and detoxifying enzymes. The aim of this study was to test the hypothesis that a deletion of Nrf2 leads to excessive oxidative stress and accelerated senescence in the brain.
Methods: To investigate whether Nrf2 plays a role in aging, we used Nrf2 wild type and knockout mice. These mice were examined twice with an age of 12 and 18 months in behavioural studies like Open-Field, Y Maze and Morris Watermaze to test behaviour and spatial memory. 12 months old Nrf2-WT and KO mice brain sections were used in transmission electron microscopy analysis of dendritic spine synapse density in the hippocampal CA1 region. Furthermore, brain tissue was analysed by immunohistochemistry and biochemical analysis.
Results: Spine synapse density and behavioural studies showed no significant differences between 12 months old Nrf2 WT and KO mice. 6 months later, significant differences were found in the Morris-Watermaze between Nrf2 WT and Nrf2 KO. 18 month old Nrf2 WT mice showed a 40% reduction of cognitive performance. Surprisingly, 18 month old Nrf2 KO mice were protected against this reduction of cognitive performance.
Conclusion: Against our initial hypothesis, we showed that Nrf2 depletion seems to protect against age-related decline in cognitive abilities.

Kategorie: Lecture

Rubrik: 8.Neuroregeneration/Neurodegeneration
Abstract Nr.:22

Titel:Deletion of a single allele of the pex11beta gene is sufficient to cause oxidative stress, delayed differentiation and neuronal death in the mouse brain.

Autoren: Ahlemeyer B.(1),Gottwald M.(1),Baumgart-Vogt E.(1),

Adressen:(1)Division of Medical Cell Biology|Institute for Anatomy und Cell Biology II|Giessen|Germany; email:Barbara.Ahlemeyer@anatomie.med.uni-giessen.de

Abstract:
Peroxisomal biogenesis disorders (PBDs) are caused by mutations in at least 12 different PEX genes and are inherited in an autosomal recessive manner. PBD patients show developmental and metabolic disturbances predominantly in the liver, kidney and brain. The clinical phenotype varies widely with Zellweger syndrome being the severest form where the patients die within the first year of life. In primary neuronal cultures from the neocortex of Pex11beta-deficient E19 mice we observed that the deletion of a single allele caused cell death although to lesser extent as compared to the homozygous animals. In corresponding brain sections, cell death was rare, but differences between the genotypes were similar as found in vitro. Similarly, neuronal development was delayed in heterozygous and to a further extent in homozygous mice as measured by changes in the levels of synaptophysin and MAP2. Moreover, oxidative stress increased gradually in brain sections and primary neuronal cultures from wild-type to heterozygous to homozygous Pex11beta-deficient mice. SOD2 was upregulated in neurons from heterozygous, but not from homozygous Pex11beta animals. Catalase remained unchanged in neurons from heterozygous and was reduced in those from homozygous Pex11beta mice, suggesting a compensation of oxidative stress in the heterozygous, but not in homozygous Pex11beta brain. In conclusion, we report for the first time about alterations in the brain caused by the deletion of a single allele of the Pex11beta gene. Our data may lead to reconsider PBD clinic and the use of knockout mice for studying autosomal recessive diseases.

Kategorie: Lecture

Rubrik: 8.Neuroregeneration/Neurodegeneration
Abstract Nr.:23

Titel:Degeneration of the cerebellum in huntington`s disease

Autoren: Rüb U.(1),Seidel K.(1),Korf H.(1),

Adressen:(1)Dr. Senckenberg Chronomedical Institute|Goethe University|Frankfurt|Germany; email:Drueb@gmx.de

Abstract:

Huntington's disease (HD) is an autosomal dominantly inherited progressive polyglutamine or CAG-repeat disease, which is characterized neuropathologically by severe degeneration of the striatum and laminar nerve cell loss in the neo-and allocortex. Since the cerebellum is among the extra-striatal brain sites whose neuropathological state in and relevance for the clinical picture of HD is still controversial we performed a systematical study of the cerebellum of eight clinically diagnosed and genetically confirmed HD patients. This comprehensive study revealed a considerable atrophy of the cerebellum in all HD patients studied, as well as a consistent loss of Purkinje cells and nerve cells of the fastigial, globose, emboliform and dentate nuclei. This cerebellar pathology was already present in HD brains assigned Vonsattel grade 2 striatal atrophy and did not correlate with the extent and distribution of striatal atrophy. Therefore, our findings suggest (1) that the cerebellum degenerates early during HD, (2) and independently from the striatal atrophy, and (3) that the onset of the pathological process of HD is polytopic or multifocal. Degeneration of the cerebellum might be the cause of poorly understood symptoms occurring in HD such as impaired rapid alternating movements and fine motor skills, dysarthria, ataxia and postural instability, gait and stance imbalance, broad-based gait and stance, while the morphological alterations observed in the majority of surviving nerve cells may represent a gateway to the unknown mechanisms of the pathological process underlying HD.

Kategorie: Lecture

Rubrik: 8.Neuroregeneration/neurodegeneration
Abstract Nr.:24

Titel:Long-term characterization of the 6-hydroxydopamine hemiparkinson mouse-model

Autoren: Rudolph A.(1),Schmitt O.(1),Wree A.(1),Haas S.(1),

Adressen:(1)Institute of Anatomy|Medical Faculty/University of Rostock|Rostock|Germany; email:stefan.haas@uni-rostock.de

Abstract:
A mouse model of Parkinson's disease (PD) by transferring the 6-OHDA-injection protocol from rats to mice offers the opportunity to use knockouts or transgenic mice and study the interactions between genes of interest and toxins in relation to PD etiology. Several groups published the successful establishment of this model. However, until today no long time observation has been described in mice. Adult male C57BL/6-mice were lesioned by stereotactic 6-OHDA-injection into the right medial forebrain bundle. Six and 13 months after lesion apomorphine-induced rotations were evaluated and forepaw preferences (cylinder test) of lesioned mice were compared with intact controls. Then, mice were perfused with 3.7% PFA and immunohistochemistry to visualize tyrosine hydroxylase (TH) immunoreactive neurons in brain sections was performed. Lesioned mice showed a robust contralateral rotation behavior over time. Six months and 13 months postlesion mice performed 11.61 (±0.65) and 12.61 (±1.1) rotations/min. This is significantly different when compared to intact controls that exhibited only 0.2 (±0.2) rotations/min. However, lesioned mice did not use their contralateral forepaws significantly less than compared to intact mice. Stereological evaluation of TH-immunoreactive neurons revealed a 89.7% unilateral dopaminergic cell loss in the substantia nigra and a dopaminergic deafferentiation, resulting in loss of TH-ir nerve terminals, in the ipsilateral striatum. However, in animals lesioned 13 months earlier we observed about 7942 (±619) TH-containing neurons in the dopaminergic deafferentiated striatum. This is in contrast to findings of other groups that observed a decrease of those, directly after lesion appearing, TH-immunoreactive striatal neurons about 7 weeks postlesion.

Kategorie: Lecture

Rubrik: 8.Neuroregeneration/Neurodegeneration
Abstract Nr.:25

Titel:Pacap-deficiency extends survival and causes a switch of microglial phenotype in the sod1(g93a) mouse model of amyotrophic lateral sclerosis

Autoren: Schütz B.(1),Ringer C.(2),Eiden L.(3),Weihe E.(2),

Adressen:(1)Institut of Anatomy and Cell Biology|Philipps-University|Marburg|Germany; email:schuetzb@staff.uni-marburg.de; (2)Institute of Anatomy and Cell Biology|Philipps-University|Marburg|Germany; (3)Laboratory on Cellular and Molecular Regulation|National Institute of Mental Health|Bethesda, Maryland|United States of America

Abstract:
Introduction: PACAP is expressed in specific neuronal circuits throughout the CNS, including some spinal and brain stem motor neurons. PACAP has pleiotropic cytoprotective and immune regulatory actions. In particular, PACAP has been found to protect rat motor neurons against glutamate-induced excitotoxicity in vitro, a mechanism discussed to cause amyotrophic lateral sclerosis (ALS). Therefore, we hypothesized a possible role of PACAP in ALS and investigated the expression pattern of PACAP and the effect of PACAP deficiency in the SOD1(G93A) mouse model of ALS.
Results: While PACAP mRNA and peptide were absent from wildtype motor neurons, an induction of PACAP expression was detected in a minor subset of SOD1(G93A) spinal and brainstem motor neurons, starting at around postnatal day (P) 60. Surprisingly, SOD1(G93A):PACAP-/- mice showed extended survival compared to SOD1(G93A):PACAP+/+ mice (+7,5 days, p = 0.002). While no differences in body weight and paw grip endurance could be observed between the two groups, loss of licking performance was delayed in SOD1(G93A):PACAP-/- mice (mean onsets: P105 vs. P112). On the morphological level, no significant differences in total motor neuron loss, astrocyte activation, and lymphocyte infiltration into brain parenchyma were evident. However, in SOD1(G93A):PACAP-/- mice, microglia was numerically increased and exhibited amoeboid morphology as compared to hypertrophic morphology in SOD1(G93A):PACAP+/+ mice.
Conclusions: Our analyses indicate that endogenous PACAP may further disease progression in the SOD1(G93A) mouse model of ALS, and that PACAP deficiency-dependent disease prolongation may be at least in part related to a switch in microglial phenotype.

Kategorie: Lecture

Rubrik: 2.Main Topic II
Abstract Nr.:26

Titel:Nuclear calcium signaling in synapse-to-nucleus communication and neuronal adaptations

Autoren: Bading H.(1),

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Abstract:
Changes in the concentration of intracellular calcium as a result of synaptic activity control virtually all adaptive responses in the adult nervous system, including neuronal survival and memory formation. Most activity-induced persistent adaptations are initiated by synaptic NMDA receptors and require changes in gene expression. The principal mediator in the dialogue between the synapse and the nucleus is calcium itself. Activity-induced calcium transients can propagate towards the cell soma and enter the cell nucleus. Nuclear calcium is a potent activator of neuronal gene expression and cntrols the expression of nearly 200 genes, many of which are CREB targets. Our studies of both acquired neuroprotection and memory formation revealed that nuclear calcium acts as a common regulator of many adaptive processes in the nervous system. The ability of nuclear calcium to promote gene expression is antagonized by another calcium signaling pathway that is stimulated by calcium entry through NMDA receptors localized outside synaptic contacts; extrasynaptic NMDA receptors couple to CREB shut-off and cell death pathways. Thus, the decision whether a neuron survives (and undergoes plasticity) or dies after glutamate exposure is dependent on the location of the NMDA receptor activated. This concept of differential signaling induced by synaptic NMDA receptor-nuclear calcium versus extrasynaptic NMDA receptors has implications for the understanding and treatment of disease- and ageing-related neurodegeneration and cognitive decline, which may be caused by a reduction in synaptic activity, malfunctioning of calcium signaling towards and within the nucleus (‘nuclear calciopathy’), or increases in death signaling by extrasynaptic NMDA receptors.

Kategorie: Lecture

Rubrik: 2.Main Topic II
Abstract Nr.:27

Titel:Coding olfaction

Autoren: Mombaerts P.(1),

Adressen:(1).|Max Planck Institute of Biophysics|Frankfurt am Main|Germany; email:peter.mombaerts@biophys.mpg.de

Abstract:
Mammalian olfactory perception is based on interaction of odorants with odorant receptors, which are G-protein coupled receptors. In the mouse the odorant receptor gene repertoire consists of ~1,200 genes and is by far the largest gene family in its genome. Each olfactory sensory neuron in the main olfactory epithelium is thought to express one allele of just one odorant receptor gene. Axons of neurons that express the same odorant receptor coalesce within the olfactory bulb into discrete neuropil structures called glomeruli, of which there are 3,600 per bulb. Odorant receptor proteins are critical determinants of axonal coalescence into glomeruli. I will summarize our progress in understanding the mechanisms of odorant receptor gene choice and axonal wiring.

Kategorie: Lecture

Rubrik: 2.Main Topic II
Abstract Nr.:28

Titel:Remodeling of hippocampal mossy fiber synapses as revealed by high-pressure freezing

Autoren: Frotscher M.(1),Studer D.(2),Graber W.(2),Young C.(3),Zhao S.(1),

Adressen:(1)Institute for Structural Neurobiology|Center for Molecular Neurobiology Hamburg|Hamburg|Germany; email:Michael.Frotscher@zmnh.uni-hamburg.de; (2)Institute of Anatomy|University of Bern|Bern|Switzerland; (3)Institute of Anatomy and Cell Biology|University of Freiburg|Freiburg|Germany

Abstract:
Despite recent progress in fluorescence microscopy techniques, electron microscopy (EM) is still superior in the simultaneous analysis of all tissue components at high resolution. However, it is unclear to what extent conventional fixation and dehydration for EM using aldehydes and ascending series of ethanol, respectively, result in tissue alteration. Here, we made an attempt to minimize tissue alteration by using rapid high-pressure freezing (HPF) of hippocampal slice cultures. We used this approach to monitor fine-structural changes at hippocampal mossy fiber synapses associated with chemically induced long-term potentiation (LTP). Synaptic plasticity in LTP has been known to involve structural remodeling at synapses including reorganization of the actin cytoskeleton and de novo formation of spines. While LTP-induced formation and growth of postsynaptic spines have been reported, little is known about associated structural changes in presynaptic boutons. Mossy fiber synapses are assumed to exhibit presynaptic LTP expression and are easily identified by EM. In slice cultures from wild-type mice, we found that chemically induced LTP increased the length of the presynaptic membrane of mossy fiber boutons, associated with a de novo formation of small spines and an increase in the number of active zones. Of note, these changes were not observed in slice cultures from Munc13-1 knock-out mutants exhibiting defective vesicle priming. These findings show that activation of hippocampal mossy fibers induces remodeling of pre- and postsynaptic structures of mossy fiber synapses that can be monitored by EM.

Kategorie: Lecture

Rubrik: 2.Main Topic II
Abstract Nr.:29

Titel:Sphingosine-1-phophate receptor signaling regulates denervation-induced dendritic remodeling of dentate granule cells in mouse hippocampal slice cultures

Autoren: Vlachos A.(1),Willems L.(1),Zahn N.(1),Scholich K.(2),Deller T.(1),

Adressen:(1)Institute of Clinical Neuroanatomy|Dr. Senckenberg Anatomy|Goethe-University Frankfurt|Frankfurt am Main|Germany; email:a.vlachos@med.uni-frankfurt.de; (2)Institute of Clinical Pharmacology|Goethe-University Frankfurt|Frankfurt am Main|Germany

Abstract:
Denervation-induced plasticity is a form of neuronal plasticity which is of particular interest in the context of neurological disease. Since neurons are highly interconnected cells the loss of a given neuronal population will subsequently lead to a loss of afferent innervation in remote areas of the brain, and thus to the denervation of target neurons. Structural and functional remodeling in these denervated areas may contribute to the progression of the disease and/or lead to disease-associated long-term complications. Although this form of plasticity has been described many years ago the molecular mechanisms underlying denervation-induced structural and functional remodeling remain unclear. Here, we have studied denervation-induced dendritic reorganization following entorhinal deafferentation of hippocampal granule cells in organotypic slice cultures of Thy1-GFP mice. Time-lapse imaging revealed a progressive reduction in total dendritic length until the end of week 2 after denervation, followed by a gradual recovery. Strikingly, treatment of denervated dentate granule cells with fingolimod (FTY720), a modulator of the sphingosine-1-phosphate receptors recently approved for the treatment of multiple sclerosis, prevented the loss of granule cell dendrites in our slice cultures. To verify this finding, we also tested the specific sphingosine-1-phosphate receptor antagonist VPC23019 and obtained similar results. Taken together, these data suggest that sphingosine-1-phosphate receptor signaling is an important component of the molecular machinery which regulates transneuronal dendrite loss after denervation.
(Supported by LOEWE LiFF).

Kategorie: Lecture

Rubrik:
Abstract Nr.:30

Titel:Mutations in the plasticity related gene PRG1 contribute to seizure susceptibility and modify the epilepsy phenotype

Autoren: Vogt J.(1), Knierim E.(2,3), Schuelke M.(2,3,5), Schmitz D.(4,5), Nitsch R.(1), Mainz, Berlin (Germany)

Adressen: (1) Institute for Microanatomy and Neurobiology|University Medical Center| Mainz| Germany; (2) Neurocure Clinical Research Center (NCRC); (3) Department of Neuropediatrics; (4) Neuroscience Research Center (NWFZ); (5) Cluster of Excellence NeuroCure|Charité – Universitätsmedizin Berlin|Berlin|Germany

Abstract:
Plasticity related gene 1 (PRG-1) is a postsynaptic membrane protein which homeostatically regulates excitatory transmission in glutamatergic neurons to physiologic values. Loss of PRG-1 results in generalized spike-wave discharges and slowing of background rhythm in the EEG leading to epileptic seizures in mice. The level of PRG-1 expression is gene-dose dependent and correlates with its regulatory activity since heterozygous deletion of *PRG1* increased the susceptibility for seizures in a kainate-model of epilepsy in mice. In a child with infantile spasms (West syndrome) we identified a heterozygous c.896C>G (p.T299S) mutation in the highly conserved third extracellular phosphatase domain of PRG1. A second mutation (c.1034G>C, p.R345T), which is located in the intracellular domain of PRG1, was shown to aggravate the phenotype of benign familial infantile seizures (BFIS). Neuronal reconstitution via i*n utero* electroporation in *Prg1*-knockout animals revealed that these mutations abolished the PRG-1 function as they, in contrast to the wild-type protein, were not able to rescue the knockout phenotype. The p.R345T mutation has a population frequency of 7.7/1,000 and was only found in individuals from Middle Europe. The influence of *PRG1* mutations on epileptogenesis reveals the presence of novel molecular pathways that would be amenable to pharmacologic intervention.

Kategorie: Lecture

Rubrik: 6.Neuroanatomy/Neurobiology
Abstract Nr.:31

Titel:Gnrh regulates hippocampal aromatase expression

Autoren: Prange-Kiel J.(1),Zheng W.(1),Halvorson L.(1),

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Abstract:
Previously, we demonstrated that gonadotropin-releasing hormone (GnRH) regulates estradiol synthesis in hippocampal neurons. Via this mechanism GnRH influences synaptic plasticity in this brain region. Here, we aim at understanding the underlying pathways by which GnRH influences estradiol synthesis. Our studies are focused on aromatase, the key enzyme in estradiol synthesis.
In the hippocampi of postnatal male and female rats, we observed a simultaneous increase in the expression of GnRH-receptor mRNA and aromatase mRNA as measured by quantitative RT-PCR. Expression levels were highest in animals at the onset of puberty and relatively low in adult animals. The treatment of hippocampal neuronal culture with GnRH resulted in a significant increase in the expression of aromatase mRNA and protein, indicating that the previously observed GnRH-induced increase in estradiol synthesis is, at least partially, mediated by an increase in aromatase expression. Interestingly, we were able to show that hippocampal neurons also express luteinizing hormone (LH)-receptors. GnRH is known to induce the synthesis and release of LH from the pituitary; LH, in turn, regulates ovarian aromatase expression. In subsequent experiments we demonstrated that treatment of hippocampal neurons with LH results in an increase in aromatase expression. Based on our data we hypothesize that GnRH regulates hippocampal estradiol synthesis by influencing the LH pathway and thereby increasing aromatase expression.

Kategorie: Lecture

Rubrik: 6.Neuroanatomy/Neurobiology
Abstract Nr.:32

Titel:Reelin induces branching of neurons and radial glial cells during corticogenesis

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Abstract:
The mammalian brain is formed through a series of intricately orchestrated events whereby neurons born in germinal zones migrate for long distances to reach their final positions and form specific connections. The postmitotic neurons use the radial glial fibers as scaffold for their migration during brain development. It has been established that the Reelin signaling pathway is crucial for neuronal migration and positioning during corticogenesis. However, the mechanism of Reelin action on migrating neurons has remained elusive. Here we successfully visualized individual migrating neurons and radial glial cells using in utero electroporation with a pCAGGFP plasmid transfected at embryonic day 14.5 (E14.5) and E17.5, respectively. Combined with immunocytochemistry against Reelin, we demonstrate that intensive branching of migrating neurons and radial glial cells was closely correlated spatiotemporally with the distribution of endogenous Reelin. Furthermore, our data show that the apical dendritic complexity increases gradually, characterized by longer total branch length and more bifurcations, as the soma approaches the Reelin-rich marginal zone and the residual leading process becomes shorter. Our results suggest that Reelin induces branching of the leading processes of migrating neurons, as well as the apical processes of radial glial cells, which may favor the terminal somal translocation and contribute to the arrest of migrating neurons and correct neuronal positioning.

Kategorie: Lecture

Rubrik: 6.Neuroanatomy/Neurobiology
Abstract Nr.:33

Titel:Functional integration of newly formed granule cells in the adult hippocampus

Autoren: Jungenitz T.(1),Al-Qaisi O.(1),Jedlicka P.(1),Deller T.(1),Schwarzacher S.(1),

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Abstract:
Adult neurogenesis of dentate gyrus granule cells has been implicated in hippocampal forms of learning and memory. In the present study, we analyzed at which stage of structural maturation and age newly formed granule cells are functionally integrated into the existing adult dentate gyrus network.

High frequency stimulation (HFS) of the perforant pathway was performed in urethane anesthetized rats. Following HFS, almost 100% of mature, Calbindin-positive granule cells were labeled with the immediate early genes c-Fos, Arc, zif268 and pCREB133. Maturing granule cells were detected with Doublecortin (DCX). Unexpectedly, both c-Fos and Arc were absent in immature DCX-positive granule cells, whereas zif268 was upregulated in only a subset of immature neurons after HFS. The expression of zif268 correlated with the stage of dendritic growth in immature neurons, revealing prominent staining after granule cell dendrites had reached the outer molecular layer, the termination zone of the perforant pathway afferents. pCREB133 was strongly expressed in mature granule cells following HFS, but again was not upregulated in immature neurons.

Following injections with BrdU to label mitotic cells, we found an increasing synaptic integration of maturing granule cells, starting with 21 days of age, but a lack of ability to respond to synaptic activation with expression of factors important for synaptic plasticity, as long as the cells are DCX-positive. Expression of markers both for maturation and synaptic activation closely correspond to age and structural maturation of granule cells.

Kategorie: Lecture

Rubrik: 2.Main Topic II
Abstract Nr.:34

Titel:An in vitro model for scar formation to study the mechanisms of scar-reducing treatments used in spinal cord injury

Autoren: Vogelaar C.(1),Krafft S.(2),Ziegler B.(3),Müller H.(3),

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Abstract:
Spinal cord injury leads to permanent damage of axon tracts and impairment of sensory and motor functions. Lesion-induced fibrous scarring is considered a major impediment for regeneration of injured axons in the CNS. The collagen-rich basement membrane acts as a scaffold to axon growth inhibitory factors, including chondroitin sulphate proteoglycans (CSPGs), semaphorins (Sema), and ephrins. In our laboratory a pharmacological treatment was developed, transiently suppressing fibrous scarring, leading to improvements in regeneration (Klapka et al, 2005). This “anti-scarring treatment” (AST) consists of an iron chelator and cyclic AMP, inhibiting collagen synthesis by invading fibroblasts. In order to study the molecular mechanisms of AST we used an in vitro model for scar formation. In this model, fibroblasts and astrocytes in co-culture form scar-like clusters after addition of TGF-beta1. Clusters were mainly formed by fibroblast proliferation. Live cell imaging showed that cluster formation also involved reorganization of the existing fibroblast layer and scar-like contraction of the cluster. Immunohistochemical analysis showed that mature clusters contain extracellular matrix (ECM) molecules and growth inhibitory proteins, like various CSPGs (NG2, neurocan, phosphacan, CS-56), sema-3A, and tenascin-C. Cultivation of cortical neurons in this scar model revealed that axon growth was reduced on the clusters. We are currently using this scar formation model to test the scar-reducing properties and mechanisms of AST and related scar-reducing treatments. We are studying various scar-reduction mechanisms, like the effects of treatment on proliferation, apoptosis, migration, inhibitor expression and axon growth.

Kategorie: Lecture

Rubrik: 10.Developmental Biology
Abstract Nr.:35

Titel:Vagus ganglion controls the ventral projection of vagus and accessory axons

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Abstract:
The accessory nerve (the XI cranial nerve) is a pure motor nerve. It displays a unique organization in that its axons exit along an extended region of the cervical spinal cord and turn then cranially, ascendant along the developing spinal cord. At the level of the medulla oblongata, they assemble into the likewise longitudinally oriented vagus axons (the X cranial nerve). Axons of both nerves bend ventrally to the periphery at the first somite level. Little is known about how this organization is achieved. Based on the observation that the vagus nerve is associated with a sensory ganglion, we investigated the function of the vagus ganglion regarding the axonal guidance of these both nerves. After ablation of the developing vagus ganglion, the common outlet of vagus and accessory axons at the first somite level was disappear. Transplantations of the vagus ganglion anlagen to the place of the third or fourth somite led to an additional ventral projection of axons. Our results reveal that the ventral turn of the vagus and accessory axon is guided by the vagus sensory ganglion.

Kategorie: Lecture

Rubrik: 1.Main Topic I
Abstract Nr.:36

Titel:Post-pneumonectomy lung tissue growth and vessel morphogenesis

Autoren: Houdek J.(1),Ackermann M.(1),Gibney B.(2),Chamoto K.(2),Tsuda A.(3),Mentzer S.(2),Konerding M.(1),

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Abstract:
Purpose. Lung growth after pneumonectomy has been observed in many mammalian species; nonetheless, the pattern and morphology of alveolar angiogenesis during compensatory growth is unknown. Here, we investigated alveolar angiogenesis in a murine model of post-pneumonectomy lung growth.

Methods. C57/B6 mice were used in all experiments. Pneumonectomies were performed through a 5th intercostal space left thoracotomy. The hilum was ligated en bloc and the entire left lung was excised. 3, 6, 9, 12 15, and 21 days after surgery the tissue was harvested and used for flow cytometry cell cycle analyses, immunohistochemistry, and corrosion casts. Casts were examined by means of SEM and synchrotron radiation.

Results. Volumes and weights of the remaining lungs returned to near-baseline levels within 21 days of pneumonectomy. The percentage increase in lobar weight was greatest in the cardiac lobe (p <0.001). Flow cytometry demonstrated a peak of lung cell proliferation (12.02 ± 1.48%) 6 days after pneumonectomy. Spatial autocorrelation analysis demonstrated clustering of similar vascular densities that consistently mapped to subpleural regions of the cardiac lobe. Scanning electron microscopy 3–6 days after pneumonectomy demonstrated subpleural vessel sprouts. The monopodial sprouts were randomly oriented along the vessel axis with interbranch distances of 11.4 ± 4.8 μm in the regions of active angiogenesis. Also in all regions of active angiogenesis holes or pillars consistent with active intussusceptive angiogenesis were observed.

Conclusions. These findings indicate that the process of alveolar construction involves discrete regions of regenerative growth, particularly in the subpleural regions of the cardiac lobe, characterized by both sprouting and intussusceptive angiogenesis.

Kategorie: Lecture

Rubrik: 9.Cell Biology
Abstract Nr.:37

Titel:Interplay between nrf2 and amphiregulin: implications for ventilator induced lung injury.

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

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Abstract:
Objectives: Oxidative stress plays a role in the development of Ventilator Induced Lung Injury (VILI), an adverse side effect in mechanically ventilated patients. VILI is characterized by disruption of the airspace walls resulting in alveolar edema, and inflammation. An interaction between Nuclear factor erythroid 2-related factor 2 (Nrf2), a transcription factor which plays a major role in cellular defence mechanisms against oxidative stress, and amphiregulin, a member of the epidermal growth factor family, seem to play an important role in the development of VILI.
Methods: Mice were confronted with high and low volume ventilation in the isolated perfused mouse lung setup or received amphiregulin intratracheally and Nrf2-activity was measured. Precision cut lung slices (PCLS) were stimulated with amphiregulin and Nrf2-activity was quantified. To investigate whether Nrf2 directly regulates amphiregulin expression, we inactivated Keap1, the cellular inhibitor of Nrf2, in HeLa cells using shRNA. Cells were then used in a dual luciferase reportergene assay utilizing an amphiregulin promoter containing luciferase vector (pGL3-AREG).
Results: Nrf2 is significantly increased in a ventilation strategy dependent manner. Intratracheally applicated amphiregulin and amphiregulin stimulated PCLS showed significant Nrf2 activation. Depletion of Keap1 in pGL3-AREG HeLa´s leads to AREG-promoter activation.
Conclusion: Mechanical stress during ventilation leads to Nrf2 activation. Nrf2 activation increases the expression of AREG that, in turn, is able to activate Nrf2. Here we provide evidence for a positive feedback loop between Nrf2 and amphiregulin during lung ventilation that might protect the lung against VILI via the upregulation of Nrf2-regulated cytoprotective genes.

Kategorie: Lecture

Rubrik: 2.Main Topic II
Abstract Nr.:38

Titel:Nrf2 protects against tissue injury and promotes regeneration of post-ischemic skeletal muscle

Autoren: Al-Sawaf O.(1),Sönmez T.(2),Weiß M.(1),Keimes N.(1),Strzelczyk E.(1),Fragoulis A.(1),Rosen C.(1),Pufe T.(1),Wruck C.(1),

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Abstract:
Reperfusion of ischemic skeletal muscles induces an extensive inflammatory response and tissue injury. NF-E2-related factor 2 (Nrf2) affects the intracellular antioxidant response by the transcriptional activation of ARE (antioxidant response element)-driven genes, which protect tissues from oxidative stress. To study the role of Nrf2 in muscle damage and regeneration, we used an in vivo model of a heavy hind limb ischemia for four hours with variable reperfusion times from six hours up to fourteen days utilizing Nrf2-wild type and -knockout mice. Histological and biochemical analysis revealed significant higher tissue damage, higher inflammation and impaired structural and functional regeneration in Nrf2-knockout mice compared to wild type. We have identified several possible places of interaction between two key players of myogenesis (MyoD and myogenin) and Nrf2. In addition, we investigated the expression of the proliferative protein Notch1 and the cell cycle inhibitor p21 as a marker for the beginning of differentiation. Nrf2-wild type mice have an extended satellite cell proliferation and differentiation of during myogenesis then Nrf2-knockout mice. Delayed muscle regeneration of Nrf2-knockout mice could be confirmed by the use of a Rotarod Performance test, which indicates differences in the actual physical condition of the post-ischemic mice. Consequently, our results suggest that an adequate intracellular regulation of Nrf2 is essential for skeletal muscle protection and regeneration of post-ischemic skeletal muscle.

Kategorie: Lecture

Rubrik: 10.Developmental Biology
Abstract Nr.:39

Titel:Neuroendoderm – a novel paradigm?

Autoren: Didilescu A.(1),Nicolescu M.(2),Pop F.(3),Motoc A.(4),Jianu A.(4),Rusu M.(5),

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Abstract:
Recently, it has been demonstrated that neurons develop de novo in cells already specified as endoderm. Endodermal neurogenesis seems mediated by factors known as required for neurogenesis in the oral ectoderm. One of the best documented endodermal derivative is the pancreas. Identification of a population of neuron-like cells of endodermal origin as possible precursors of the islets of Langerhans addresses a long-standing controversy regarding the embryological origin of the pancreatic islet cells. In guinea pig, S-100-positive cells were identified in the islets of Langerhans and were classified into glial and endocrine, insulin-producing cell types. The insulin-producing cells also express nestin, which is expressed in neuroepithelial stem cells during embryogenesis. Thus, immature pancreatic endocrine cells share characteristics with developing neuronal cells. Further studies are needed in order to support the idea that, in mammals, not only the ectoderm, but also the endoderm, can give rise to cells expressing morphological, biochemical, and physiological features characteristic of neurons. The discussion here is supported by the results of our study which identified in human midterm fetuses (N=3) a distinctive S-100-positive cell population within the pancreas; additional samples were collected, evaluated in TEM, and also immunostained for CD117/c-kit, CD34, CD63, smooth muscle actin, and Tau protein.

Kategorie: Lecture

Rubrik: 2.Main Topic II
Abstract Nr.:40

Titel:Platelet rich plasma (prp) induces osteoanabolic factors in human osteoblasts

Autoren: Tohidnezhad(1),Kweider(1),Lippross(2),Beckmann(1),Varoga(2),Pufe(1),

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Abstract:
Purpose:Vascular-endothelial-growth-factor(VEGF) is a mitogen for vascular-endothelial cells and is major promoter of angiogenesis. VEGF is involved in the process of bone regeneration and-healing.
Platelet-released-growth-factors(PRGF) is a mixture of autologous growth-factors, prepared from a determined volume of Platelet-rich-plasma(PRP). Platelet-concentrates are used for various surgical procedures to support healingprozess. The exact mode of action of PRP is not investigated sufficiently. The aim of this study is to elucidate if the platelets regulate the VEGFexpression in osteoblasts.
Methods:PRGF was obtained from healthy human donors. Primary human-osteoblast were used for this study. Cell proliferation of osteoblasts during the treatment with PRGF was dertermied using CyQuant and BrdU assay. The expression of VEGF after PRGF treatment was determined using real-time RT PCR. The VEGF-protein expression in cytosol of osteoblast and released VGF amount in supernatant of osteoblasts were measured using ELISA. Also the amount of VEGF in PRGF was dertermined by ELISA. The fluorescence labelling of VEGF was used to verify the results.
Rsults:Increased cell proliferation was detectable using CyQuant and BrdU-assay. We could demonstrate that PRGF leads to expression of VEGF in osteoblasts. Using real-time RT PCR we showed the gene expression of VEGF in Osteoblast after incubation with PRGF. VEGF-level in cytoplasm of osteoblast and released-VEGF from osteoblasts were analysed by ELISA. Immune-fluorescence shows the localisation of expressed VEGF after treatment with PRGF-treatment.
Discussion:Since VEGF is an essential anabolic factor for osteoblasts the increase of VEGF due to PRGF stimulation may be beneficial in fracture healing.

Kategorie: Lecture

Rubrik: 2.Main Topic II
Abstract Nr.:41

Titel:Platelet mediator concentrates (pmc): beneficial in tendon healing?

Autoren: Salin(1),Jaeger(2),Huebner(2),Pufe(1),Tohidnezhad(1),

Adressen:(1)Anatomy and Cellbiology|RWTH Aachen University|Aachen|Germany; (2)curasan AG|curasan AG|Kleinostheim|Germany; email:mtohidnezhad@ukaachen.de

Abstract:
Purpose:
Little is know about the pathophysiology of acute and degenerative tendon injuries. Although most lesions are uncomplicated, treatment is long and unsatisfactory due to the poor vascularity of this tissue. Platelet mediator concentrate(PMC) (Curasan AG; Kleinostheim, Germany) contains many growth factors derived from platelets, which can promote wound healing. In this study, we investigate the effect of PMC on tenocytes. Aim of this present study was the investigation of effects of PMC on tendon-healing and to explore the mechanism of action in order to provide the experimental basis for tissue regeneration and to develop new treatment concepts.
Methods:
Using ELISA we could quantified the growth factors, BMPs and cytokines in PMC. Tenocytes were isolated from human-Achilles-tendons and stimulated with PMC. CyQuant and Cell-Titer-Blue assay were carried out to analyse tendon growth and viability in different concentration of PMC. Real-time-RT-PCR was used to analyse tenocytes gene-expression with or without PMC treatment.
Results:
We could demonstrate that PMC include numerous growth factors and BMPs like BMP-2 and BMP-4. A positive effect of PMC on tendon cell growth and viability could be shown in a dose-dependent manner. Furthermore PMC leads to an induction of gene expression like scleraxis (Scx) and typ I-collagen (Col1-a1) by tenocytes after treatment with PMC.

Discussion:
PMC can serve as a source of growth factors and can improve tenocyte growth and viability. We suggest that the use of autologous PMC in order to increase tendon healing can be a suitable addition to conventional therapy.

Kategorie: Lecture

Rubrik: 5.Experimental Morphology
Abstract Nr.:42

Titel:Enoxaparin prevents steroid-related avascular necrosis of the femoral head.

Autoren: Shaheen H.(1),Drescher W.(2),Beckmann R.(3),Kweider N.(3),Ghassemi A.(4),Pufe T.(3),Kadyrov M.(3),

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Abstract:
Objectives: Avascular femoral head osteonecrosis is a common complication with disabling effect for young patients after high-dose corticosteroid treatment.Prevention of osteonecrosis after corticosteroid administration would be important. In this study we investigated the preventive effect of enoxaparin on steroid-related bone necrosis in a rabbit model.
Methods: New Zealand White rabbits (male; 3-4.5 kg bodyweight) were injected with 20 mg/kg bodyweight methylprednisolone (GC group; n = 6). Control animals (n = 6) were treated with phosphate-buffered saline. A third group (GC + E; n = 6) additionally received enoxaparin (20 mg/kg). Four weeks after i.m. methylprednisolone injection the animals were sacrificed. After decalcification, the femurs were embedded in paraffin. 4-ìm-thick coronal sections of the femurs were prepared and stained with ladewig and hematoxylin-eosin staining. Empty lacunae, a histologic sign of FHN, where determined by histomorphometry.
Results and Conclusion: The signs of osteonecrosis were determined based on the diffuse presence of empty lacunae or pyknotic nuclei of osteocytes. Histomorphometry revealed a significant increase of pyknosis and empty lacunae-number in glucocorticoid-treated animals compared to controls and GC + E-treated animals. No significant difference in empty lacunae count was detected between the GC + E and control groups. HE staining revealed the more preserved osteocytes in the GC and enoxaparin-treated versus the GC groups.
This study demonstrates an increased number of empty osteocyte lacunae representing a pathologic feature of osteonecrosis, in the GC group which could be reversed by administration of enoxaparin. Our findings may offer a new approach for the prevention of corticosteroid-induced osteonecrosis.

Kategorie: Lecture

Rubrik: 2.Main Topic II
Abstract Nr.:43

Titel:Telocytes – cells or particular cell morphologies?

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Abstract:
Telocytes (TCs) were described as a distinctive type of stromal cells. Formerly descried as interstitial Cajal-like cells (ICLCs), these cells were defined by their long, slender, moniliform prolongations called telopodes (Tp). Transmission electron microscopy (TEM) is the only reliable tool to identify TCs/Tp. We evaluated in TEM various tissues: (a) human tongue, larynx, greater omentum, heart, eye iris, trigeminal ganglion, stellate ganglion, major salivary glands, pancreas, and skin; (b) rat trachea, esophagus, thymus, and heart. We performed additional immunohistochemistry using antibodies for CD117/c-kit, CD34, vimentin, and alpha-smooth muscle actin. We got ultrastructural evidences of TCs/Tp in the examined samples. The results indicate that the telopodial morphology could be just the appearance of an elongated fibroblastic process. As it was previously described for fibroblasts, telocytes: (a) build stromal networks, shed vesicles and exosomes; (b) build cellular tandems with other stromal cells; (c) associate with blood vessels, as vascular and perivascular TCs; (d) build concentric perineurial layers. There are markers of absolute specificity to distinguish between fibroblasts, ICLCs and TCs. Identification of such markers would be of benefit in understanding the lineage issues. The presence of telopodes is definitory for diagnosing a cell to be a telocyte, but it is equally reasonable to consider telopodes to be just elongated processes of various subtypes of fibroblasts or other interstitial cells. Funding: POSDRU/89/1.5/S/64153.

Kategorie: Lecture

Rubrik: 1.Main Topic I
Abstract Nr.:44

Titel:Venous valves and their rhythm - its (non-)correlation with the heart beat and breathing

Autoren: Brenner E.(1),Strauß A.(1),Glodny B.(2),

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Abstract:
Venous valves are not just plain check valves but follow a rather complex closing mechanism which influences venous flow and vice-versa. Findings suggest that the turbulent flushing of valve sinuses during a closure cycle is necessary for sufficient endothelial oxygen supply in valve pockets. Accordingly, endothelial hypoxia could lead to the formation of thrombi.
We investigated the closure rates of the most proximal valves of the great saphenous vein (GSV) and the cephalic vein (CV) in 24 healthy subjects resting in supine position. Furthermore, we determined the venous diameters, the respiratory and heart rates, age, body mass index (BMI) and waist-hip-ratio (WHR).
The closure rates within the GSV correlated negatively with the heart rate ($r=-0.466$; $p=0.022$). Within the CV there was a correlation with the respiratory rate ($r=0.787$; $p=0.004$) and with the heart rate ($r=0.558$; $p=0.074$). GSV- and CV-frequencies did not correlate. Flows as well as median frequencies of both valves (CV: 64; GSV: 5) differed considerably. We found a gender difference, but no correlations of the valves' closure rates with the venous diameter, age, BMI, or WHR.
The negative relation between the valves' closure rate within the GSV and the heart rate was quite surprising. This fact, together with the negative correlation with the respiratory rate, does not fit into the popular model of venous flow. On the other hand, the frequency of the CV's most proximal valve follows a positive linear correlation with the respiratory rate and - up to 70 bpm - with the heart rate, too.

Kategorie: Lecture

Rubrik: 7.Neuroimmunology
Abstract Nr.:45

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

Autoren: Jansen S.(1),Leib S.(2),Podschun R.(3),Lucius R.(4),Pufe T.(1),Brandenburg L.(1),

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Abstract:
Antimicrobial peptides are part of the innate immune system on 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, Western Blot 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 demonstrate the occurrence of antimicrobial peptides in the cerebrospinal fluid of meningitis patients. Also we detected the secretion of biological active Psoriasin in glial cells and meningeal cells. We could show an involvement of Psoriasin in the rat meningitis model pointing to a role of Psoriasin in the pathogenesis of this disease. Furthermore we examined Psoriasin as signal peptide. We are able to show that Psoriasin induced a signal pathway in glial cells and meningeal cells. Our results suggest that Psoriasin is an important part of the innate immunity in the brain against bacterial CNS pathogens.

Keywords: antimicrobial peptides, Psoriasin

Kategorie: Lecture

Rubrik: 7.Neuroimmunology
Abstract Nr.:46

Titel:Involvement of mas-related gene receptor mrgd in the inflammatory response during intestinal schistosomiasis

Autoren: Avula L.(1),Buckinx R.(1),Adriaensen D.(2),Van Nassauw L.(3),Timmermans J.(1),

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Abstract:
Members of the Mas-related gene receptor (Mrg) family have been suggested to play a role in nociception, in mediating IgE-independent mast cell activation, and in neuroimmune communication. In a previous study, we have observed an increased expression of some Mrgs, such as MrgD, in the murine ileum during intestinal inflammation. To further unravel the expression and role of MrgD in intestinal inflammation, we compared the ileum of non-inflamed and Schistosoma mansoni-infected wild-type (WT) and MrgD-/- mice. In WT mice, in the non-inflamed ileum, immunohistochemistry revealed no MrgD immunoreactivity (IR), whereas in the inflamed ileum, MrgD IR was detected in 5% of the myenteric neurons. Neurochemical coding revealed that the MrgD-expressing neurons were intrinsic primary afferents. Moreover, MrgD IR was detected in mucosal mast cells (MMCs) in the inflamed ileum. In MrgD-/- mice, no MrgD IR was detected in any tissue, while increased infiltration of MMCs and increased calcitonin gene related peptide (CGRP) expression in enteric neurons was observed in the inflamed ileum. Expression of MrgD in sensory neurons and MMCs in the inflamed ileum of WT mice, and increased MMC infiltration, in conjunction with increased CGRP expression in the inflamed ileum of MrgD-/- mice, suggest that MrgD is involved in mast cell and nociceptive responses, thereby in the inflammatory response during intestinal schistosomiasis. Future work should aim at elucidating the mechanisms underlying MrgD-mediated effects during intestinal inflammation. Supported by FWO grant G.0179.08 and TOP-BOF grant of the University of Antwerp.

Kategorie: Lecture

Rubrik: 13.Pheripheral and vegetative nervous system
Abstract Nr.:47

Titel:Serotonin immunoreactive neurons in the mouse esophagus. enteric production or extrinsic uptake?

Autoren: Hempfling C.(1),Neuhuber W.(1),Wörl J.(1),

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Abstract:
Serotonin (5-HT) is a major transmitter in the gastrointestinal tract. The existence of several 5-HT receptor subtypes has enabled the development of drugs for treatment of gastrointestinal motility disorders. In contrast to pharmacological and functional studies, little is known about the morphological organization of serotoninergic neurons in the esophagus. This study aimed at identifying serotonin-containing neurons in this organ. Antibodies against 5-HT, choline acetyltransferase (ChAT), protein gene product 9.5 (PGP 9.5), tryptophan hydroxylase (TPH), tyrosine hydroxylase (TH) and fluorchrome-tagged α-bungarotoxin (α-BT) were used in multilabel immunoflourescence. Ten percent of PGP 9.5 positive myenteric perikarya were also 5-HT immunoreactive. Varicose nerve terminals positive for 5-HT were present on 13% of α-BT labeled motor endplates and in the muscularis mucosae. As ChAT positive neurons of the nucleus ambiguus were negative for 5-HT, serotoninergic varicosities at motor endplates are presumably of enteric origin. As 5-HT positive nerve fibers around blood vessels in the esophagus and its adventitia were also TH immunoreactive, thus presumed sympathetic neurons which had taken up 5-HT from non-neuronal sources, e.g., platelets, the question arose, if serotonin immunoreactive neurons in the tunica muscularis and muscularis mucosae actually produced 5-HT or also took it up from external sources. However, TPH antibodies showed cross reactivity with TH and specific antibodies against TPH2, although demonstrating neurons in raphe nuclei and small intestine, did not display adequate results in the esophagus. Thus, this issue can hardly be solved by immunofluorescence alone.

Kategorie: Lecture

Rubrik: 13.Pheripheral and vegetative nervous system
Abstract Nr.:48

Titel:Massive versus moderate neurodegeneration in myenteric and submucosal plexus of human chagasic megacolon

Autoren: Jabari S.(1),da Silveira A.(2),Neuhuber W.(1),Brehmer A.(1),

Adressen:(1)Friedrich- Alexander- Universität Erlangen- Nürnberg|Institute for Anatomy Department 1|Erlangen|Germany; (2)Universidade Federal de Uberlândia|Human Anatomy Sector|Minas Gerais|Brazil

Abstract:
Massive myenteric neuron loss is regarded as key factor for the development of chagasic megacolon being the morphological sign of a severely and irreversibly impaired colonic motility. In our samples, neuron numbers decreased down to 20 % of controls. Additionally, we found a partial, selective survival of nitrergic myenteric neurons. The resulting preponderance of nitrergic, inhibitory nerve elements could explain why neuron loss in Chagas' disease results in chronic dilation rather than constriction of involved intestinal segments as in congenital Hirschsprung's disease. In contrast to the myenteric plexus, chagasic neurodegeneration in the submucosal nerve networks appeared moderate; neuron numbers were about 50 % of controls. However, mainly in the internal submucosal plexus, we found a drastic loss of somatostatin(SOM)-immunoreactive neurons (10% in controls versus 2% in chagasic patients). Accordingly, SOM-reactive nerve fibres, abundantly present in control mucosa (20% of stained mucosal nerve fibres), were almost absent from megacolonic mucosa (1%). In contrast, calretinin(CALR)-positive neurons and nerve fibres were present at comparative frequency in both control and megacolonic submucosal plexus and mucosa, respectively. Additionally, we found extensive colocalization of vasoactive intestinal peptide (VIP) in CALR-positive neurons and fibres. It has been shown that VIP has both neuroprotective and neuroeffector functions, one of the latter are regulatory effects on epithelial cells and barrier function. Thus, this peptide may allow both survival of VIP-containing neurons and patient survival for decades by maintaining mucosal barrier despite complete loss of colonic motility.

Kategorie: Lecture

Rubrik: 4.Gross Anatomy/Clinical Anatomy
Abstract Nr.:49

Titel:Drycon hpcs© - a new method for preservation and mummification of whole bodies in a closed system without formalin

Autoren: Weber G.(1),Weber G.(1),

Adressen:(1)Managing Director|MEDIS Medical Technology GmbH|Buseck|Germany; email:karin.hooker@medisgmbh.com

Abstract:
This new anatomical technology, a first worldwide, was designed to eliminate the use of formalin and to create a closed system for the processing of whole bodies for teaching purposes in anatomy. Consisting of modular units, the system allows the preservation of an unlimited number of cadavers.

A special advantage is the processing not just of the whole body but also of organs and body parts in a fully closed process chamber, whereby the infiltration solution is pumped into the chamber until the body / organ is fully covered (akin to a closed Tissue Processor in pathology). After this procedure, the body maybe “mummified” for longterm storage in a dry environment on a simple shelf. If the body is to be used for a student dissecting course, it can be readily restored to its original “soft” condition and kept soft by leaving the body in a hermetically sealed plastic body bag on the dissecting table.

This unit consists of a solid, and self-carrying construction with automatic electronic control functions to enable one-man operation. Specially developed to guarantee pollution-free fixation and conservation of whole bodies without the use of formalin in a fully closed system that is pressurized with 3 bars in order to enhance the infiltration process.

The infiltration / preservation solution (DIS) is a special, quality controlled solutionbased on a harmless, none-toxic, organic product called “Shellac” (with low concentrations of ethanol). With the approval of leading anatomists, because of the use of Shellac, the method is termed “Lacservation”.

Kategorie: Lecture

Rubrik: 10.Developmental Biology
Abstract Nr.:50

Titel:Motoneurons from human ips cells

Autoren: Liebau S.(1),Stockmann M.(1),Boeckers T.(1),

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Abstract:
Human iPS cells and their differentiated progeny represent a valuable tool to investigate developmental mechanisms. The developmental processes including neuronal maturation and synaptogenesis of human neurons in vitro have been poorly investigated up to now. During formation of the human motoneuron system, neuronal maturation and synaptogenesis are major hallmarks of the system´s functionality. Understanding the distinct developmental steps of in vitro neurogenesis requires the thorough analyses of temporal and spatial patterning of the cellular compartments, especially their synaptic apparatus. Motoneuron degeneration in various diseases includes denervation and degradation of synaptic contacts. If pathogenetic studies are including comparisons between physiological and pathological developmental steps, the differential time points of maturation steps must be well established. We analyzed time-dependent maturation steps of human induced pluripotent stem cell derived motoneurons and found that iPS cells developed into mature neuronal cells displaying all hallmarks of fully functional motoneurons.

Kategorie: Lecture

Rubrik: 6.Neuroanatomy/Neurobiology
Abstract Nr.:51

Titel:Neuronal ikk/nfkappab signaling regulates synapse formation via insulin-like growth factor 2

Autoren: Schmeisser M.(1),Baumann B.(2),Johannsen S.(1),Jensen V.(3),Hvalby O.(3),Sprengel R.(4),Oswald F.(5),Wirth T.(2),Boeckers T.(1),

Adressen:(1)Institute for Anatomy and Cell Biology|Ulm University|Ulm|Germany; email:michael.schmeisser@uni-ulm.de; (2)Institute for Physiological Chemistry|Ulm University|Ulm|Germany; (3)Institute of Basic Medical Sciences|University of Oslo|Oslo|Norway; (4)Molecular Neurobiology|Max Planck Institue for Medical Research|Heidelberg|Germany; (5)Internal Medicine I|Ulm University Medical Center|Ulm|Germany

Abstract:
Impaired neuronal NF-kappaB activity has previously been shown to result in the disruption of synaptogenesis and synaptic plasticity. However, there is still limited information on the exact molecular mechanisms which regulate the plastic changes of synaptic contacts downstream from NF-kappaB.
Here, we report that the NF-kappaB inducing IkappaB-Kinase(IKK)-Complex is localized and activated in the postsynaptic density of mouse forebrain. We further show that conditional genetic inactivation of IKK-2 function in mouse principal neurons leads to a loss of synapses and mature dendritic spines, impaired AMPA-mediated basal synaptic transmission and corresponding molecular and behavioral changes. Synaptic deficits can be restored in adult animals by in-vivo re-activation of the NF-kappaB signaling cascade within one week indicating a highly dynamic regulation process. We further identified the insulin-like growth factor 2 (Igf2) gene as novel NF-kappaB target and found that exogenous Igf2 is able to restore synapse density in IKK/NF-kappaB deficient neurons within 24 hours.
Our findings therefore illustrate a fundamental role of IKK/NF-kappaB-Igf2 signaling in synapse formation and provide an intriguing link between the molecular actions of IKK/NF-kappaB in neurons and the recently identified memory enhancement factor Igf2.

Kategorie: Lecture

Rubrik:
Abstract Nr.:52

Titel:Spine densities of hippocampal neurons in vitro are regulated by ADAM-10-dependent processing of amyloidprecursor protein (APP)

Autoren: Gruber M.(1), Beyer M.(1), Grimm I.(1), Copanaki E.(1), Altmann C.(1), Samanta A.(3), Jäschke A.(3), Weyer S.(2), Tsankova A.(2), Tschäpe J.(2), Müller U.(2), Deller T.(1),

Adressen:(1)Institute of Clinical Neuroanatomy|Dr. Senckenberg Anatomy|Goethe-University Frankfurt|NeuroScience Center|Frankfurt am Main|Germany
(2) Institute of Pharmacy and Molecular Biotechnology |Heidelberg University|Germany
(3) Department of Pharmaceutical Chemistry|Institute of Pharmacy and Molecular Biotechnology|Heidelberg University|Germany

Abstract:
Processing of the amyloid precursor protein (APP) by α-secretase results in the secretion of the soluble ectodomain sAPPα. Since sAPPα is known to be involved in synaptic plasticity, we speculated that sAPPα might also have an effect on dendritic spines, postsynaptic structures involved in synaptic plasticity, learning and memory. To address this issue, mature (days in vitro 18-21) organotypic entorhino-hippocampal slice cultures of Thy1-GFP mice were generated and individual dentate granule cells as well as CA1 pyramidal neurons were visualized under control conditions and after treatment with an α-secretase inhibitor (ADAM-10-inhibitor). Time-lapse imaging of individual dendritic segments was employed to study spine density and dynamics for 6-7 days in vitro. Whereas control neurons did not show significant changes in their spine densities, neurons treated with the ADAM-10 inhibitor lost a major portion of their spines. Preliminary data indicate that this effect could be caused by a reduced formation of new spines after ADAM-10-inhibition. The effect of ADAM-10-inhibition on granule cell spine densities could be largely rescued with exogenously applied sAPPα. In contrast, application of the soluble ectodomain sAPPβ, which is generated by β-secretase cleavage, did not have an effect on granule cell spine densities. Taken together, these data suggest that sAPPα is involved in the regulation of spine formation in the hippocampus and, possibly, in Alzheimer´s disease patients. (Supported by DFG; *contributed equally).

Kategorie: Lecture

Rubrik: 8.Neuroregeneration/Neurodegeneration
Abstract Nr.:53

Titel:Tgf beta signaling protects retinal neurons from ontogenetic cell death during development

Autoren: Braunger B.(1),Pielmeier S.(1),Demmer C.(1),Landstorfer V.(1),Kawall D.(1),Jägle H.(2),Fischer D.(3),Tamm E.(1),

Adressen:(1)Institute of Human Anatomy and Embryology|University of Regensburg|Regensburg|Germany; email:Barbara.Braunger@vkl.uni-regensburg.de; (2)Department of Ophthalmology|University Clinic|Regensburg|Germany; (3)Experimental Neurology|Heinrich-Heine-University Düsseldorf|Düsseldorf|Germany

Abstract:
Purpose: To investigate the role of TGF-beta signaling during ontogenetic cell death in the mouse retina.

Methods: Floxed TGF-beta receptor type 2 (TGFbr2) mice were crossed with alpha-Cre mice, expressing Cre recombinase in retinal neurons. As control, floxed Smad7 mice were used. Smad7 is an endogenous inhibitor of TGF-beta signaling. Apoptotic cell death of retinal neurons was analyzed at embryonic days (E) 12.5, 14.5, 16.5 and at postnatal days (P) 4, 7, 9 by TUNEL labeling and an ELISA for free nucleosomes. Retinal ganglion cells (RGCs) were isolated from newborn mice, cultured in the presence of TGF-beta or SIS3, an inhibitor of Smad3 phosphorylation, and the cell number was determined 24h after treatment.

Results: Western blot analyses (TGFR-2, Smad7) and immunohistochemistry confirmed the conditional knock out in the retina in both mouse lines. TUNEL analysis showed significantly (p < 0.01 – 0.05) more TUNEL positive cells in the retina of TGFbr2flox/flox/alpha-Cre mice at each time point when compared to their wild-type littermates. This resulted in a significantly thinner inner nuclear layer in adult mice and significantly fewer axons (p < 0.01) in the optic nerve. Standard flash-ERG indicated functional changes. In cultured RGCs, TGF-beta showed a dose-dependent positive effect on cell survival and could be antagonized with SIS3. In contrast, Smad7flox/flox/alpha-Cre mice showed significantly (p < 0.01 – 0.02) less apoptotic cell death in the retina when compared to wild-types.

Conclusion: TGF-beta signaling protects retinal neurons from apoptotic cell death during development and may have comparable effects following retinal injury in adult animals.

Kategorie: Lecture

Rubrik: 6.Neuroanatomy/Neurobiology
Abstract Nr.:54

Titel:Functional morphology of reelin-mediated glia-synaptic interaction

Autoren: Brunkhorst R.(1),Bock H.(2),Haseleu J.(3),Rizzoli S.(4),May P.(2),Zurhove K.(2),Bouché E.(2),Derouiche A.(1),

Adressen:(1)Dr. Senckenbergische Anatomie, Institut für Anatomie II|Goethe-Universität, Frankfurt am Main, Germany|Frankfurt/ M.|Germany; (2)Centre for Neurosciences|University of Freiburg|Freiburg|Germany; (3)Institute of Cellular Neurosciences|Univ. of Bonn|Bonn|Germany; (4)STED Microscopy of Synaptic Function|European Neuroscience Institute|Göttingen|Germany

Abstract:
In the adult rodent hippocampus, the glycoprotein reelin is synthesized in and released from interneurones. It is believed that reelin diffuses in the extracellular matrix (ECM) to the glutamatergic synapse, where it has been shown to enhance long term potentiation (LTP). The extraneuronal localization of reelin in the adult rodent hippocampus displays a punctate pattern. However, until now the evidence for the localization of this extraneuronal reelin in the ECM has been scarce. We therefore analyzed the extraneuronal distribution of reelin in detail using immunofluorescence, high-resolution deconvolution microscopy and immuno-electron microscopy, in rat and mouse hippocampus and in freshly dissociated, morphologically intact glial cells (DIMIGs).
We unexpectedly found that extraneuronal reelin is not localized in the ECM but in astrocyte processes. We confirmed this observation on the mRNA and protein levels in primary astrocyte cultures. As demonstrated electron microscopically, reelin is significantly associated with vesicular organelles and small vesicle clusters localized to restricted, near-membrane sites within peripheral astrocyte processes. We further investigated possible exocytosis routes of reelin-positive organelles applying STED microscopy analysis of DIMIGs. We found a strong association of reelin with Rab6 and its effector protein ERC1.
Together with our observation that reelin is preferentially localized at restricted membrane sites, this is strong evidence for a spatially controlled, constitutive secretion of reelin by astrocytes. Since we did not observe a localization of reelin in the ECM, our results may show that the proposed action of reelin at the glutamatergic synapse might represent an example of glia-synaptic interaction.

Kategorie: Lecture

Rubrik: 8.Neuroregeneration/Neurodegeneration
Abstract Nr.:55

Titel:Functions of neuronal and glial key proteins in reorganization of the deafferented adult cochlear nucleus

Autoren: Fredrich M.(1),Zeber A.(2),Illing R.(2),

Adressen:(1)Dr. Senckenbergische Anatomie, Institute of Anatomy II|Goethe-University Frankfurt|Frankfurt am Main|Germany; email:Fredrich@med.uni-frankfurt.de; (2)Neurobiological Research Laboratory, Department of Otorhinolaryngology|University of Freiburg|Freiburg|Germany

Abstract:
We deafferented the rat cochlear nucleus (CN) by cochlear ablation (CA) and investigated the subsequent rearrangements of the extracellular matrix (ECM), axonal growth and synaptogenesis, applying immunocytochemistry on different postlesional days.
Key modulators of ECM, the matrix metalloproteinases MMP-9 and MMP-2, were secreted from neuronal somata into the neuropil, and their expression increased in activated CN astrocytes. Likewise, polysialic acid (PSA), a key player in axonal growth, was increased in activated astrocytes. We further investigated whether the spatio-temporal dynamics of MMP-9, MMP-2, and PSA are causally related to initiation of compensatory CN reinnervation originating from axons of the ventral nucleus of the trapezoid body (VNTB). VNTB neurons were destroyed by stereotaxic injection of kainic acid, preventing postlesion CN reinnervation. MMP-9 dynamics were found to be unaffected, but MMP-2 and PSA returned to control pattern when reinnervation was prevented. Thus, MMP-9 might be associated with degeneration, and both, MMP-2 and PSA with reinnervation, including axonal growth and synaptogenesis. A possible reciprocal dependence of reinnervation with PSA expression was examined by combining CA with intraventricular endo-neuraminidase injection. This enzyme removes PSA from its carrier, the neural cell adhesion molecule (NCAM). We observed a reduced reinnervation, spatially limited to the ventral third of CN. Astrocytic PSA, thus, provides an environment permissive for sprouting and guidance of axons.
Altogether, reinnervating axons and nascent synapses appear to change the molecular profile of neurons and astrocytes, which jointly support completion of compensatory tissue reorganization after deafferention.

Kategorie: Lecture

Rubrik: 6.Neuroanatomy/Neurobiology
Abstract Nr.:56

Titel:Distribution of corticosteroid-binding globulin and glucocorticoid receptor-immunoreactivity in the rat brain

Autoren: Sivukhina E.(1),Grinevich V.(2),Jirikowski G.(1),

Adressen:(1)Department of Anatomy II|Friedrich-Schiller University|Jena|Germany; email:elena.sivukhina@mti.uni-jena.de; (2)Department of Molecular Neurobiology|Max-Planck-Institute for Medical Research|Heidelberg|Germany

Abstract:
Endocrine regulation of stress response is controlled by systemic glucocorticoid levels. So far steroids have been thought to act on the brain exclusively through nuclear receptors. However, many of brain systems known to respond to glucocorticoids seem to be devoid of respective receptor proteins. Recently we described an intrinsic expression of corticosteroid-binding globulin (CBG) in rat and human brains. Cerebral CBG as well as glucocorticoid receptor (GR) expression seems to be linked to serum glucocorticoid levels. Here we report a comprehensive mapping study on the co-localization of immunoreactive CBG and of GR in the rat brain. Both proteins are abundant throughout the brain; however we observed them mostly in separate sets of neurons. In the nucleus accumbens, septum, hippocampus, globus pallidus, medial and basolateral amygdale nuclei, magnocellular preoptic nuclei, diagonal band of Broca the high intensity of CBG-immunoreactivity was accompanied by weak or moderate GR staining, and vice versa. In several strongly GR-positive brain regions (caudate putamen, bed nucleus of stria terminalis, septohypothalamic nucleus, parvocellular subdivision of the paraventricular nucleus), CBG-immunoreactivity was almost undetectable. In contrast, throughout the supraoptic nucleus and magnocellular subdivision of the paraventricular nucleus numerous strongly CBG-positive cells were observed while these cells were devoid of specific GR-immunoreactivity. It is likely that known central actions of adrenal steroids may not be always confined to genomic effects through a nuclear receptor but should also include the involvement of intracellular signaling pathways, mediated - at least in some types of cells - by the interaction between steroids and intrinsic binding globulin.

Kategorie: Lecture

Rubrik: 6.Neuroanatomy/Neurobiology
Abstract Nr.:57

Titel:Organizational principles of the human inferior parietal lobule

Autoren: Caspers S.(1),Eickhoff S.(2),Schleicher A.(2),Bacha-Trams M.(1),Palomero-Gallagher N.(1),Amunts K.(2),Zilles K.(1),

Adressen:(1)Institute of Neuroscience and Medicine, INM-2|Research Centre Jülich|Jülich|Germany; email:s.caspers@fz-juelich.de; (2)Institute of Neuroscience and Medicine, INM-1|Research Centre Jülich|Jülich|Germany

Abstract:
The human inferior parietal lobule (IPL) is still an enigmatic brain region, with such diverse cognitive functions as language, attention, and action processing. This obvious functional diversity is reflected by cytoarchitectonical heterogeneity, with seven distinct areas. How can the different functions of human IPL assigned to a specific receptor and structural organization?
We assessed receptor architecture of human IPL using quantitative in-vitro receptor autoradiography of all classical neurotransmitter systems and analyzed similarities in receptor-based organization using hierarchical cluster analysis. Structural connectivity was studied in vivo using diffusion tensor imaging, which detects water diffusion along white matter fibre bundles, and diffusion modelling of fibre bundles originating in different IPL areas. Functional connectivity was analysed using meta-analytic connectivity modelling. Selecting functional neuroimaging studies with at least one activation focus within the IPL enabled modelling of co-activation patterns of the IPL areas.
The heterogeneous multi-receptor balance of the IPL demonstrated a tripartite subdivision into a rostral, intermediate, and caudal IPL cluster, most prominently distinguished by serotoninergic 5-HT2 and glutamatergic kainate receptors. Receptor, structural, and functional connectivity analysis together revealed an onion-like organisation of the IPL clusters and connected regions, particularly in the frontal lobe: Rostral IPL (behaviourally assigned to action processing) was connected to caudal-most aspects of inferior frontal gyrus, frontal operculum, and medial frontal cortex, thus forming the inner shell of the onion. More caudal IPL clusters (cognitive tasks) were connected with more rostral aspects of these three frontal regions and with superior parietal and temporal cortex, thus occupying the outer shells.

Kategorie: Lecture

Rubrik: 7.Neuroimmunology
Abstract Nr.:58

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

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

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

Kategorie: Lecture

Abstract Nr.:59

Misgeld T., Munich (Germany)

Imaging axonal recovery in the spinal cord

Abstract Nr.:60

Acker-Palmer A., Frankfurt am Main (Germany)

Neuronal and vascular guidance

Rubrik: 2.Main Topic II
Abstract Nr.:61

Titel:Regeneration of the newt heart:structural reconstitution is accomplished by dedifferentiation, proliferation and migration of cardiac cells

Autoren: Piatkowski T.(1),Mühlfeld C.(2),Braun T.(1),Borchardt T.(1),

Adressen:(1)Cardiac Development and Remodelling|Max-Planck-Institute for Heart and Lung Research|Bad Nauheim|Germany; email:Tanja.Piatkowski@mpi-bn.mpg.de; (2)Institute of Functional and Applied Anatomy|Hannover Medical School|Hannover|Germany

Abstract:
The newt heart has a remarkable capacity to regenerate after an induced injury. To elucidate the regenerative process, hearts were injured by squeezing the right half of the ventricle with a forceps and investigated by electron microscopy and immunohistochemistry at various stages post injury.
One day after injury, markers of different cardiac cells were reduced in the damaged region. Cardiomyocytes in the border zone of the infarct showed a disorganized pattern concomitant with a substantial decline in size of the residual branches of the trabecular network. The re-patterning proceeded by migration of dedifferentiated cardiomyocytes from the border zone into the damaged region. The residual branches of the trabecular network, mainly composed of endothelial cells around a core of cellular debris seemed to serve as a scaffold. To reorganize the trabecular network, existing trabeculae divided into thinner branches, which increased in size and showed an organized pattern of mature cardiomyocytes at 200 days post injury. Remarkable proliferative events could be observed during the first and fifth week of the regeneration process. In parallel, extracellular matrix proteins were first deposited and degraded during later stages of regeneration until a normal level was reached. Macrophages infiltrated the injured region during the first 24 hours and phagocytotic events could be observed during the whole regeneration process.
Taken together, heart regeneration in the newt is achieved by dedifferentiation, proliferation and migration of the different cardiac cell types. Moreover, the deposition of extracellular matrix proteins is only transient without formation of a scar.

Kategorie: Lecture

Rubrik: 2.Main Topic II
Abstract Nr.:62

Titel:Desmoglein 2 mutant mice show a distinct sequence of myocardial alterations from adolescence to adulthood

Autoren: Krusche C.(1),Krull P.(1),Kant S.(1),Denecke B.(2),Leube R.(1),

Adressen:(1)Molecular and Cellular Anatomy|RWTH Aachen University|Aachen|Germany; email:ckrusche@ukaachen.de ; (2)IZKF Chip facility|RWTH Aachen University|Aachen|Germany

Abstract:
Mice carrying a deletion of the adhesive extracellular domain of the desmosomal cadherin desmoglein 2 develop a cardiomyopathy characterized by ventricular dilation, local and interstitial fibrosis and susceptibility to arrhythmia. Development of the disease phenotype starts at 2 weeks after birth in homozygous mutants with the appearance of cell-rich but collagen-poor lesions that subsequently accumulate abundant amounts of collagen fibers. Cardiac alterations are detectable in all animals by 4 weeks. The focus of the current study was on the formation and progression of the prominent infarct-like lesions.
mRNA expression of the panleukocyte marker CD45 and the murine macrophage marker F4/80 were significantly elevated in 2 week old mutants with visible cardiac lesions but not in mutants without pathology. By immunohistochemistry high amounts of CD45+ immune cells and F4/80+ macrophages were detected within the lesions. At the same time, increased mRNA expression of the chemotactic cytokines Ccl2, Ccl3 and OPN was detected by RT-PCR in lesioned myocardium. At 8-12 weeks lesions of mutant hearts present with low cell densities and high amounts of collagen. Accordingly, CD45+ immune cells as well as F4/80+ macrophages were reduced but still significantly elevated in comparison to the wildtype.
Taken together, the desmoglein 2 mutation induces myocardial lesions at an age when cardiac load increases. Scar formation starts with aseptic inflammation and subsequent collagen deposition. A continuous myocardial remodeling follows which leads to heart failure or sudden cardiac death.

Kategorie: Lecture

Rubrik: 2.Main Topic II
Abstract Nr.:63

Titel:Desmoglein 2 mutant mice lack typical desmosome-like structures and develop changes in actin expression and localization

Autoren: Kant S.(1),Krusche C.(1),Eisner S.(1),Leube R.(1),

Adressen:(1)Institute of Molecular and Cellular Anatomy|RWTH Aachen University|Aachen|Germany; email:skant@ukaachen.de

Abstract:
Desmosomes are important components of intercalated discs (IDs) which couple cardiomyocytes mechanically and electrically. Furthermore, mutations in desmosomal proteins have been implicated in dilated and arrhythmogenic cardiomyopathy in human. To identify early cellular and ultrastructural changes at disease onset, we examined mice that express a mutant desmoglein 2 (Dsg2) protein with a deletion in the adhesive extracellular domain and develop cardiac dilation, pronounced fibrosis and arrhythmia. Quantification of the ID proteins plakoglobin, desmoplakin, desmocollin 2, N-cadherin and beta-catenin by confocal microscopy in 2 and 12-week-old animals revealed no alterations in the amount or distribution of these proteins in mutant mice. However, the amount of mutant Dsg2 protein in IDs was significantly reduced compared to the wildtype. These data were verified by immunoblotting.
Typical desmosome-like structures were not detectable in IDs of mutant mice by transmission electron microscopy. In addition, gene expression of intercalated disc-anchored skeletal muscle actin (ACTA1), which is usually abundant in fetal but not in adult murine heart, was increased 3±0.21-fold in 2-week-old DSG2 mutant mice. In 8 and 12-week-old mutants ACTA1 mRNA expression was further increased (8 weeks: 4.8±1-fold; 12 weeks: 8.9±0.6-fold). Immunofluorescence microscopy of 2 and 8-week-old DSG2 mutant mice further showed that actin-staining was increased at IDs, especially in cardiomyocytes close to focal fibrosis.
Taken together, it is concluded that the DSG2 mutation disturbs ID structure, increases cardiac ACTA1 expression and alters actin distribution. We hypothesize that these changes are indicative of an impaired force distribution eventually leading to adverse cardiac remodeling.

Kategorie: Lecture

Rubrik: 2.Main Topic II
Abstract Nr.:64

Titel:Sodiumnitrit/pdlla coating could enhance angiogenesis and remodelling of â-tcp scaffolds

Autoren: Beckmann R.(1),Janzen N.(2),Raupach K.(3),Pufe T.(4),Fischer H.(3),

Adressen:(1)Anatomie and Cellbiology|University hospital Aachen, RWTH|Aachen|Germany; email:rbeckmann@ukaachen.de; (2)Dental Materials and Biomaterial Research (ZWBF),|University hospital Aachen, RWTH|Aachen|Germany; (3)Dental Materials and Biomaterial Research (ZWBF)|University hospital Aachen, RWTH|Aachen|Germany; (4)Anatomy and Cellbiology|University hospital Aachen, RWTH|Aachen|Germany

Abstract:
The use of autografts for bone-defects is still the gold-standard among graft materials, but it is limited by tissue availability, bleeding, infection or donor-site morbidity. Alternatively, grafts of biomaterials can be used. A perfect bone-graft-substitute has to provide osteoconductive, osteoinductive, and osteogenic properties. Further important for successful implantation is the supply with oxygen and nutrients to the implanted-cells. Oxygene supply is of crucial importance since its diffusion is limited to approximately 150 µm from capillaries. Besides known factors of angiogensis like BMP2 and VEGF there are hints for the impact of NO in reperfusion-models of heart, lever or kidney. In order to create functionized scaffolds, we investigated the influence of natriumnitrit on endothelial progenitor cells and osteoblasts.

Methods:
Sodiumnitrite-release of sodiumnitrit/poly-(d,l)-lactide (PDLLA) coated beta-tricalciumphosphate(âTCP) scaffolds was monitored over a period of 35d. Primary human endothelial progenitor cells (EPCs) and osteoblast-like SAOS-2 cells were incubated with sodiumnitrit for 24h. Proliferation and cell viability assays were performed with Cell-titerblue-assay and CyQUANT® Direct Cell Proliferation-assay. VEGF quantification was measured using ELISA.

Results and Conclusion:
The sodiumnitrit release was constant for 5 weeks. The released dose was able to increase the proliferation of EPCs. Furthermore, sodiumnitrit lead to an increase of VEGF expression in osteoblasts, which is a potent angiogenic factor. VEGF is known to differentiate EPCs to endothelial cells and VEGF can lead to endothelial cell migration. Both could improve rapid vascularisation of the scaffold subsequently in vivo. Thus sodiumnitrit/PDLLA-beta-TCP scaffolds could be a more qualified alternative compared to current bone substitutes.

Kategorie: Lecture

Rubrik: 1.Main Topic I
Abstract Nr.:65

Titel:Improvement of wound healing by boosting angiogenesis: studies in normoglycemic and diabetic mice

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Abstract:
Purpose. Angiogenesis is one of the most crucial processes involved in tissue repair and is considered to be an adaptive response to hypoxia. Diminished production of proangiogenic growth factors and decreased wound angiogenesis are thought to contribute to impaired wound healing in diabetic patients. The aim of our studies in normoglycemic and diabetic mice was to elucidate the effects of proangiogenic therapeutic strategies on wound closure and functional outcome.
Methods. For proangiogenic pre-treatment, a mixture of VEGF, bFGF, and PDGF was administered subcutaneously 3, 5, and 7 days prior to wounding in the first group, whereas the second group received three doses of PDGF alone. Wound sizes were assessed daily and the repaired tissues were harvested 7 days after wound closure. For the stretching experiments, a custom computer-controlled stretch device was designed and applied to the backs of C57BL/6 mice. Corrosion casting and three-dimensional scanning electron microscopy and CD31 staining were performed to analyze microvascular architecture.
Results. Subcutaneous priming with different growth factors as well as microdeformational wound therapy displayed a higher wound neovascularisation and faster time-to-closure. Analysis of microvascular architecture by corrosion casting highlighted the positive impact of subcutaneous proangiogenic priming on wound healing. Skin stretching was associated with increased angiogenesis as demonstrated by CD31 staining and corrosion casting where intervascular distances and vessel diameters were significantly decreased.
Conclusions. These results suggest a beneficial effect of pretreatment with combinatory growth factors and microdeformational wound therapy in murine wound healing.

Kategorie: Lecture

Rubrik: 5.Experimental Morphology
Abstract Nr.:66

Titel:The new lymphangiogenesis inhibitor esvegfr2 is expressed in embryonic tissues and down-regulated in advanced stage neuroblastoma.

Autoren: Becker J.(1),Wilting J.(1),

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Abstract:
Tumor metastasis is facilitated by hemangiogensis and lymphangiogenesis. Key regulators of angiogenesis are the members of the vascular endothelial growth factor (VEGF) family and their receptors (VEGFR). Recently, an endogenous soluble VEGFR-2 splice variant (esVEGFR-2) was described (Albuquerque et al. 2009). It does not bind VEGF-A, the hemangiogenic ligand of the membrane-bound variant, but rather VEGF-C, the key inducer of lymphangiogenesis.
We investigated esVEGFR2 in embryonic tissues and neuroblastoma (NB), a tumor of early childhood. NB is an embryonic tumour, derived from neural crest descending sympatho-adrenal progenitor cells. In clinical NB staging, infestation of distant lymph nodes is a critical sign, demanding classification into the most advanced stage 4.
We present that in human embryonic tissue esVEGFR-2 is expressed in sympathetic ganglia and the adrenal medulla, indicating its contribution to normal development of sympatho-adrenal organs. In NB expression is lower in the progressed stages 3 and 4. We also found that MYCN amplification, the most adverse prognostic marker in NB, correlates with lower esVEGFR-2 expression. Examination of esVEGFR-2 in NB cells after treatment with the differentiating agent all-trans retinoic acid (ATRA) reveals that ATRA-induced differentiation enhances esVEGFR-2 expression.
Tumor angiogenesis is not only achieved by the up-regulation of pro-angiogenic molecules (VEGF-A, VEGF-C) but also by the down-regulation of inhibitory molecules like esVEGFR-2. Additionally, esVEGFR-2 is associated with normal development of sympatho-adrenal tissues and can be induced in NB cell lines by differentiating ATRA treatment. Therefore sVEGFR-2 may be a potent regulator of lymphangiogensis in both normal development and tumors.

Kategorie: Lecture

Rubrik: 10.Developmental Biology
Abstract Nr.:67

Titel:Wnt5a-knock-out mice possess modified morphology of dermal lymphatic vessels

Autoren: Buttler K.(1),Pukrop T.(2),Wilting J.(1),

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Abstract:
The maintenance of tissue homeostasis and immune surveillance are important functions of the lymphatic vascular system. Lymphatic vessels are lined by lymphatic endothelial cells (LECs). Their defects may lead to functional disorders with poor physical condition and quality of life for patients. Our gene microarray analyses of isolated LECs in comparison with human umbilical vein ECs revealed regulation of approximately 1,200 genes. Among typical lymphendothelial markers, we observed Wnt5a highly up-regulated in LECs. About 20 Wnt ligands signal via specific receptors and intra-cellular pathways and are crucially involved in embryonic development. In the human, mutations in the WNT5a gene, and its receptor ROR2, are associated with the Robinow syndrome, characterized by similar abnormalities as the Wnt5a-knock-out (k.o.)-mice. Recent studies proved an important role of the Wnt signalling in the morphogenesis and differentiation of blood vessels. However, Wnt5a has not been investigated in lymphangiogenesis. Here, we studied lymphatic vessels of Wnt5a-k.o.-mouse embryos in comparison with wild-type mice. Immunohistological staining was performed to characterize the morphology and frequency of lymphatics in the skin and other organs. We show that size and shape of lymphatics and the structure of the skin are altered in k.o.-mice. We did not observe differences in the gut. Our studies suggest that Wnt5a may play a role in the development of superficial but not deep lymphantics, and indicate a function for Wnt5a in sprouting lymphangiogenesis in the dermis.

Kategorie: Lecture

Rubrik: 2.Main Topic II
Abstract Nr.:68

Titel:Nrf2 protects hepatocytes against oxidative liver injury and has a role in liver regeneration via stem cell activation

Autoren: Schellenberg T.(1),Fragoulis A.(1),Streetz K.(2),Rosen C.(1),Pufe T.(1),Wruck C.(1),

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Abstract:
Aim of this study was to elucidate the hepatic defence mechanisms employed against oxidative stress. In particular, the specific role of the redox-sensitive transcription factor Nrf2, the major regulator implicated in the endogenous defence system against oxidative stress, was investigated. We used the DDC-model that leads to oxidative liver damage resulting in chronic cholestatic liver injury and therefore resembles human diseases like sclerosing cholangitis and forms of metabolic liver diseases. Therefore, Nrf2-knockout and hepatic Keap1-knockout mice, which have a hepatocyte specific overactive Nrf2, were feed on 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC) containing diet and analysed over time by oxyblot-technique, TBARS-assay, western-blot, real time-PCR and immunohistochemistry. Mice deficient in Nrf2 showed significant more lymphocyte infiltration as wild type mice. Over time significantly more necrosis, apoptosis and cholestasis became evident in Nrf2-knockout mice. This was associated with stronger periportal oval cell activation. In contrast, mice with hepatocyte specific knockout of Keap1, the inhibitor of Nrf2, showed significant less oxidative liver damage, and less lymphocyte infiltration. Interestingly, Keap1-liver knockout leads to enhanced stem cell proliferation in response to DDC feeding. We show that Nrf2-dependent signalling pathway is crucial to protect liver from oxidative damage induced by DDC feeding. These findings indicate that the use of Nrf2-inducers might be considered as a novel therapeutic strategy to induce liver regeneration.

Kategorie: Lecture

Rubrik: 7.Neuroimmunology
Abstract Nr.:69

Titel:Gene expression profile of transforming growth factor-beta1 treated primary microglia

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Abstract:
TGF-beta has been described as a cytokine with potent anti-inflammatory functions in a variety of immune cells including T cells, macrophages and microglia. Microglia are the resident immune cells of the central nervous system (CNS) and are involved in physiological and pathophysiological processes. Several CNS pathologies, such as Alzheimer´s disease or Parkinson´s disease, are characterized by a strong microglia activation which has been shown to promote disease progression and inflammation-mediated neurotoxicity. Thus, the activation of microglia during the course of neurodegenerative diseases has to be tightly regulated and terminated to prevent additional degeneration of otherwise uninjured neurons. TGF-beta has been shown to inhibit LPS-induced microglia activation, thereby promoting survival of midbrain dopaminergic (mDA) neurons in vitro. In order to understand the molecular mechanisms underlying the TGF-beta-mediated regulation of microglial activity, we used cDNA microarrays to monitor gene expression profiles of primary microglia. In accordance with our hypothesis that TGF-beta acts as an inhibitor of microglia activation, we identified several TGF-beta-regulated genes that are involved in cell cycle regulation, interferon signalling, cytokine/chemokine signalling, inflammasome formation and extracellular matrix composition. Moreover, TGF-beta treatment resulted in regulation of genes that increase the sensitivity of microglia for TGF-beta and trigger the activation of latent TGF-beta. Taken together, these data strongly support the anti-inflammatory function of TGF-beta in microglia and introduce new TGF-beta-regulated genes involved in silencing of microglia activation.

Kategorie: Lecture

Rubrik: 7.Neuroimmunology
Abstract Nr.:70

Titel:Oligodendrocyte-derived cxcl10 in multiple sclerosis lesion formation

Autoren: Kipp M.(1),Berger K.(1),Amor S.(2),Harald N.(3),Cordian B.(1),

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Abstract:
Studies using human multiple sclerosis (MS) tissue displayed activated oligodendrocytes in close vicinity to activated microglia. Studies from our lab suggest that oligodendrocytes are implicated in microglia accumulation by the secretion of chemokines and thus actively participate in MS lesion formation. In this study we aim to identify oligodendrocyte-derived factors which are functionally involved in early microglia activation.
Stimulated OLN93 cells and primary oligodendrocyte cultures were analyzed for chemokine expression and release. Transwell chemotaxis and phagocytosis assays as well as gene expression studies were performed to investigate the effect of CXCL10 on microglia cells in vitro. Dynamic expression and cellular distribution of chemokines was analyzed in cuprizone-treated animals by Affymetrix® microarray, rt-PCR, in situ hybridization (ISH) and ELISA. Loss-of-function studies were performed to study the role of CXCL10 for microglia accumulation. MS lesions were analyzed for the presence of CXCL10-expressing oligodendrocytes.
Stressed oligodendrocytes express and secrete different chemokines in vitro, among CXCL10. In parallel, oligodendrocytes express CXCL10 mRNA/protein in a well defined toxic demyelination in vivo model. In this model, CXCL10 deficiency led to a significant decrease in the accumulation of Iba1-positive cells. While CXCL10 induces a pro-inflammatory microglia phenotype and microglia attraction, it does not affect microglial phagocytosis activity. The relevance of our findings was further demonstrated by the expression of CXCL10 by oligodendrocytes in MS lesions.
We showed that oligodendrocytes are indeed a source of chemokines under stress conditions and that they are functionally involved in microglia activation. We assume that oligodendrocytes are active modulators of neuroinflammatory processes.

Kategorie: Lecture

Rubrik: 8.Neuroregeneration/Neurodegeneration
Abstract Nr.:71

Titel:The role of nrf2 in myelin phagocytosis

Autoren: Rosen C.(1),Hilverling A.(1),Fragoulis A.(1),Dijkstra C.(2),Beyer C.(3),Kipp M.(3),Pufe T.(1),Wruck C.(1),

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Abstract:
Background: Microglia and macrophages are able to phagocytise myelin-debris, a prerequisite for remyelination in multiple sclerosis lesions. Nuclear factor-erythroid 2-related factor 2 (Nrf2) is a transcription factor maintaining the cellular defence against oxidative stress via binding to the antioxidant response element (ARE) within the promoter region of antioxidative genes. We tested the hypothesis that a deletion of Nrf2 leads to exorbitant oxidative stress in microglia cells and macrophages, leading to an impaired myelin phagocytosis and thus to an impaired remyelination capacity.
Methods: To examine in vitro whether Nrf2 plays a role in myelin phagocytosis, we used the murine microglia cell line BV2 and isolated primary macrophages from Nrf2-wild type and knockout mice. Nrf2 expression in BV2 cells was suppressed by RNAi. Myelin phagocytosis was observed by microscopy. Reactive oxygene species (ROS) production in cells after myelin phagocytosis was analysed with CM-H2DCF-DA method and Nrf2 activation was measured by ARE-luciferase reporter gene assay. Cell viability was detected with different cell viability tests. Remyelination properties of Nrf2-WT and KO mice were investigated immunohistological in a cuprizone-induced demyelination model.
Results: Nrf2-KO microglia and macrophages showed decreased cell viability and decreased phagocytosis compared to WT cells after myelin stimulation. Nrf2-KO mice showed a decreased content of myelin density and a disorder in myelin structure in corpus callosum compaired with WT mice.
Conclusion: Our data suggest that Nrf2 is necessary for proper myelin phagocytosis in vitro and remyelination in MS lesions.

Kategorie: Lecture

Rubrik: 6.Neuroanatomy/Neurobiology
Abstract Nr.:72

Titel:Corticosteroids impair remyelination in the corpus callosum of cuprizone-treated mice

Autoren: Clarner T.(1),Parabucki A.(2),Beyer C.(1),Kipp M.(1),

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Abstract:
Acute demyelinating relapses in multiple sclerosis (MS) are clinically treated by a short-term high dose corticosteroid (CS) therapy to suppress auto-aggressive inflammation, thereby improving symptoms and severity of disability. Despite this anti-inflammatory potency, the influence of CS on myelination/remyelination as clinical outcome is not known. Endogenous remyelination is tremendously important for neuroprotection of damaged axons and often fails during the course of MS.
In the present study, we aimed to elucidate the role of CS on spontaneous endogenous remyelination in the adult mouse brain using the well-characterized cuprizone demyelination animal model. Additionally, we investigated CS effects on astrocytes and oligodendrocyte progenitors (OPC) in primary cultures with respect to the expression of myelination-stimulating factors and myelin proteins.
Dexamethasone (Dex) and methylprednisolone (MP) caused a significant acceleration of OPC differentiation. CS increased the expression of genes involved in OPC proliferation such as basic fibroblast growth factor (FGF2) and platelet-derived growth factor-αα (PDGF-αα) and reduced levels of the pro-maturation factor insulin-like growth factor 1 (IGF1) in astroglia. OPC maturation stimulated through CS was completely blocked by FGF2 and PDGF-αα. MP treatment in vivo attenuated endogenous remyelination, whereas the re-population of the demyelinated corpus callosum with adenomatous polyposis coli-expressing oligodendrocytes was not altered. Numbers of microglial cells and astrocytes during remyelination were similar in placebo and MP-treated animals.
Our findings suggest that the exposure to CS has, in contrast to the well-known beneficial anti-inflammatory effect, a negative influence on spontaneous remyelination. These findings question the non-constraining therapeutic use of CS during MS.

Kategorie: Lecture