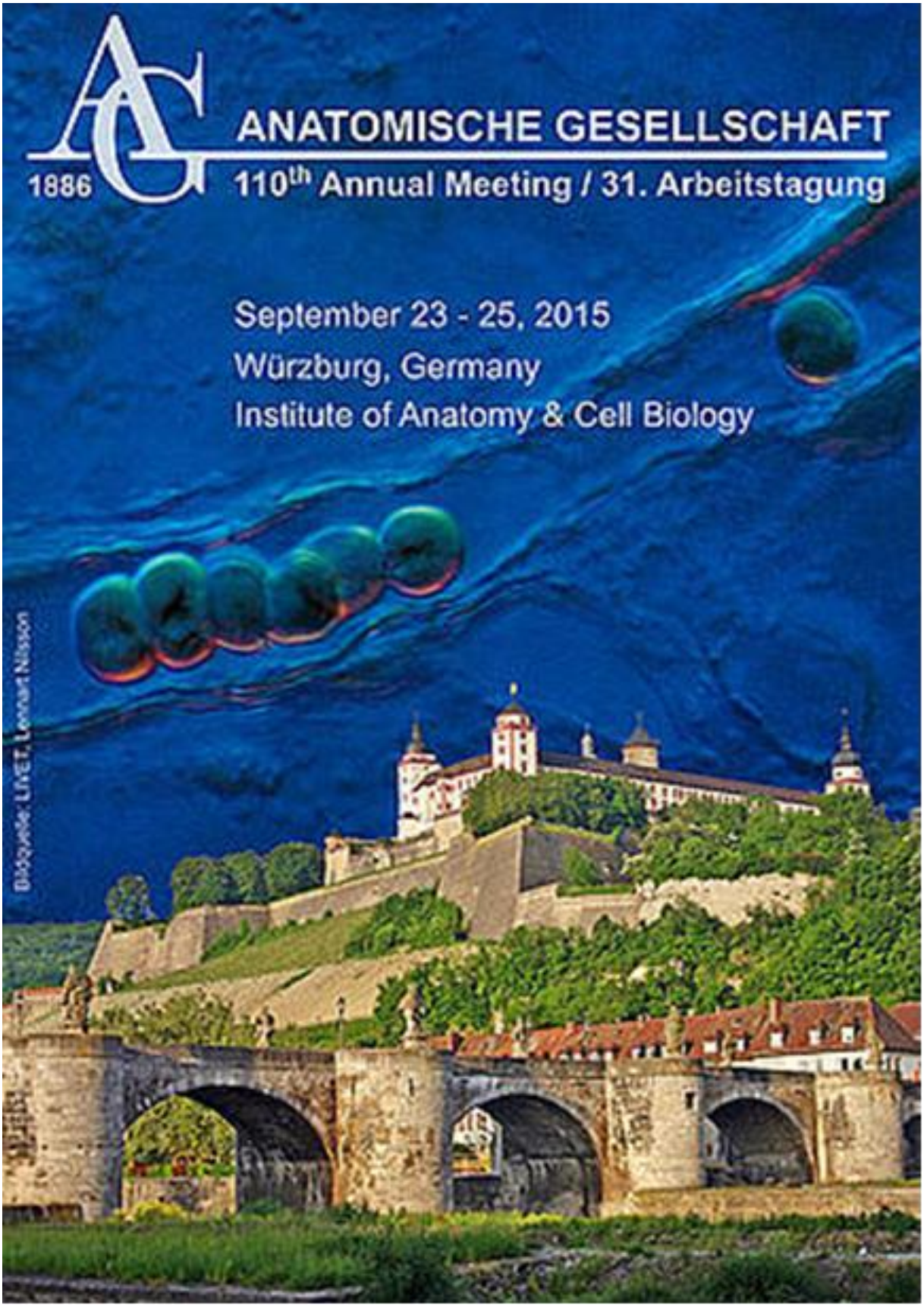
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Bildquelle: Lennart Nilsson

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Vortrag 1

Titel:Nrf2 promotes skeletal muscle regeneration

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Abstract:
Skeletal muscles harbour a resident population of stem cells termed satellite cells (SC). After trauma, SCs leave their quiescent state to enter the cell-cycle and undergo multiple rounds of proliferation, a process regulated by MyoD. For differentiation, fusion and maturation to new skeletal muscle fibers, SCs upregulate myogenin. However, the regulation of these myogenic factors (MRFs) is not fully understood. Here we demonstrate that Nrf2 plays a vital role in the expression of MRFs. In-silico analysis of the MyoD-promoter revealed an ARE-sequence. To assess its functionality, myoblasts were stimulated with the Nrf2-activator methysticin. Via Luciferase and ChIP assay, we detected a significant upregulation of an Nrf2-dependent MyoD-promoter activity. Knockdown of Nrf2 and Keap1 was performed to confirm these findings. To verify our in-vitro findings, we established a murine model of sterile hindlimb ischemia-reperfusion injury with Nrf2-wildtype and knockout mice. MyoD-mRNA and protein was significantly fainter expressed in Nrf2-knockout mice compared to wildtype. Consequently, Nrf2-deficient mice showed delayed muscle regeneration 14 days after ischemia compared to wild type. Hence, our results suggest a possible role of Nrf2 in muscle regeneration. Beside the well defined cytoprotective effect of Nrf2, we show here for the first time that Nrf2 has also a regenerative influence on skeletal muscle. The interaction of Nrf2 with MyoD leads to enhanced proliferation and adds to Nrf2’s regenerative capacities, providing new implications for pharmacological Nrf2 activators in skeletal muscle diseases.

Kategorie: Lecture

Vortrag 2:

Titel:Control of inhibitory circuit formation in the cerebellum by neurod2

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Abstract:
The cerebellum plays an important role in motor control. NeuroD2, a neuronal basic helix-loop-helix (bHLH) transcription factor, is expressed in cerebellar granule cells and molecular layer interneurons (MLI), suggesting a function in cerebellar network formation. To test this hypothesis, we examined Neurod2 null mutants using histological, electrophysiological, and behavioral approaches. These studies revealed that NeuroD2 is not essential for the functional integration of granule cells into cerebellar circuitry, most likely due to functional compensation by NeuroD1, a closely related bHLH protein. The investigation of inhibitory circuits showed that MLI (basket and stellate cells) in Neurod2 null mutants maintained expression of Pax2, a marker of immature interneurons, and failed to induce expression of parvalbumin, a marker of differentiated cells. Consistent with impaired differentiation, we observed that basket cell axons followed irregular trajectories in the ML. As a result, the formation of basket cell terminals and inhibitory input onto Purkinje cells were strongly reduced in Neurod2 mutants. We conclude that NeuroD2 controls a terminal differentiation program in basket cells required for directed axon growth and inhibitory synapse formation. Motor learning was impaired in Neurod2 mutants, consistent with the hypothesis that motor learning depends on MLI-mediated Purkinje cell inhibition.

Kategorie: Lecture

Vortrag 3:

Titel:Communication between distant epithelial cells by filopodia-like protrusions during embryonic development

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Abstract:
Long-range intercellular communication is essential for the regulation of embryonic development. Apart from simple diffusion, various modes of signal transfer have been described in the literature. Here, we describe a novel type of cellular extensions found in epithelial cells of the somites in chicken embryos. These filopodia-like protrusions span the subectodermal space overlying the dorsal surface of the somites and contact the ectoderm. We show that these protrusions are actin- and tubulin-positive and require Rac1 for their formation. The presence of glycophosphatidylinositol-anchored proteins and net retrograde trafficking of the transmembrane Wnt-receptor Frizzled-7 along the protrusions indicate their role in signal transport and distribution. Taken together, our data suggest a role of filopodia-like protrusions in mediating signaling events between distant epithelial cells during embryonic development.

Kategorie: Lecture

Vortrag 4:

Titel:Par-6 regulates apical-basal epithelial polarity by preventing the degradation of sdt/pals1

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Abstract:
Cell polarity is one of the key prerequisites for the establishment of multicellular organisms. The PDZ- and PB1-domain containing protein PAR-6 associates with PAR-3, aPKC and Cdc42 in a quaternary complex (PAR-aPKC complex), modulating the kinase activity of aPKC and activating Cdc42, thus controlling cell polarity in various tissues and organisms. Furthermore, PAR-6 has been described to regulate the positioning of a second apical complex, consisting of Crumbs and Stardust/Pals1. However, in Drosophila epithelial cells we found no robust association of PAR-6 with Crb/Sdt under endogenous conditions. We demonstrate that instead of a direct binding, PAR-6 modulates the stability of the Crumbs/Stardust complex. In PAR-6-mutant cells, Stardust is degraded, resulting in an intracellular mislocalization of Crumbs and subsequent disturbance of apical-basal polarity. These defects are independent of aPKC activity. In contrast, PAR-6 binds the proteasomal receptor Rpn13, thereby preventing the proteasomal degradation of Stardust. Downregulation of Rpn13 or a core component of the proteasome in PAR-6-mutant epithelial cells restores Crb/Sdt accumulation at the apical junctions. Similar, enhanced Rpn13-expression in cultured mammalian cells results in degradation of Pals1, leading to increased cell migration. Notably, overexpression of Rpn13 and loss of PAR-6 is observed in different types of carcinoma, suggesting a novel mechanism for tumor formation and enhanced metastasis. Indeed, we found in many biopsies of colon carcinoma a significant decrease in Pals1 expression. Thus prevention of proteasomal degradation is a new mechanism for the establishment and maintenance of apical-basal polarity in epithelial cells and a misregulation might be associated with cancer and enhanced cell motility during epithelial-mesenchymal transition.

Kategorie: Lecture

Vortrag 5:

Titel:Role for reelin in adult neurogenesis in the dentate gyrus

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Abstract:
The glycoprotein Reelin is significantly decreased in schizophrenia and major depression (Fatemi, 2008, review). Of note, adult neurogenesis in the dentate gyrus is reduced in depressive disorders, and we have shown some time ago that adult neurogenesis in the dentate gyrus is also reduced in reeler mutant mice deficient in Reelin (Zhao et al., 2007). Is Reelin required for adult neurogenesis in the dentate gyrus? This question cannot be addressed to reeler mice since these animals show severe malformations, including an abnormal architecture of the dentate stem cell niche. To address this issue, we established a conditional Reelin knockout mouse in collaboration with Hans Bock and Joachim Herz. To switch off Reelin expression selectively in the adult brain, we took advantage of the different expression patterns of Reelin. While Cajal-Retzius cells are the main Reelin source during embryonic development, at early postnatal stages interneurons take over. Hence, we used a cre mouse line, which expresses cre recombinase under the control of an interneuron specific promotor (Dlx5/6 cre). We report indeed that our conditional mouse model showed a normally developed dentate gyrus, including its stem cell niche. However, cell proliferation within the stem cell niche was significantly decreased. The data point to a direct role of Reelin in the regulation of adult neurogenesis and not to an indirect effect resulting from malformation of the stem cell niche. (Supported by the DFG: BR 4888/2-1; SPP 1757)

Kategorie: Lecture

Vortrag 6:

Titel:The cytokine leukemia inhibitory factor, lif, impairs cortical neuron development by counteracting neurotrophin signaling

Autoren: Engelhardt M.(1),Grabert J.(2),Patz S.(2),Wirth M.(2),Koenig J.(2),Jamann N.(1),Jack A.(2),Hamad M.(2),di Cristo G.(3),Berardi N.(4),Wahle P.(2),

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Abstract:
The actions of neurotrophins during the development of the mammalian visual cortex are well characterized, but whether the cytokine LIF plays a role during postnatal development remains elusive. LIF and neurotrophins employ overlapping signaling pathways like MAP kinase, yet the cellular consequences can be opposing. Here, we aimed at elucidating the action of LIF on neuronal differentiation and its potential modulation of neurotrophin signaling in rat visual cortex, utilizing PCR, immunoblots, immunofluorescence and morphometry in vitro in organotypic cultures (OTC) and with LIF infusions in vivo. Our data indicate that LIF has profound effects on phenotype specification and maturation of visual pathway neurons. LIF infusion into area 18 inhibits somatic growth of interneurons and pyramidal cells in area 17/18a, of projections neurons of the LGN and (transsynaptically) of tectal Calbindin-immunoreactive neurons. In OTC, interneuronal soma and dendrite growth is impaired. NPY is massively upregulated while GAD-65, Parvalbumin and Kv3.2 mRNA and protein are reduced. In pyramidal cells, maturation of dendritic spines and of the axon initial segment is impaired. Acute LIF injections in vivo and LIF stimulation in OTC immediately decrease NT4, but not BDNF mRNA. Despite of a massive compensatory increase of NT4 mRNA that occurred subsequently, LIF prevents NT4 and BDNF from activating c-fos expression and the MAP kinase pathway. Furthermore, NT4 fails to promote dendritic growth in the presence of LIF. Our results suggest LIF as a negative upstream modulator of NT4-trkB signaling, this way counteracting neurochemical and structural maturation of visual cortex neurons.

Kategorie: Lecture

Vortrag 7:

Titel:Synaptic integration of adult newborn dentate granule cells in the adult rat brain

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Abstract:
Adult neurogenesis of dentate gyrus granule cells (GCs) has been implicated in hippocampal forms of learning and memory. We combined viral techniques to label adult newborn granule cells (ANGCs) with high frequency stimulation (HFS) of the perforant path to analyze morphological properties and structural plasticity of ANGCs during integration into the adult rat hippocampus. We have shown previously that HFS elicited long-term synaptic potentiation which was accompanied by an age-related increase in the expression of activity-associated immediate early genes (IEGs) in ANGCs. We performed HFS of the medial perforant path after intrahippocampal injection of a GFP expressing murine leukemia virus to label maturing ANGCs or an adeno-associated virus to label mature GCs. To obtain complete dendritic trees 300µm thick frontal hippocampal sections were cut and GFP labeled GCs were imaged with a two-photon microscope. Dendritic trees were reconstructed from volume stacks using TREES Toolbox. The dendritic morphology of single identified GCs was analyzed in a layer-specific manner. Subsequently, sections were resliced to 50µm for immunostaining of IEGs and confocal imaging of dendritic spines. Our data revealed an age dependent gradual increase of dendritic arborization and spine density. Furthermore, we found a layer-specific increase in spine size in the stimulated middle molecular layer. Spine size is known to be correlated with synaptic weight indicating that a layer-specific long-term potentiation occurred in response to HFS. In summary, our data show that synaptic activity and plasticity is gradually achieved in the majority of ANGCs between the third and the 11th week.

Kategorie: Lecture

Vortrag 8:

Titel:Nuclear pore complex remodeling by p75ntr cleavage controls tgf-beta signaling and astrocyte functions

Autoren:Schachtrup C.(1),Ryu JK.(2),Mammadzada K.(1),Khan A.(2),Carlton P.(3),Perez A.(4),Christian F.(5),Le Moan N.(6),Vagena E.(7),Baeza-Raja B.(2),Rafalski V.(2),Chan J.(2),Nitschke R.(8),Houslay M.(9),Ellisman M.H.(10),Wyss-Coray T.(11),Palop J.(2),Akassoglou K.(2),

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Abstract:
Astrocytes play critical roles in neuronal activity and inhibition of regeneration. Here, we show that loss of the p75 neurotrophin receptor (p75NTR) rescues TGF-beta-induced hydrocephaly and astrocyte activation, decreased gamma oscillations and altered locomotor activity. Mechanistic analysis revealed that p75NTR regulated TGF-beta signaling by controlling P-Smad2 nucleocytoplasmic shuttling. Super-resolution microscopy and live cell imaging revealed that the cleaved p75NTR was a component of the nuclear pore complex (NPC) and that TGF-beta induces a dynamic redistribution of the cleaved p75NTR at the NPC. The cleaved p75NTR binds to FG-repeat-containing nucleoporins to facilitate Smad2 translocation into the nucleus. Loss of p75NTR or inhibition of gamma-secretase reduces TGF-beta-dependent nuclear accumulation of P-Smad2 and the secretion of proteoglycans that inhibit neurite outgrowth. Thus, NPC remodeling by regulated intramembrane cleavage of p75NTR controls astrocyte neuronal communication in response to profibrotic factors.

Kategorie: Lecture

Vortrag 9:

Titel:Muscarinic receptors 2 and 5 mediate a negative autocrine feedback mechanism in urethral brush cells activated by bitter stimuli

Autoren: Deckmann K.(1),Krasteva-Christ G.(2),Rafiq A.(1),Scholz P.(3),Baumgart S.(3),Bschleipfer T.(4),Kummer W.(1),

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Abstract:
Urethral brush cells (UBC) are cholinergic chemosensory cells functionally expressing the canonical bitter and umami taste transduction signaling cascade (α-gustducin, PLCbeta2, TRPM5). Recently, we described them as sentinels initiating local or reflexive protective responses at the entrance to the urogenital tract, communicating via acetylcholine (ACh) release. They also respond to ACh by themselves in that the bitter (denatonium) evoked increase in [Ca2+]i is enhanced in presence of a muscarinic/nicotinic blocker cocktail. Consecutively, an autocrine feedback mechanism seems to be involved in the regulation of the system sensitivity. Expression levels of muscarinic receptors (MR) were determined with RT-PCR, qPCR, and Deep Sequencing of murine UBC. Confocal laser scanning microscopy based intracellular [Ca2+]-imaging was used to record the specific response of UBC isolated from MR knockout animals after stimulation with denatonium without and in presence of a muscarinic/nicotinic blocker cocktail. In expression studies, mRNAs coding for all MR were detected, although only those coding for subtypes 2, 3, and 4 were consistently found. Inconsistent detection of M1R and M5R might be due to expression below the detection threshold. Comparable to control animals, M1R, M3R and M4R knockout animals showed an increase in intracellular calcium concentration in response to denatonium. In UBC isolated from M2/3R double knockouts or M5R knockouts, however, the inhibitors did not enhance the bitter response. Our results show an autocrine, cholinergic negative feedback loop on activated UBC mediated via M2R and M5R.

Kategorie: Lecture

Vortrag 10:

Titel:Tumor angiogenesis in the limelight: xct-mediated glutamate signalling takes center stage

Autoren:Savaskan N.(1),Fan Z.(1),Buchfelder M.(1),Broggini T.(2),Stampanoni M.(3),Meyer E.(4),Nitsch R.(5),Yakubov E.(1),Sehm T.(1),Eyüpoglu I.(1),

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Abstract:
Brain tumors are hallmarked by the increased angiogenesis, neuronal destruction and brain swelling. We could show that interference with the glutamate antiporter xCT/SLC7A11 reduces neuronal cell death and alleviates tumor-associated brain edema. However, the underlying mechanisms of xCT mediated brain swelling, i.e. cytotoxic or vasogenic effects remain to be uncovered. Here we show that diminished glutamate secretion through xCT silencing normalizes tumor vasculature and tumor-induced angiogenesis. Consistent with this, xCT overexpressing brain tumors enhance tumor vessels and increase cell death. Moreover, glioma-derived glutamate impacts directly on endothelial cells in a glutamate receptor-dependent manner. Glutamate attenuates Avastin-mediated anti-angiogenesis. xCT expressing tumors form functional vessels whereas xCT knock down in gliomas normalizes vessel function as revealed by intravital microscopy. By using inducible loss-of-function genetics in vivo, we demonstrate that endothelial-cell-specific glutamate knockout mice show suppressed endothelial sprout formation and vascular density. Thus, our data reveal that the transporter xCT and its substrate glutamate operate on endothelial cells promoting angiogenesis. We propose that targeting glutamate receptors in endothelial cells and xCT in gliomas provide a therapeutic roadmap for normalizing the tumor microenvironment and angiogenesis.

Kategorie: Lecture

Vortrag 11:

Titel:Age-dependent cellular and molecular changes in the nigrostriatal system

Autoren: Spittau B.(1),Zöller T.(1),Schädler S.(1),Krieglstein K.(1)
,
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Abstract:
The nigrostriatal system, which is composed of midbrain dopaminergic (mDA) neurons and their projections to the basal ganglia is involved in the control of motor behavior. Degeneration of mDA neurons and subsequent decrease in basal ganglia dopamine levels are the hallmarks of Parkinson's disease (PD). In a majority of cases, the reasons and underlying molecular mechanisms for degeneration of mDA neurons are not known, However, aging seems to be one of the most important risk factors for PD. Here, we used 6 months-old (young) and 24 months-old (aged) mice to analyse changes in the cellular composition of the nigrostriatal system and describe an age-dependent loss of mDA neurons and microglia, whereas the numbers of GFAP+ astrocytes increased in aged mice. Gene expression analysis of total brain tissues and functional analysis revealed activation of pathways related to inflammatory responses, immune cell trafficking and metabolic as well as neurological diseases in aged mice. However, gene expression analysis of CD11b+/CD45low microglia, acutely isolated from the nigrostriatal system of young and aged mice only partially resembled expression patterns from total tissues. In contrast, several microglial genes involved in alternative priming and sensing of pathogen-associated danger signals were upregulated in aged mice. Moreover, analysis of cell-surface activation markers CD36, CD206 and CD86 suggest that aged microglia are not primarily activated to trigger neuroinflammation-induced degeneration of mDA neurons, but rather adopt an alternative and neuroprotective phenotype in the aged nigrostriatal system.

Kategorie: Lecture

Vortrag 12:

Titel:Connectivity of cajal-retzius cells in the hippocampus

Autoren: Anstoetz M.(1),Haumann I.(2),Maccaferri G.(3),Luebke J.(4),

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Abstract:
Connectivity of Cajal-Retzius cells in the hippocampus Connectivity of hippocampal Cajal-Retzius cells, (CR cells) the function of which is well established in the context of cortical development, is far from being understood. To this end, we have combined patch-clamp recordings with intracellular biocytin-fillings in hippocampal slices of EGFP-tagged (CXCR4-EGFP) mice. According to their axonal projections, we found 3 subpopulations of CR cells cells in the hippocampus: non-projecting, local-projecting, and long-range projecting cells. Hence, the projections that target distant regions reach the subiculum, presubiculum, parasubiculum and even the entorhinal cortex. Furthermore, we found that synapses predominantly terminate on dendritic shafts of GABAergic neurons and rarely on spines. Paired recordings from presynaptic CR cells and postsynaptic GABAergic interneurons reveal large-amplitude glutamatergic unitary postsynaptic potentials. Our data show that (1) unlike in the neocortex, CR cells are able to project to more distant targets than previously believed; (2) CR cells are glutamatergic; (3) CR cells terminate at GABAergic interneurons. The input of a single CR cell on interneurons, which project to pyramidal cells, may have an impact on the susceptibility of the immature brain to epilepsy.

Kategorie: Lecture

Vortrag 13:

Titel: Optogenetics in the eye – development of a light-inducible gene therapy for pathological neovascularization

Autoren: Cambridge S.(1),Brandhorst E.(1),Hammes H.(2),

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Abstract:
Vascular complications due to abnormal growth of vessels or excessive leakage are the most common cause of vision loss. Progress in characterizing the molecular basis of diseases such as age-related macular degeneration (AMD) or diabetic retinopathy has led to major therapeutic advances. Treatments include regular injections of vascularization inhibitors such as antibodies against VEGF, but recently, gene therapies based on viral expression of antiangiogenic proteins are being evaluated in clinical trials. However, in the affected eye, neovascularization occurs not in all retinal regions so that global inhibition also impairs ongoing angiogenesis in the remaining healthy retinal tissue. We sought to overcome this limitation by developing a photoactivated gene expression paradigm for induction of anti-angiogenic transgenes by irradiation with light. This method is based on the Tamoxifen-inducible Cre/lox system and a reversibly inhibited, photo-sensitive (‘caged’) Tamoxifen analog. The idea is that locally restricted irradiation induces anti-angiogenic transgenes only in the diseased but not healthy regions of the retina. Importantly, after treatment, caged Tamoxifen washes out of the system thereby rendering the eye insensitive to unspecific photoactivation through normal daylight. We could readily induce transgenes with light in vitro and ex vivo while preliminary results also show successful in vivo photoactivation in the illuminated but not the control eye. After further optimization, we plan to use established mouse models of neovascularization to test the effects of localized induction of anti-angiogenic transgenes. Clearly, such an optogenetic approach offers exciting opportunities for gene therapeutic intervention in the eye.

Kategorie: Lecture

Vortrag 14:

Titel:The disorganized visual cortex in reelin-deficient mice is functional and allows for enhanced plasticity

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Abstract:
A hallmark of neocortical circuits is the segregation of processing streams into six distinct layers. The importance of this layered organization for cortical processing and plasticity is little understood. The so called reeler mouse, is a mouse line that is deficient for the glycoprotein reelin. Reelin is a key player in the establishment of cortical layers, thus absence of reelin results in a substantial disorganization of the vertical layering pattern of the cortex. We investigated the structure, function and plasticity of primary visual cortex (V1) of adult reeler mice and their wild-type littermates. We found that in V1 of the reeler cortex, cells with different laminar fates show a complex pattern of laminar disorganization with severe intermingling of different neuronal groups. Interestingly, the (vertically) disorganized cortex maintains a precise retinotopic (horizontal) organization. We could demonstrate that reeler mice have normal basic visual capabilities, but are compromised in more challenging perceptual tasks, such as orientation discrimination. However, reeler animals were able to learn and memorize a visual task. Surprisingly, we showed that reelin deficiency enhances visual cortical plasticity: juvenile-like ocular dominance plasticity is preserved into late adulthood. In the present study we combined histological, molecular and functional approaches in order to analyze the disorganized visual cortex and to demonstrate its basic functional properties. We found that substantial developmental plasticity compensated for the cellular disorganization, restoring substantial, however not normal function.

Kategorie: Lecture

Vortrag 15:

Titel:A general homeostatic principle following lesion induced dendritic remodeling

Autoren: Jedlicka P.(1),Platschek S.(2),Vuksic M.(3),Cuntz H.(4),Deller T.(1),

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Abstract:
Neuronal death and subsequent denervation of target areas are hallmarks of many neurological disorders including brain trauma, ischemia and neurodegeneration. In response to this loss of innervation, denervated neurons retract and remodel their dendritic tree. Although this appears to be a fundamental neuropathological process, the functional relevance of these dendritic alterations and their impact on neuronal electrical properties are poorly understood. Based on experimental data, we studied the electrotonic structure and excitability of dentate granule cells after entorhinal cortex lesion, a classical model system for studying denervation. Our computational models show that the experimentally observed morphological changes alone suffice to (1) precisely adjust the excitability enabling the remaining synapses to drive the neuron and (2) selectively enhance action potential backpropagation in the retracted dendritic area, which is likely to affect neuronal plasticity. Both these features are exquisitely homeostatically tuned as a result of general electrotonic passive properties of neurons. These principles are shown to be true for any given dendritic tree undergoing structural changes. Our results suggest that in addition to functional forms of homeostatic plasticity a novel form of structural homeostatic plasticity, i.e. homeostatic dendritic remodeling, is operating in denervated neurons. Supported by grants of Goethe-University, DFG, BMBF and Croatian Science Foundation.

Kategorie: Lecture

Vortrag 16:

Titel:Imaging of mucus clearance in the trachea of living mice by optical coherence microscopy

Autoren: Pieper M.(1,2),Schulz-Hildebrandt H.(2,3), Hüttmann G.(2,3), König P.(1,2),

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Abstract:
Mucus protects the airway epithelium by trapping inhaled particles and is removed by mucociliary transport or via coughing. Often mice are used to study lung disease with impaired mucus transport although they are thought not to cough. We examined if mice are capable to remove large amounts of fluid or mucus that need coughing to be removed in other species by using a newly developed optical coherence microscope (OCM).
We developed a custom-built OCM device providing a resolution around 1 µm with a field of view of 2 mm at up to 150 images/s. Images of the intact trachea and its mucus transport were recorded in anesthetized spontaneously breathing mice. NaCl solution (0.9% und 7%) or LPS were applied intranasally.
OCM resolved the detailed structure of the trachea and enabled measuring the airway surface liquid (ASL) through the tracheal wall. Before stimulation, the amount of ASL was only a few µm above the epithelium and remained constant. After application of 30 µl saline irrespective of its concentration, an early fast fluid removal with velocities higher than 1 mm/s was observed that removed the bulk amount of liquid. The ASL thickness increased transiently and quickly returned to prestimulation levels. LPS induced mucus release and an additional slow mucus transport by ciliary beating (100 µm/s) towards the larynx was observed.
Our results demonstrate that mice exhibit an effective mechanism of fluid removal that is equivalent to coughing and OCM is a suitable tool to study mechanisms of mucus transport in living animals.

Kategorie: Lecture

Vortrag 17:

Titel:Cyclosporine inhibits the neuronal k-cl co-transporter

Autoren:Zessin M.(1),Boldt C.(1),Deisz R.(1),Loyola S.(1),Strauss U.(1),Boehm M.(1),Blankenstein K.(1),Borschewski A.(1),Bachmann S.(1),Mutig K.(1),

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Abstract:
The calcineurin inhibitor cyclosporine is widely used for immunosuppression after solid organ transplantation, although it has in part serious side effects at the cardiovascular and neurological levels. Seizures may occur. Calcineurin interferes with neuronal excitability by modulating cellular chloride homeostasis. We hypothesized that cyclosporine affects the function of the major neuronal cation-coupled chloride cotransporter KCC2, thereby inducing hyperexcitability of neocortical neurons. Cytochemical localization revealed the co-expression of calcineurin and KCC2 in rat neocortical neurons. In rat brain, both products may interact with each other as shown by co-immunoprecipitation. Short term administration of cyclosporine (20 mg/kg; 1 to 4h) to rats increased KCC2 phosphorylation at the activating serine and tyrosine residues by approximately two-fold, which suggested increased activity of KCC2. In contrast, intracellular recordings of chloride homeostasis after iontophoretic Cl- loading revealed strong cyclosporine-induced prolongation of the Cl- extrusion time (+3.4s) which rather suggested a blockade of KCC2. To resolve this discrepancy we further evaluated the KCC2 inhibiting SPAK kinase. In concert with reduced KCC2 activity, substantially increased expression of SPAK was detected after cyclosporine application (+59%). In sum, our data suggest that inhibition of calcineurin by cyclosporine attenuates KCC2 function probably due to stimulation of the SPAK kinase. Taken into account the broad clinical use of cyclosporine our data have clinical implications.

Kategorie: Lecture

Vortrag 18:

Titel:The tumour suppressor lkb1 controls efficient replication by phosphorylating histone 2b

Autoren: Cidlinsky N.(1),Dogliotti G.(1),Reinders J.(2),Hallstein I.(1),Krahn M.(1),

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Abstract:
Liver Kinase B1 (LKB1) is a serine-threonine kinase, which functions as a “master” kinase, activating various downstream kinases, including MARK3, AMPK and SAD-Kinases. Thereby LKB1 regulates various cellular processes, in particular energy metabolism, cell proliferation and cell cycle control. Mutations in the gene encoding LKB1 (stk11) are linked to the “Peutz-Jeghers-Syndrome”, an autosomal dominant disorder reflected by the development of intestinal polyps and a high risk for intestinal and extraintestinal cancer. Moreover LKB1 is frequently inactivated in several cancer specimens and –cell lines. We have now identified a new mechanism of LKB1 regulating replication and chromosomal stability: LKB1 interacts with and directly phosphorylates Histone 2B (H2B). Phosphorylated H2B colocalizes with origins of replication and enhances binding of Minichromosome-maintenance (Mcm) complex proteins, which play a crucial role in the initiation of replication. Overexpression of a non-phosphorylatable H2B in cultured mammalian cells results in prolonged S-phase. Inhibition of G2/M check-point releases G2-phase delay but results in replication defects and chromosomal abnormalities which are observed in LKB1-deficient cancer biopsies, too. In Drosophila, expression of the non-phosphorylatable H2B-variant delays development and growth in larval stages (low dose expression) or produces early lethality (high dose expression). Taken together, we describe a new pathway how LKB1 regulates efficient replication by phosphorylation of H2B and binding of Mcm proteins. In context of carcinogenesis this mechanism might play a crucial role to control chromosomal stability which is frequently lost in cancer.

Kategorie: Lecture

Vortrg 19:

Titel:New aspects of central regulation of food intake

Autoren: Koch M.(1),Elmquist J.(2),Morozov Y.(3),Rakic P.(3),Bechmann I.(4),Cowley M.(5),Horvath T.(6),

Adressen:(1)University of Leipzig, Medical Faculty|Institute of Anatomy|Leipzig|Germany; email:marco.koch@medizin.uni-leipzig.de; (2)The University of Texas Southwestern Medical Center, Department of Internal Medicine|Division of Endocrinology & Metabolism|Dallas|USA; (3)Yale university, School of Medicine|Kavli Institute for Neuroscience|New Haven|USA; (3)Yale University, School of Medicine|Kavli Institute for Neuroscience|New Haven|USA; (4)University of Leipzig, Medical Faculty|Instutite of Anatomy|Leipzig|Germany; (5)Monash University, Department of Physiology|Obesity & Diabetes Institute|Clayton, VIC|Australia; (6)Yale University, School of Medicine, Section of Comparative Medicine|Program in Integrative Cell Signaling and Neurobiology of Metabolism|New Haven|USA

Abstract:
Specific groups of neurons in the hypothalamic arcuate nucleus regulate feeding behavior and energy metabolism by translation of nutritional signals and integration of this metabolic information into neuronal circuit activity. When activated, Agouti-related protein (AgRP)/neuropeptide Y (NPY) neurons rapidly induce feeding, while pro-opiomelanocortin (POMC) cells promote gradual onset of satiety. Cannabinoid receptor 1 (CB1) is a key regulator of central control of food intake. We have previously shown that pharmacological activation of CB1 increased feeding, and strikingly, CB1 activation also promoted neuronal activity of POMC cells (Koch et al. 2015; Nature 519:45-50). This paradoxical increase in POMC activity was essential for CB1-mediated feeding, since pharmacogenetic inhibition of POMC neurons abolished CB1-induced food intake. Here, we ask for the potential mechanisms behind CB1-driven POMC activation. The POMC gene encodes for the anorexigenic peptide, alpha-melanocyte-stimulating hormone, and the peptide, beta-endorphin. However, CB1 activation only affected and increased beta-endorphin levels by up regulation of specific prohormone convertases. Moreover, CB1-dependent mitochondrial adjustments in POMC cells, such as increased mitochondria/ER fusion and modulation of mitochondrial respiration, were observed. Within these adaptations, uncoupling protein 2 (UCP2) seems to play a major role since genetic ablation of UCP2 blocked CB1-induced POMC activation, beta-endorphin release and feeding. Taken together, our findings unmasked a novel role for POMC neurons in cannabinoid-driven feeding.
US-National Institutes of Health (DP1-DK098058, R01-DK097566, R01-AG040236 and P01-NS062686), American Diabetes Association, Klarmann Family Foundation, Helmholtz Society (ICEMED) and Deutsche Forschungsgemeinschaft SFB 1052/1 (Obesity Mechanisms).

Kategorie: Lecture

Vortrag 20:

Titel:Local proliferation of adipose tissue macrophages is driven by th2 cytokines

Autoren:Gericke M.(1),Braune J.(1),Weyer U.(2),Eilers J.(3),Bechmann I.(1),

Adressen:(1)Institute of Anatomy|Leipzig University|Leipzig|Germany; email:martin.gericke@medizin.uni-leipzig.de; (2)Institute of Anatomy|Leipzig University| Leipzig|Germany; (3)Carl-Ludwig-Institute for Physiology|Leipzig University|Leipzig|Germany

Abstract:
Obesity is frequently associated with a low-grade inflammation within adipose tissue (AT) and the increase of adipose tissue macrophages (ATMs) is linked to the onset of type 2 diabetes. We used three different mouse models of obesity and human samples to study the origin of ATMs in obesity. We show that besides monocyte recruitment from the blood stream, focal sites of inflammation around dying adipocytes, so-called crown-like structures (CLS), exhibit a microenvironment for macrophage proliferation. Interestingly, locally proliferating macrophages are not classically-activated (M1), but exhibit an immune phenotype, which is rather found after tissue damage (alternatively-activated; M2). In line, expression of the interleukin (IL)-4 receptor and its ligand IL-13 is elevated in AT from obese mice. By stimulation of living AT ex vivo with several cytokines, we can show that Th2 cytokines, such as IL-4, IL-13 and GM-CSF stimulate ATM proliferation. In contrast, Th1 cytokines, such as TNFa inhibit local ATM proliferation. Finally, AT from obese mice also exhibits an increased susceptibility to Th2 cytokine stimulation, shown by an increased phosphorylation of STAT6. We conclude that although the total number of ATMs increases dramatically in obesity, there are different mechanisms for the two main macrophage populations, M1 and M2. While is known that M1 macrophages are blood-derived and infiltrate the AT in a chemokine receptor 2 dependent manner, M2 macrophages proliferate on the site of adipocyte death due to local stimulation via the IL13-IL4 receptor axis. This work was supported by the DFG-SFB 1052 and the Helmholtz alliance ICEMED.

Kategorie: Lecture

Vortrag 21:

Titel:Vertebrate-specific presynaptic protein mover controls release probability at the calyx of held

Autoren: Körber C.(1),Horstmann H.(1),Venkataramani V.(1),Herrmannsdörfer F.(1),Dresbach T.(2),Kuner T.(1),

Adressen:(1)Functional Neuroanatomy|University of Heidelberg|Heidelberg|Germany; email:koerber@ana.uni-heidelberg.de; (2)Anatomy and Embryology|University of Göttingen|Göttingen|Germany

Abstract:
Mover is a vertebrate-specific presynaptic protein previously discovered in a yeast-two-hybrid screen of bassoon-interacting proteins. Mover is expressed in subsets of excitatory and inhibitory synapses throughout the brain, including hippocampus, cerebellar cortex and brain stem. While a more global expression in all terminals would suggest an essential contribution to synaptic transmission, the pronounced subset-specificity of Mover expression points towards a synapse-specific modulatory function. Biochemical characterization revealed that Mover is attached to the surface of synaptic vesicles and binds to calmodulin. However, the function of this protein remains unknown. We have previously shown that Mover is expressed in the rat calyx of Held, a giant terminal in the auditory brain stem. Here, we used shRNA mediated knock-down of Mover, to assess its function. Knock-down of Mover was highly effective as revealed by 3D immunohistochemistry. Calyceal Mover knock-down increased the amplitude of evoked EPSCs and accelerated and enhanced short-term depression. Direct measurements of presynaptic calcium currents as well as action potential waveform confirmed that these effects are not caused by altered calcium influx. Instead, using Ca2+-uncaging to generate defined Ca2+ concentrations in the calyx, we found that Mover knock-down increases the calcium sensitivity of neurotransmitter release. These findings are in line with a model depicting Mover as a negative regulator of release probability by decreasing the calcium sensitivity of the presynaptic release machinery. The expression of Mover in certain subsets of synapses may thus constitute a novel mechanism to tune the bandwith of synaptic transmission by regulating release probability.

Kategorie: Lecture

Vortrag 22:

Titel:Egfl7 as a therapeutic agent for the treatment of malignant brain tumors

Autoren:Schmidt M.(1),Dudvarski Stankovic N.(2),

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Abstract:
Patients with malignant brain tumors have a poor prognosis of survival. The most lethal form, glioblastoma multiforme (GBM), is non-curable despite of multimodal treatments. Patients have a median survival time of less than a year. Therefore new additional treatments are desirable. Recently, we characterized the protein epidermal growth factor-like domain 7 (EGFL7) as a regulator of stem cells (NCB 2009). EGFL7 is a proangiogenic factor that acts as a matricellular protein in the extracellular matrix to promote angiogenesis by its interaction with integrins (Blood 2013). Our findings identified EGFL7 as a putative modulator of the tumor microenvironment with the potential to promote tumor neoangiogenesis. This study aims to unravel the role of EGFL7 and the miR-126 localized within the gene in glioblastoma multiforme in order to explore its potential application as a novel medication of malignant glioma. The expression of EGFL7 in glioma biopsies was evaluated by microarray, qRT-PCR and promotor methylation studies. To determine the impact of EGFL7 and miR-126 on glioma in vivo, intracranial implantation models were performed and tumor neovascularization assessed by immunohistochemistry. Mechanistical data was compiled by biochemical methods, e.g. western blot or FACS. Our data unravel a tumor grade dependent expression of EGFL7 in glioma. EGFL7 has a severe effect on glioma growth in vivo, which is driven by its effect on blood vessel formation and morphology within the tumor. Molecularly, this effect is mediated by blood vessel integrins. Our findings provide evidence that EGFL7 can be exploited as a target to treat malignant glioma.

Kategorie: Lecture

Vortrag 23:

Titel:The enteric nervous system as an immune target in multiple sclerosis

Autoren:Wunsch M.(1),Weyer L.(2),Schwarz A.(2),Rodi M.(1),Wagner N.(1),Asan E.(1),Erguen S.(1),Kuerten S.(1),

Adressen:(1)Department of Anatomy and Cell Biology|University of Wuerzburg|Wuerzburg|Germany; (2)Department of Anatomy I|University of Cologne|Cologne|Germany; email:stefanie.kuerten@uni-wuerzburg.de

Abstract:
Multiple sclerosis (MS) is an autoimmune disease caused by the infiltration of autoreactive lymphocytes into the central nervous system (CNS). How these lymphocytes become activated remains unclear. Several experimental studies have shown that the gut is a key player in the pathogenesis of MS. Furthermore there is clinical evidence of bowel dysfunction like obstipation in a high proportion of MS-patients. This study aims at investigating the background of these clinical symptoms in a mouse model of MS, the MP4-induced experimental autoimmune encephalomyelitis (EAE). In order to determine if the enteric nervous system (ENS) in the small intestine is a possible immune target in EAE, we performed ultrastructural and immunohistochemical analysis of the intestinal wall in a total of 10 EAE and 10 control mice. In addition, we quantified serum antibody titers against enteric neuronal proteins. Our data demonstrate the infiltration of macrophages and lymphocytes into the myenteric plexus of the ENS and a progressive gliosis in chronic and acute EAE mice. Ultrastructural analysis showed neurodegeneration of the myenteric plexus accompanied by atrophy of the intestinal muscular layer in EAE. Furthermore, serum antibodies directed against enteric neuronal proteins were detected in EAE mice, but were absent in the controls. To our knowledge this is the first study providing evidence that the ENS is a possible immune target in MS. Future research will have to identify specific autoantigens of the ENS. It also remains to be elucidated whether MS patients themselves display ENS-reactive B and T cells in the blood.

Kategorie: Lecture

Vortrag 24:

Titel:Activation of the tumor suppressor kinase lkb1 is mediated by plasmamembrane targeting

Autoren: Kullmann L.(1),Dogliotti G.(1),Thiele C.(1),Dhumale P.(2),Mendel G.(1),Püschel A.(2),Krahn M.(1),

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Abstract:
The serine/threonine kinase Liver kinase B1 (Lkb1) regulates various cellular processes such as cell proliferation, energy homeostasis and cell polarity. Mutations in the human lkb1 gene are found in diverse cancers and are the cause of the Peutz-Jeghers syndrome, an autosomal dominant cancer disease. Many downstream processes have been described to mediate Lkb1's function but little is known about its upstream regulatory mechanisms, activating Lkb1 or targeting its kinase activity. Using Drosophila as an in vivo system as well as cultured mammalian cells we found that transient targeting of the kinase to the plasmamembrane via its farnesylation motif is sufficient for a residual functionality in vivo. However for its full activation a direct binding of Lkb1 to the plasmamembrane stably recruits the kinase to the (lateral) cortex of epithelia and neural stem cells of Drosophila to ensure its physiological function. Furthermore, in vitro assays demonstrate that the kinase activity of membrane-targeting deficient Lkb1 is almost abolished. Vice versa, addition of phosphatidic acid to recombinant Lkb1-complex strongly increases its catalytic activity. In mammalian cells, Lkb1-variants which are deficient of membrane-binding are not capable to fully activate the downstream target AMPK to prevent cells from apoptosis under energetic stress. Similar, primary neurons fail to determine multiple axons upon overexpression of membrane-targeting deficient Lkb1.

Kategorie: Lecture

Vortrag 25:

Titel:Mechanisms of posttranslational regulation of the renal na-k-2cl cotransporter

Autoren:Mutig K.(1),Borschewski A.(1),Boldt C.(1),Himmerkus N.(2),Bleich M.(2),Bachmann S.(1),

Adressen:(1)Institut für vegatative Anatomie|Charité Berlin|Berlin|Germany; email:kerim.mutig@charite.de; (2)Physiology| Christian-Albrechts-Universität zu Kiel|Kiel|Germany

Abstract:
The furosemide-sensitive Na-K-2Cl cotransporter (NKCC2) mediates NaCl reabsorption in kidney thick ascending limb (TAL) thereby promoting the urinary concentration. Its activity is crucially modulated by N-terminal phosphorylation and dephosphorylation reactions but the underlying mechanisms are still unclear. In this study we evaluated phosphorylation-dependent changes of NKCC2 surface expression and transport activity. Stimulation of NKCC2 phosphorylation in AVP-deficient Brattleboro rats using a vasopressin V2 agonist desmopressin (1 ng/g i.p. for 30 to 60 min) was associated with reduced ubiquitination and clathrin-mediated internalization of the transporter eventually increasing its surface expression. Similar results were observed in cultured TAL cells after stimulation of NKCC2 phosphorylation by low chloride hypotonic stress. GST pull down assays in rat kidney lysates using N-terminal NKCC2 mutants mimicking its constitutive (de)phosphorylation at functionally important residues (T96, T101, and S126) also suggested that NKCC2 phosphorylation attenuates its association with clathrin-coated pits. Electrophysiological evaluation of NKCC2 function in isolated perfused mouse TAL treated with either desmopressin or cyclosporine to stimulate NKCC2 phosphorylation revealed parallel increases of NKCC2 transport activity and affinity to chloride. These effects were blunted in TAL obtained from mice with constitutively low baseline NKCC2 phosphorylation due to genetic deletion of the sorting-related receptor with A-type repeats. In summary, our data suggest that NKCC2 phosphorylation may facilitate its function by two different mechanisms: (i) stabilizing the transporter within the plasma membrane via inhibition of its clathrin-mediated internalization and (ii) increasing the affinity of NKCC2 to chloride thereby stimulating its transport activity.

Kategorie: Lecture

Vortrag 26:

Titel:Evidence for an intrinsic catecholaminergic system in the murine bladder wall

Autoren: Pfeil U.(1),Goldenberg A.(1),Renno L.(1),Papadakis T.(1),Kummer W.(1),

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Abstract:
Voluntary control of micturition is complex and requires the coordinated action of smooth and striated muscles regulated by neuronal and non-neuronal mechanisms. Storage of urine depends on relaxation of the detrusor muscle and closure of the external urethral sphincter caused by norepinephrine released from postganglionic sympathetic nerves acting on inhibitory beta-adrenergic receptors in the bladder wall and excitatory alpha-adrenergic receptors in the upper urethra and bladder neck, respectively, as well as closure of the external urethral sphincter by somatic cholinergic motor nerve innervation. In contrast, voiding requires contraction of the detrusor muscle caused by acetylcholine released by parasympathetic postganglionic nerves acting on M3 receptors and opening of the internal and external urethral sphincter. Although long regarded as a passive barrier protecting the underlying smooth muscle from various substances of the urine, recent findings suggests an active involvement of the urothelium in regulation of bladder function. In this study we showed the expression of the catecholamine synthesizing enzymes in abraded urothelium and in smooth muscle cells in the bladder wall of mice and rats by RT-PCR. These results could be confirmed on protein level by immunohistochemistry where TH, the rate limiting enzyme in catecholamine synthesis, was demonstrated in urothelium and smooth muscle cells of the detrusor muscle. These findings provide first evidence for an intrinsic, non-neuronal catecholaminergic system in the bladder wall. Impairment of this intrinsic catecholaminergic system in the bladder may result in lower urinary tract symptoms such as urgency, frequency, and urge incontinence.

Kategorie: Lecture

Vortrag: 27

Titel:Ceacam1 interaction with enos and its role in regulation of endothelial barrier function

Autoren: Ghavampour S.(1), Boemmel H.(1),Wagner N.(1),Niedermeier N.(1),Lorey S.(1),Najjar S.(2), Erguen S.(1),

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Abstract:
Ceacam1 plays an important role in vascular hemostasis. Mice with Ceacam1−/− exhibit small atherosclerotic lesions in the aorta accompanied by impaired endothelial integrity and reduced NO level. One of the earliest steps of atherosclerosis initiation is endothelial dysfunction. We therefore, aimed to study a potential direct interaction between Ceacam1 and eNOS and its role in regulation of endothelial barrier function. Immunoprecipitation experiments using the Ceacam1 specific anti-body mCC1 could pull down the eNOS from lysate of WT mouse myocardial endothelial cell line (MyEnd) which express Ceacam1 at a high level. As control we used MyEnd cells from Ceacam1−/−. Then we performed eNOS and Ceacam1 co-localization on cultured WT and Ceacam1−/− MyEnd cells using immunocytochemistry and DuolinkR in situ technique. These analyses revealed a close co-localization of Ceacam1 and eNOS in WT MyEnd cells. Furthermore, immune electron microscopic studies showed a Ceacam1 accumulation in the caveolae of WT MyEnd cells. This finding further underlines the eNOS and Ceacam1 interaction. In addition, immunofluorescence staining revealed strong perinuclear eNOS signals in Ceacam1−/− MyEnd and en-face stained Ceacam1−/− aortic explants. Ceacam1−/− Myend cells displayed a significantly reduced levels of total and phosphorylated eNOS (Ser 1177). Additionally, eNOS tyrosine phosphorylation in response to TNF- α altered differently in WT and Ceacam1−/− MyEnds. Taken together, our data show for the first time a co-localization and direct interaction of eNOS and Ceacam1 in vascular endothelial cells and suggest that this interaction plays a crucial role in vascular endothelial integrity and barrier function.

Kategorie: Lecture

Vortrag 28:

Titel:Increasing cardiomyocyte cohesion is a novel function of sympathetic signaling

Autoren: Schinner C.(1),Schipp A.(1),Roetzer V.(1),Hartlieb E.(1),Vielmuth F.(1),Spindler V.(1),Waschke J.(1),

Adressen:(1)Institute of Anatomy and Cell Biology|Ludwig-Maximilians-Universität München LMU|München|Deutschland; email:camilla.schinner@med.uni-muenchen.de

Abstract:
Cardiomyocytes are electrically and mechanically coupled via intercalated discs (ICDs) composed of desmosomes, adherens junctions and gap junctions. The desmosomal cadherins desmoglein 2 (Dsg2) and desmocollin 2 and the classical cadherin N-cadherin are the transmembrane adhesion molecules of the ICD and provide intercellular adhesive strength. Gap junctions dynamically remodel to adapt to beta-adrenergic stimulation. It is unknown whether such rapid adaption also is evident for the adhesive function of the ICD. Atomic force microscopy revealed that symapthetic signaling enhanced the number of Dsg2-specific interactions along cell junctions and elevated their binding forces. This was accompanied by increased cell cohesion in cardiomyocyte cultures and murine heart slices. Enhanced Dsg2 staining along cell borders and increased junction length reflected reorganization of ICDs induced by beta-adrenergic stimulation. Symapthetic signaling induced PKA-dependent phosphorylation of the ICD plaque protein plakoglobin (Pg). In line with this, Pg deficiency abrogated junctional remodeling by adrenergic stimulation and prevented increased cohesion in cardiac slices. Here we provide evidence that symapthetic signaling strengthens cardiomyocyte cohesion in a Pg-dependent manner. This mechanism may be of high medical relevance because reduced Pg levels at intercalated discs was shown to be a consistent feature of arrythmogenic cardiomyopathy.

Kategorie: Lecture

Vortrag 29:

Titel:Wnt5a controls morphogenesis and differentiation of the embryonic lymphatic vascular system in the murine dermis

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

Adressen:(1)Institute of Anatomy and Cell Biology|University of Goettingen|Goettingen|Germany; email:buttler.kerstin@med.uni-goettingen.de; (2)Institute of Hematology and Oncology|University of Goettingen|Goettingen|Germany

Abstract:
Normal development and function of lymphatic networks are of great importance for tissue homeostasis and immune surveillance. To identify new regulators of lymphangiogenesis, we compared human lymphatic endothelial cells (LECs) and umbilical vein endothelial cells (HUVECs) with gene microarrays. Besides well-known LEC markers, we identified differentially expressed WNT signaling components, which play important roles in the morphogenesis of various organs, including the cardio-vascular system. WNT signaling has rarely been addressed in lymphangiogenesis. We found high expression of FZD3, FZD5 and DKK2 mRNA in HUVECs, and WNT5A in lymphangioma-derived LECs. The latter was verified in normal skin-derived LECs. With immunohistological methods we detected WNT5A in LECs, as well as the co-receptors ROR1, ROR2 and RYK in both LECs and HUVECs. We studied Wnt5a-knock-out-mouse embryos and observed major defects of the dermal lymphatics. Instead of regular lymphatic networks, isolated and dilated lymphatics developed. The mean size of ko-lymphatics and the LEC number per vessel were significantly greater, but the total area covered by lymphatics in the dermis and the total number of LECs were not significantly altered. The reduced number but increased size of initial lymphatics indicates a morphogenesis defect rather than a proliferation defect. Fluorescence micro-lymphangiography with FITC-dextran revealed a delay of lymph uptake into the initial lymphatics and the absence of a centripetal transport by lymphatic collectors. Ex vivo assays confirmed a morphogenetic function of Wnt5a in dermal lymphangiogenesis. Treatment with recombinant Wnt5a-protein of cultured embryonic Wnt5a-ko-dermis rescued the lymphatic networks. Our studies identify Wnt5a as a regulator of i) morphogenesis of initial lymphatics and ii) differentiation of lymphatic collectors in the murine dermis.

Kategorie: Lecture

Vortrag 30:

Titel:Mitochondrial malfunction caused by protein mistargeting

Autoren: Reichold M.(1),Broeker C.(1),Klootwijk E.(2),Stanescu H.(2),Renner K.(3),Reinders J.(4),Assmann N.(4),Oefner P.(4),Kleta R.(5),Warth R.(1),

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Abstract:
Renal Fanconi syndromes are a generalized inherited or acquired dysfunction of the renal proximal tubules. In a family with an inherited autosomal dominant form of renal Fanconi syndrome, we identified a heterozygous mutation in a peroxisomal protein called enoyl-CoA hydratase/L-3-hydroxyacyl-CoA dehydrogenase (EHHADH). This mutation leads to a de-novo formation of a mitochondrial targeting motif and, as a consequence, to an impaired mitochondrial function. To assess the pathomechanism of this renal disease, an inducible proximal tubular cell model was generated. Immunohistochemical analysis of these cells revealed mistargeting of the mutant EHHADH into mitochondria. Respirometric measurements exhibited a 35% decrease in O2 consumption when cells over-expressing the mutated protein were stimulated with octanoyl-carnitine as a specific substrate for mitochondrial beta-oxidation. In addition measurement of ATP content in these cells showed a significantly lower ATP/ADP ratio. EHHADH has a 30% homology with HADHA, which is involved in mitochondrial beta-oxidation as a part of the mitochondrial trifunctional protein complex (MTP). As confirmed by immunoprecipitation mutated EHHADH is incorporated into the MTP complex, replacing one or more of its alpha-subunits. The resulting MTP deficiency entails a characteristic accumulation of hydroxyacyl- and acylcarnitines. According to our hypothesis EHHADH is imported into the mitochondria where it is integrated into the MTP, thereby interfering with normal mitochondrial beta-oxidation. As a consequence, ATP generation in proximal tubular cells is impaired leading to a defect in energy dependent transport and thus to a renal Fanconi syndrome.

Kategorie: Lecture

Vortrag 31:

Titel:Repetitive magnetic stimulation induces plasticity of inhibitory synapses in mouse organotypic entorhino-hippocampal slice cultures

Autoren:Lenz M.(1),Muller-Dahlhaus F.(2),Wierenga C.(3),Szabo G.(4),Ziemann U.(2),Deller T.(1),Funke K.(5),Vlachos A.(1),

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Abstract:
Repetitive transcranial magnetic stimulation (rTMS) is a non-invasive brain stimulation technique that is used as a therapeutic tool in neurology and psychiatry. However, the cellular and molecular mechanisms underlying rTMS-based therapies remain not well understood. Using repetitive magnetic stimulation (rMS) of organotypic entorhino-hippocampal slice cultures we were recently able to demonstrate that 10 Hz rMS in vitro induces long-term potentiation of excitatory synapses and enlargement of dendritic spines on CA1 pyramidal neurons. To learn more about the effects of rTMS on network connectivity and excitation/inhibition balance, we here tested for the effects of the same 10 Hz protocol on structural, functional and molecular changes of inhibitory synapses. We used (1) immunohistochemistry for GABA-receptors and gephyrin scaffolds, (2) whole-cell patch-clamp recordings of inhibitory synaptic currents, including paired recordings of inhibitory interneurons and CA1 pyramidal neurons, (3) time-lapse microscopy of GFP-tagged gephyrin and (4) GABA-uncaging experiments, to demonstrate that rMS in vitro induces a Ca2+-dependent reduction in inhibitory synaptic strength, which accompanies the potentiation of excitatory synapses (2-4 h after rMS). Together with our previous work we conclude that rTMS is a potent tool for modulating excitation/inhibition balance in neuronal networks, through the induction of coordinated structural, functional and molecular changes of excitatory and inhibitory synapses. These findings disclose a mechanism how rTMS could assert its beneficial effects seen in patients with neurological and psychiatric diseases (supported by Federal Ministry of Education and Research, Germany; GCBS-WP1).

Kategorie: Lecture

Vortrag 32:

Titel:The role of the cxcl12 system in skeletal muscle during physical exercise

Autoren: Puchert M.(1),Adams V.(2),Engele J.(1),

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Abstract:
The chemokine CXCL12 and its primary receptor, CXCR4, not only promote myogenesis, but also muscle regeneration. CXCL12 chemoattracts CXCR4-positive satellite cells/blood-borne progenitors to the injured muscle and induces their proliferation. CXCL12 further promotes myoblast fusion, partially with existing myofibers, and induces angiogenesis in the regenerating muscle. Interestingly, the mechanisms underlying muscle regeneration are in part identical to that involved in muscular adaptation to intensive exercise. These similarities now prompted us to determine whether exercise would impact the CXCL12 system in skeletal muscle. We found that CXCL12 and CXCR4 are upregulated in the gastrocnemius muscle of rats that underwent a four week period of constrained daily running exercise on a treadmill. Double-staining experiments confirmed that CXCL12 and CXCR4 are expressed in MyHC positive muscle fibers. Moreover, these increases in CXCL12 and CXCR4 expression also occurred in rats with surgical coronary artery occlusion (CAO), implying that the muscular CXCL12 system is still active in patients with chronic heart failure and subsequently impaired physical performance. Expression of the second CXCL12 receptor, CXCR7, which presumably acts as a scavenger receptor in muscle, was not affected by training. Attempts to dissect the molecular events underlying the training-dependent effects of CXCL12 revealed that the CXCL12-CXCR4 axis activates anabolic mTOR-p70S6K signalling and prevents upregulation of the catabolic ubiquitin ligase MurF-1 in C2C12 myotubes; mechanisms known to promote muscle hypertrophy and to prevent atrophy of muscle fibers. Together, these findings suggest that the CXCL12-CXCR4 axis acts as a mediator of exercise-induced muscle growth and/or maintenance.

Kategorie: Lecture

Vortrag 33:

Titel:Embalming of cadavers with pyrrolidine derivatives: substitution of formaldehyde is possible.

Autoren: Hirt B.(1),Zeyher C.(1),Mack A.(1),Just L.(1),Gleiser C.(1),

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Abstract:
In the anatomy environment, formaldehyde is the most used chemical for fixing, preserving and storing human tissues and cadavers. However, since 2004 formaldehyde is classified as a human carcinogen by the International Agency for Research on Cancer (IARC), which means there is a need to survey alternative fixation agents. In this study we investigated a self-made pyrolidine derivative (PD) as a new fixative, which easily penetrates the tissue, inhibits the autolysis of the tissue, stabilizes the fatty tissue, provides good tissue qualities, is low in costs, not toxic, easy to handle and has a high antimicrobial effect. Using animal and human tissue the morphological quality of tissues and cadavers was examined by standardized questionnaires and cutometer measurements. Further the inhibition of enzymatic activities with FRET-based techniques and the antimicrobial inhibition of the PD-fixative were tested. Tissue sections were used for detailed morphological and immunohistochemical analysis. Fixation with PD provides cadavers and tissue with realistic visual and haptic properties which are suitable for both, macroscopic preparations and histological investigations. The fixation is based on the complete inhibition of autolysis enzymes and microorganisms. Fixation of tissue and cadavers with PD offer a non-toxic, cheap and easy to handle alternative to formaldehyde.

Kategorie: Lecture

Vortrag 34:

Titel:Why Wolfgang Bargmann lost his job as secretary of the Anatomische Gesellschaft in 1950

Autoren: Winkelmann A.(1),

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Abstract:
The list of past secretaries (Schriftführer) of the Anatomische Gesellschaft, founded in 1886, is not very long because four out of five served around 30 years each. The only exception is Wolfgang Bargmann (1906-1978), elected at the post-war re-foundation meeting in Bonn in 1949. He stepped down in 1950 after only one year in office, officially "due to a longer stay abroad". Newly available archival material, including Bargmann's correspondence of the time, reveals that this was not the true reason. As the first post-war secretary, Bargmann took over a difficult job, but was very successful in re-establishing links to the international scientific community at a time when German science had a very low reputation after the Nazi era. Nevertheless, there were quarrels with his predecessor Heinrich von Eggeling, who had started a new journal as the "official organ of the Anatomische Gesellschaft", but did not want to admit Bargmann as a co-editor. And there was a fierce, if covert debate about whether or not members with a particular role in Nazi Germany, including Max Clara and Eugen Fischer, should be re-admitted to the society. These arguments brought Bargmann into opposition to Otto Veit, the first acting president after the war. Eventually, Veit and other members of the board were unwilling to tolerate Bargmann's management of these disputes any longer, as his approach was felt to be dishonest and autocratic. These new aspects add to the picture of Wolfgang Bargmann, whose historical role has been controversial in recent years.

Kategorie: Lecture

Vortrag 35:

Titel:Anatomical landmarks to optimize catheter tip position in preterm and term neonates

Autoren: Eifinger F.(1),Vierzig A.(1),Koerber F.(2),Dötsch J.(1),Roth B.(1),Scaal M.(3),

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Abstract:
Insertion of a central venous catheter (CVC) is widely used in neonatology. Complications occur significantly more frequently in infants, especially neonates, than in adults. Pericardial effusion and life-threatening cardiac tamponade are both still associated with the presence of the catheter tip in the right atrium or the nearby pericardium. Since no anatomical studies of the pericardium in neonates are available and conventional chest radiography (CXR) shows that the heart shadow is not identical with the pericardial reflection, we seeked to determine the characteristics, topography, regional relationships and scale of the neonatal pericardium. The study was approved by the local ethics committee (No:14_288). In preterm and term cadavers (24-42 gestational weeks, n=20) preparation of the intrathoracic situs was performed with care and the pericardium lightly contrasted. CXR analysis after injection of contrast medium was used to compare key thoracic osseous structures with the contrasted pericardium. We demonstrated that below the 30th gestational week (GW) the 3rd costa describes the upper end of the right atrium and pericardium and corresponds with the insertion depth of a CVC; between the 30th and 42nd GW the 4th costa respectively. In conclusion, we identify an optimized catheter tip position with respect to fewer fatal complications caused by pericardial perforation in preterm and term neonates.

Kategorie: Lecture

Vortrag 36:

Titel:Fix for life, the development of a new embalming method to preserve lifelike morphology

Autoren:van Dam A.(1),van Munsteren C.(2),de Ruiter M.(2),

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Abstract:
In medicine, the use of embalmed bodies is essential for studying anatomy and for training surgical skills. Almost all embalming fluids worldwide in use contain formalin and phenol of which formalin is responsible for fixation and phenol for preservation of the cadaver. As the anatomical community is more aware of the occupational risks involved and of the high costs to reduce levels of exposure to these toxic substances, the interest for low-hazardous alternatives grows. Furthermore, in surgical training there is a growing demand for embalmed cadavers with lifelike morphology as a safer and more durable alternative for fresh (frozen) cadavers. The Fix for Life project aims to develop a low-hazardous embalming method preserving lifelike morphology. To achieve this, surplus rats were used to test 12 experimental embalming recipes/methods. For comparison, 2 rats were embalmed by applying conventional methods. A fresh frozen rat served as a control. After 2-3 months the morphological properties (consistency, colour, flexibility and suitability for dissection and/or surgical techniques) of the rats were rated by an expert panel. Embalming experiments performed on surplus rats show that the newly developed Fix for Life method can provide in well preserved cadavers with lifelike morphology for education and training uses without the risk of exposure to pathogens when using fresh cadavers or to toxic levels of formaldehyde and phenol when applying conventional embalming methods.

Kategorie: Lecture

Vortrag 37:

Titel:Combining time-lapse imaging and Fourier analysis - new tools to visualize contractile cell function under near-physiological conditions

Autoren:Mietens A.(1),Tasch S.(1),Eichner G.(2),Kügler R.(1),Middendorff R.(1),

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Abstract:
The function of contractile cells in their regular environment is difficult to assess and the study of isolated single contractile cells may not reflect their function within the different tissues. We developed a new and versatile approach to assess and characterize contractile cell function ex vivo by combining time-lapse imaging with Fourier analysis. Epididymis and testis tissue were immobilized in a collagen lattice and a time stack was acquired. By placing a virtual section through this image stack tubular wall movements were tracked over time. With each contraction translating into a little spike, the contractile pattern and frequency could be visualized and allowed further statistical analysis. In the epididymal duct, regular phasic contractile activity and movement of intraluminal contents could be observed. In contrast, seminiferous tubules showed a pattern of irregular and undulating wall movements which was further characterized by Fourier analysis. Fourier analysis assumes that any irregular curve can be expressed as a sum of sine curves with distinct frequencies. It allows to decompose a given irregular curve into a characteristic array of sine curves with specific frequencies that have different contributions to the original curve. By this technique, we could reveal a link between changing contractile patterns in seminiferous tubules which are associated to different spermatogenic stages. In general, time-lapse imaging in combination with Fourier analysis also allowed us to visualize and evaluate the contribution of different signaling pathways to the different (irregular) contraction patterns under nearly physiological conditions and to systematically investigate the influence of various drugs.

Kategorie: Lecture

Vortrag 38:

Titel:Resin-free scanning electron microscopy: preserved cytokeleton and immunogold labeling in an intact ultrastructural landscape

Autoren: Kuner T.(1),Horstmann H.(1),Venkataramani V.(1),

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Abstract:
Conventional transmission electron microscopy (TEM) of chemically fixed tissue embedded in resin suffers from at least two fundamental drawbacks: poor visualisation of fine cytoskeletal structures and limited morphological detail in antigen detection by immunogold-labeling. Here, we introduce a resin-free preparation of tissues in conjunction with scanning electron microscopy (SEM) revealing intact cellular cytoskeleton and immunogold labeling in a highly detailed ultrastructural landscape.

Kategorie: Lecture

Vortrag 39:

Titel:Synapses show differential plasticity depending on their history – monitoring individual living hippocampal mossy fiber synapses

Autoren:Drakew A.(1),Maier U.(1),Frotscher M.(1),

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Abstract:
Synaptic plasticity is generally studied using stimulation of afferent fiber tracts and somatic intracellular recording or extracellular recording of bulk synaptic responses. However, the various synapses on a single neuron have not experienced identical activation and as a consequence may be encountered in different states. In order to analyze the structure and function of individual hippocampal mossy fiber synapses, we combined single-bouton stimulation and 2-photon imaging of spines postsynaptic to the stimulated mossy fiber bouton. We visualized hilar mossy cells in entorhino-hippocampal slice cultures using patch clamp pipettes filled with Alexa 594. Fluo-4FF served to report calcium transients in individual postsynaptic spines. A second pipette containing Alexa 488 was advanced under 2-photon excitation towards the already labelled spines. Mossy fiber boutons presynaptic to these spines emerged as shadows covering the spines, contrasting against the stained extracellular space. Stimulation of the attached bouton evoked excitatory postsynaptic potentials (EPSPs) and calcium transients in the spine. We found a striking variability in the EPSP amplitudes as well as in the calcium responses, pointing to a varying contribution of different glutamate receptors. Furthermore, individual synapses were quite differently potentiated following a defined stimulation protocol. The morphology of the spines changed also to a varying extent following stimulation. These results suggest that hippocampal mossy fiber synapses reside in individual states that reflect previous activity at these synapses. M.F. is Senior Research Professor for Neuroscience of the Hertie Foundation.

Kategorie: Lecture

Vortrag 40:

Titel:Synaptic properties and subcellular targeting in dis-/inhibitory cortical circuits

Autoren: Witte M.(1),Walker F.(1),Feyerabend M.(1),Moeck M.(2),Staiger J.(1),

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Abstract:
GABA-releasing interneurons play a crucial role in the information processing within the primary somatosensory cortex, also known as the barrel cortex. Martinotti cells, a well-defined subclass of GABAergic interneurons, modulate the dendritic excitation of local pyramidal cells, thereby having direct influence on cortical output structures. In vivo data showed that Martinotti cells also receive strong and temporally precise inhibition during active whisking, which could be attributed to upstream connected vasoactive intestinal polypeptide (VIP)-expressing or parvalbumin (PV)-expressing interneurons. Using paired patch-clamp recordings we studied the synaptic properties, especially short term plasticity, of the two disinhibitory connections between VIP- or PV-expressing interneurons and Martinotti cells of layer 2/3 and 5 in the barrel cortex. In an optogenetic approach, we investigated the specific subcellular target region of VIP- and PV-fibers on Martinotti cells. PV-expressing interneurons target Martinotti cells in a dominant perisomatic pattern and show pronounced short term depression even at low frequencies. However, VIP-expressing interneurons innervate the complete dendritic tree of Martinotti cells and strongly facilitate at higher frequencies. Additionally, we show that PV-fibers innervate Martinotti cells in an intralaminar manner, in contrast to the more translaminar orientated VIP-fibers innervation. This argues for two fully distinct disinhibitory circuits, which should have a profound impact on the contribution of GABAergic interneurons to the processing of sensory information and the function of state dependent inhibition of Martinotti cells.

Kategorie: Lecture

Vortrag 41:

Titel:Axonal prg2 regulates thalamocortical circuit formation via phospholipid signaling

Autoren: Vogt J.(1),Cheng J.(1),Sahani S.(1),Nitsch R.(1),

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Abstract:
In the rodent, vibrissae provide input to specific thalamic nuclei, and these nuclei have well-defined connections with the somatosensory cortical region where sensory information is processed. These connections have a somatotopic organization where somatosensory information from each vibrissal reaches the thalamic ventrobasal complex and is relayed to a specific cortical barrel field in the primary somatosensory cortex. Here we show, that bioactive phospholipids and the phospholipid interacting molecule PRG-2 play an important role in the precise targeting of the thalamocortical projection. We applied axonal tracing in PRG-2-/- mice and found that developing thalamocortical projections ectopically entered the cortical plate and eventually innervated non-corresponding barrel fields in postnatal animals. Using organotypic slice cultures we could show that this defect was mediated by lysophosphatidic acid (LPA) which failed to repell PRG-2 deficient thalamocortical axons. However, PRG-2 electroporation in PRG-2-/- organotypic slice cultures was able to rescue the observed ectopic cortical innervation. Using a Y2H search and immunoprecipitation studies we identified radixin (RDX) as a PRG-2 interaction partner, and found both molecules expressed in thalamocortical axons. Furthermore, RDX was phosphorylated upon LPA stimulation and accumulated in growth cones of wild type but not of PRG-2 deficient neurons. To prove for the functional relevance of the thalamocortical misrouting, we performed electrophysiologic recordings and whisker-specific behavior tests finding a vibrissal-related learning deficit while the learning ability per se was preserved. In sum, our data show, that bioactive phospholipids and PRG-2 play an important role in correct targeting of thalamocortical fibers.

Kategorie: Lecture

Vortrag 42:

Titel:Bcl11a (ctip1) controls migration of cortical projection neurons through regulation of sema3c

Autoren:Wiegreffe C.(1),Simon R.(1),Peschkes K.(1),Kling C.(1),Strehle M.(2),Cheng J.(3),Srivatsa S.(4),Pentao L.(5),Jenkins N.(6),Copeland N.(6),Tarabykin V.(4),Britsch S.(1),

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Abstract:
During neocortical development, neurons undergo polarization, oriented migration, and layer type-specific differentiation. The transcriptional programs underlying these processes are not completely understood. Here we show that the transcription factor Bcl11a regulates polarity and migration of upper layer neurons. Bcl11a-deficient late-born neurons fail to correctly switch from multipolar to bipolar morphology resulting in impaired radial migration. We show that the expression of Sema3c is increased in migrating Bcl11a-deficient neurons and that Bcl11a is a direct negative regulator of Sema3c transcription. In vivo gain-of-function and rescue experiments demonstrate that Sema3c is a major downstream effector of Bcl11a required for the cell polarity switch and for the migration of upper layer neurons. Our data uncover a novel Bcl11a/Sema3c-dependent regulatory pathway used by migrating cortical neurons.

Kategorie: Lecture

Vortrag 43:

Titel:Connectomics of the nervous system of rats

Autoren: Schmitt O.(1),Eipert P.(1),Wree A.(1),

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Abstract:
The totality of connections of one or all components of the nervous system is called a connectome. Since the introduction of tract-tracing substances more than 40 years ago about 6000 publications are available, where tract-tracing findings were described in the peripheral and central nervous system of the laboratory rat. This connection data has been transferred as part of a meta-study in a database (http://neuroviisas.med.uni-rostock.de/connectome/index.php). The networks of the isocortex, hippocampus, thalamus, hypothalamus, basal ganglia, cerebellum, spinal cord and peripheral nervous system were studied by applying graph theory and statistical methods. Mono-, multisynaptic and collaterals connectivity has been detected ipsi- and contralaterally with corresponding semiquantitative connection densities. The connection data have been integrated into the conventional atlases of Paxinos and Watson or Swanson. The total number of detected monosynaptic connections is about 540000, 5500 multisynaptic and 2700 collaterals connections. Approximately 44000 connections are reciprocal. The lateral hypothalamus has most efferent (1382) and afferent connections (812) followed by the locus coeruleus and the gigantocellular reticular formation. In the connectomes mentioned above network properties were found that were previously unknown: a high but not full reciprocity, a large number of redundant shortest connections, a high selectivity of contralateral connections, characteristic patterns of connectivity of cortical layers and the spinal cord. Finally, special microcircuits have been identified in the networks that statistically occur not accidentally and represent a necessary network feature. For the first time a consistent database of tract-tracing data from all published tract-tracing studies is available. In addition to conventional network analysis the network data can directly transferred through a workflow for modeling population-based simulations.

Kategorie: Lecture

Vortrag 44:

Titel:The afferent connectome of vip expressing gabaergic interneurons in the mouse barrel cortex: a brain-wide atlas using monosynaptic retrograde rabies virus tracing

Autoren: Hafner G.(1),Wagener R.(1),Guy J.(1),Staiger J.(1),

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Abstract:
The very diverse population of inhibitory interneurons plays a fundamental role in shaping cortical activity. Vasoactive intestinal polypeptide (VIP) expressing interneurons have received attention as integrators of long-range input into the local cortical network. However, from which areas they receive projections has not been studied on a brain-wide scale. We visualized the brain-wide monosynaptic afferent inputs to VIP interneurons in the mouse barrel cortex using retrograde rabies virus tracing. This technique utilizes a triple virus strategy in conjuncture with a VIP interneuron-specific Cre driver line. Cre-dependent helper viruses allow to restrict the subsequent transduction with modified rabies virus specifically to Cre-expressing VIP interneurons. Rabies virus then spreads specifically to presynaptic cells but not any further and labels them with enhanced green fluorescent protein. More than 90% of cells presynaptic to VIP neurons were found locally within the barrel cortex. Other reliably labeled cortical areas included ipsilateral primary somatosensory (outside of whisker representation) and secondary somatosensory, visual, auditory, motor and cingulate cortex as well as contralateral barrel cortex. Subcortical projections originated from several thalamic nuclei, especially the ventral posteromedial nucleus, and from the basal forebrain. This work strengthens the concept of VIP interneurons as integrators of local- and long range input. The identification of cell type specific anatomical circuits will serve as a framework for future physiological studies dissecting the role of VIP interneurons.

Kategorie: Lecture

Vortrag 45:

Titel:Amyloid-precursor-protein maintains stability of neuronal dendrites in mouse organotypic entorhino-hippocampal slice cultures

Autoren:Vlachos A.(1),Willems L.(1),Lenz M.(1),Galanis C.(1),Jungblut D.(2),Wittum G.(2),Muller U.(3),Deller T.(1),

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Abstract:
The physiological role of the Amyloid-Precursor-Protein (APP) is not well understood. Recent work has implicated APP in the regulation of neural plasticity: changes in dendritic arborization, altered dendritic spine counts, and defects in LTP have been reported in APP-deficient mice. To learn more about the physiological role of APP in structural plasticity, we here assessed dendritic stability, i.e., the dynamic remodeling of dentate granule cell dendrites in organotypic entorhino-hippocampal slice cultures. We employed time-lapse imaging of slice cultures prepared from APP-deficient mice crossed with Thy1-GFP mice and compared them to their age- and time-matched GFP-expressing wildtype littermates. In these preparations absence of APP profoundly altered the stability of dentate granule cell dendrites. An increase in dendritic elongation and retraction was observed, while no major alterations in total dendritic length and dendritic arborization could be detected. To describe these findings, we propose a new classification of dendritic segments that takes their dynamics into account. Taken together, our data suggest that APP is important for the stability of granule cell dendrites. Alterations in APP or in APP processing may thus affect the structural stability of neuronal networks. (supported by Deutsche Forschungsgemeinschaft; FOR1332)

Kategorie: Lecture

Vortrag 46:

Titel:The role of plvap for the transendothelial transport in endothelial cells

Autoren: Herrnberger L.(1),Tamm E.(1),Hennig R.(2),Kremer W.(3),Hellerbrand C.(4),Göpferich A.(2),Kalbitzer H.(3),

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Abstract:
Purpose: Sinusoidal endothelial cells of the liver (LSEC) are characterized by the presence of open fenestrations. The molecular mechanisms that control the formation of sinusoidal fenestrations are unclear. Here we report that mice deficient in plasmalemma vesicle-associated protein (PLVAP) develop a distinct phenotype that is caused by the lack of fenestrations in liver sinusoids. Methods: Plvap-deficient mice were generated and sinusoidal endothelial openings were investigated by electron microscopy, portal vein perfusion with fluorescent FITC-dextran, clinical parameters and NMR. Results: PLVAP was expressed and localized in LSEC in wild-type mice, but not in Plvap-/- littermates. Plvap-/- mice suffered from a pronounced and highly significant reduction in the number of sinusoidal fenestrations, an observation that was seen by transmission and scanning electron microscopy. The lack of fenestrations was associated with an impaired passage of macromolecules such as fluorescein isothiocyanate (FITC)- dextran and quantum dot nanoparticles from the lumen of the sinusoids into Disse's space. Plvap-/- mice showed a pronounced hyperlipoproteinemia. By nuclear magnetic resonance (NMR) spectroscopy of plasma, the nature of hyperlipoproteinemia was identified as massive accumulation of chylomicron remnants, while the amounts of low and high density lipoproteins (LDL and HDL) were reduced. At around three weeks of life, Plvap-/- livers developed extensive multivesicular steatosis, steatohepatitis, and fibrosis. Conclusion: We conclude that PLVAP is critically required for the formation of fenestrations in LSEC. Lack of fenestrations caused by PLVAP deficiency substantially impairs the passage of chylomicron remnants between the lumen of liver sinusoids and hepatocytes, and finally leads to liver damage.

Kategorie: Lecture

Vortrag 47:

Titel:Mrna/mir microarrays from prostate cancer xenograft tumors reveal cd46 as a novel biomarker and suppressor of emt

Autoren: Lange T.(1),Samatov T.(2),Wicklein D.(1),Kupfernagel K.(1),Riecken K.(3),Schlomm T.(4),Sauter G.(5),Tonevitsky A.(2),Schumacher U.(1),

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Abstract:
The prognosis of prostate cancer (PCa) patients crucially depends on the development of metastases. The underlying mechanisms of metastasis formation, however, remain poorly understood. We therefore developed spontaneous metastasis xenograft models of human PCa in immunodeficient mice, which reflect the entire metastatic cascade with divergent metastatic potential. By performing genome-wide expression analyses of mRNAs and micro-RNAs (miRs) isolated from the xenograft primary tumors, we were able to correlate gene and miR expression levels with the metastatic potential. By this, we established a metastasis-related gene/miR network for human PCa. Our network strongly substantiates the common hypothesis that epithelial-to-mesenchymal transition (EMT) drives metastasis in PCa by means of several EMT-related genes/miRs up- or down-regulated with rising metastatic potential. In addition, we identified different genes not associated with metastasis so far such as the complement co-factor CD46. CD46 was down-regulated with rising metastatic potential and was particularly absent in lung metastases. In accordance, we identified decreased CD46 protein expression in clinical prostatectomy specimens as an adverse prognostic marker (n=1,800). shRNA-mediated depletion of CD46 in non-metastatic PCa cell lines induced the expression of different EMT-markers (N-cadherin, Slug, SPARC) and the invasive and migratory potential of the cells in vitro. In vivo, CD46 depletion abrogated the proliferative phenotype at the primary tumor site, whereas the migratory/invasive phenotype was enhanced as indicated by increased lung colonization of CD46-depleted PCa cells after intravenous injection. Hence, based on mRNA/miR analyses of PCa xenograft models, we identified CD46 as a novel biomarker and potential suppressor of EMT in PCa.

Kategorie: Lecture

Vortrag 48:

Titel:Plasma gelsolin stimulates corneal epithelial cell proliferation and supports corneal wound healing

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Abstract:
Introduction: Gelsolin is an actin modulator supporting epithelial regeneration and apoptosis. Intention of this project was to identify possible functions of plasma Gelsolin (p-GSN) at the ocular surface. Methods: Different ocular surface tissues, cells and tear fluid from healthy volunteers and patients suffering from dry eye disease (DES) were analyzed by means of western blotting, ELISA, RT-PCR and immunohistochemistry. The effect of recombinant (r) p-GSN on corneal epithelial cell proliferation and corneal wound healing were analyzed in vivo and in vitro by FACS-analysis as well as using a corneal defect model in mice. Results: p-GSN was expressed in all analyzed tissues. Significant concentration differences were measured between tear fluid from healthy volunteers as well as between different forms of DES. Stimulation of cultured corneal epithelial cells with rp-GSN led to significant higher cell proliferation rates after incubation with LPS or TNF-alpha. The in vitro and in vivo wound healing models indicated a significantly increased healing rate when treated with rp-GSN. Conclusion: Gelsolin is produced at the ocular surface and becomes part of the tear film/fluid. Here, it supports corneal epithelial cell proliferation and wound healing. Therefore, it has broad implications for developing novel therapeutic strategies for treating nonhealing wounds.

Kategorie: Lecture

Vortrag 49:

Titel:Structural insights into the serotonin- and muscarinic-induced smooth muscle constriction of murine caveolin-3 in airways

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Abstract:
Serotonin and acetylcholine are clinically relevant bronchoconstrictors involved in airway disease pathogenesis. We previously showed that cholesterol depletion attenuates muscarinic and fully abrogates serotonergic bronchoconstriction in mice. Cholesterol, together with caveolin proteins (cav-1, -2 and -3) which are binding partners for receptors and enzymes, is a structural component of caveolae. The process of cav assembling into caveolae requires cytoplasmic adapter proteins (cavin-1 to -4). We here addressed the role of cav-3, cavin-1 and -4 in bronchoconstriction utilizing, immunofluorescence, RT-PCR, electron microscopy, organ bath and videomicroscopic analysis of bronchial diameter in cav-3-/- and cav-3+/+ mice. Cavin-1 and -4 co-distribute with cav-1 and cav-3 in lung and airways, respectively. Loss of cav-3 cause increase in expression of cavin-1 and -4 in airway smooth muscle cells. Interestingly, caveolae abundance, assessed by electron microscopy and expression of EHD-2 (caveolae marker), did not significantly differ between cav-3-/- and cav-3+/+ mice. Muscarine-induced airway constriction was not impaired in cav-3-/- mice. Serotonin-induced constrictions, however, were lost in the trachea and partly decreased in intrapulmonary bronchi as evaluated by videomicroscopic analysis of precision-cut lung slices from cav-3-/- mice. In conclusion, cav-3 is essential for serotonin-induced tracheal constriction. In contrast, muscarine-induced tracheal and bronchoconstriction are neither exclusively coupled to cav-1, as we have shown previously, nor to cav-3. The increased expression of caveolae stabilizers, cavins, may explain the unaltered caveolar abundance in cav-3 deficient mice.

Kategorie: Lecture

Vortrag 50:

Titel:Decorin deficiency leads to glaucomatous changes in mice

Autoren: Schneider M.(1),Fuchshofer R.(1),Tamm E.(1),Iozzo R.(2),

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Abstract:
Purpose: An elevated intraocular pressure (IOP), caused by an increased outflow resistance to the aqueous humor in the trabecular meshwork (TM), is the major risk factor to develop glaucoma. TGF-beta2 and CTGF are involved in the molecular mechanisms causing an increased outflow resistance. In this study we investigate the influence of Decorin (DCN), a potential endogenous antagonist of TGF-beta2 and CTGF, on the tissues of the outflow pathway in vitro and in vivo. Material and Method: Anterior chamber angles of the eyes from human glaucomatous and healthy donors were stained for DCN. DCN-deficient mice and their wild-type littermates were analyzed at different ages. IOP was measured by tonometry. Total number of optic nerve axons was counted. Human TM cells were treated with DCN, TGF-beta2 and CTGF. In vivo and in vitro samples were analyzed by real-time-RT-PCR, immunoblotting and immunohistochemistry. Results: Staining intensity for DCN is reduced in the anterior chamber angle of glaucomatous donors compared to healthy donors. In mice, DCN deficiency causes an induction of CTGF and ECM proteins in the TM, leading to an increased IOP and a loss of axons in the optic nerve. DCN treatment leads to a decreased synthesis of ECM proteins, CTGF and TGF-beta in TM cells, while treatments with TGF-beta2 and CTGF reduce DCN expression. Conclusion: Our results strongly indicate that DCN is a negative modulator of TGF-beta2 and CTGF in the TM. Therefore therapeutic modulation of DCN expression might be a feasible approach to treat glaucoma.

Kategorie: Lecture

Vortrag 51:

Titel: Meprin beta degrades ctgf and attenuates its transcription via beta1-integrin shedding

Autoren: Arnold P.(1),Donandt J.(1),Prox J.(2),Schneppenheim J.(1),Koudelka T.(3),Wilms H.(4),Böttcher R.(5),Tholey A.(3),Fässler R.(6),Lucius R.(1),Becker-Pauly C.(2),

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Abstract:
Assembly and degradation of the extra cellular matrix (ECM) is tightly regulated by proteolytic enzymes, and anomalies result in pathological conditions such as fibrosis or Ehlers-Dahnlos Syndrome. Metalloproteases play an important role in ECM turnover and we have recently shown that the metalloprotease meprin beta cleaves off the C- and N-pro-domains of collagens I+III thereby inducing collagen deposition. Consequently, increased expression of meprin beta is associated with skin and lung fibrosis. Using an unbiased proteomics approach we were able to identify several other ECM substrates for meprin beta. Among these were the connective tissue growth factor (CTGF) and the transmembrane receptor beta1-integrin. CTGF plays an important role as a major regulator of ECM homeostasis and beta1-integrin interacts with various alpha-integrin receptors and mediates ECM to intracellular signaling. We now show that meprin beta degrades CTGF in the extracellular environment of fibroblasts and - at the same time - sheds beta1-integrin from the cell surface. When co-expressing meprin beta and beta1-integrin both were co-localized at the cell surface and this co-localization could be enhanced by the addition of the meprin beta inhibitor actinonin. In a next step we were interested if the shedding of beta1-integrin by meprin beta leads to transcriptional alterations of fibrosis associated genes. Interestingly we found CTGF to be the most down regulated gene (~30fold) revealing a dual way of CTGF regulation by meprin beta, where the protease acts (i) proteolytically on extracellular CTGF and simultaneously (ii) influences CTGF transcription via beta1-integrin shedding.

Kategorie: Lecture

Vortrag 52:

Titel:Deletion of ocular tgf-beta signaling mimics essential characteristics of diabetic retinopathy

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Abstract:
Purpose: The vascular phenotypes of diabetic retinopathy are important causative or contributing factors of vision loss. However, its molecular causes are not sufficiently clear. To identify the role of TGF-beta signaling for maintenance and proliferation of retinal vessels, we generated mice with a conditional deletion of the TGF-beta type II (Tgfbr2) receptor which is essential for TGF-beta signaling. Methods: Floxed Tgfbr2 mice were crossed with CAG-Cre mice, expressing the Cre recombinase under control of a tamoxifen-responsive chicken actin promoter. The successful deletion of Tgfbr2 was confirmed. Retinal structure and function were studied by light and electron microscopy, immunohistochemistry, fluorescence angiography, real time RT-PCR, and electroretinography. Results: Lack of TGF-beta signaling led to the formation of microaneurysms, leaky capillaries and hemorrhages in the retina. Retinal capillaries were not covered by differentiated pericytes and were surrounded by reactive microglia. In older animals, loss of endothelial cells and ghost vessels were observed, findings that correlated with the induction of angiogenic molecules such as VEGF-A, FGF-2, ANGPT2 and IGF and the accumulation of HIF-1 alpha. Consequently, retinal and vitreal neovascularization occurred, leading to retinal detachment, vitreal hemorrhages, neuronal apoptosis and impairment of the sensory function. Conclusion: TGF-beta signaling is required for the differentiation of retinal pericytes. Their lack initiates a scenario of structural and functional changes that mimics those of diabetic retinopathy. We conclude that Tgfbr2-/-;CAG-Cre mice constitute an animal model to study the molecular pathogenesis of retinal diseases associated with neo-angiogenesis such as diabetic retinopathy or age related macular degeneration.

Kategorie: Lecture

Vortrag 53:

Titel:A dual role of bmp antagonism during eye morphogenesis: implications for coloboma formation

Autoren:Heermann S.(1), Schütz L.(2),Lemke S.(2),Mateo J.(2),Rahhal B.(3),Krieglstein K.(3),Wittbrodt J.(2),

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Abstract:
The optic cup forms from an oval optic vesicle during vertebrate eye development. Using zebrafish as a model we show by 4D imaging that the lens-averted epithelium functions as a reservoir for the growing neuroretina through epithelial flow around the distal rims of the optic cup. Further this epithelial flow is essential for the development of the ventral optic cup and the generation of the optic fissure, a transient gap in the developing eye. BMP-mediated inhibition of the epithelial flow results in ectopic neuroretina in the RPE domain and severely affects optic fissure development resulting in coloboma, a persistent optic fissure. Furthermore we find a prominent coloboma phenotype in mice with a targeted inactivation of a TGFb ligand. Microarray analysis of wildtype and mutant eyes reveal a TGFb dependent regulation of BMP antagonists and in addition indicate remodeling of the extracellular matrix (ECM) during optic fissure closure. Using a newly developed TGFb signaling reporter in zebrafish, we demonstrate active TGFb signaling in the margins of the optic fissure, the region where we also find defined expression of the BMP antagonists. We hypothesize that TGFb is important for ECM remodeling during optic fissure closure, however, inhibited by BMP. To unleash its activity, TGFb locally induces BMP antagonists. Taken together we propose a dual role of BMP antagonism during eye development. First BMP signaling must be suppressed to enable tissue flow during cup formation. Secondly BMP signaling must be suppressed to allow TGFb mediated fusion of the fissure margins.

Kategorie: Lecture

Vortrag 54:

Titel:Norrin inhibits the development of glaucoma in dba/2j mice.

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Abstract:
Purpose: Norrin protects retinal ganglion cells (RGC) against an excitotoxic NMDA-mediated damage. Here we investigated if Norrin has similar neuroprotective properties on chronic RGC death in glaucoma. Methods: Transgenic mice with an overexpression of Norrin in cells derived from the optic cup under the specific control of a Pax6 promoter fragment (Pax6-Norrin) were generated in the genetic background of DBA/2J mice. Intraocular pressure (IOP) was measured, and morphological changes were investigated by light microscopy. Retinal expression of IGF-1 and phosphorylation of AKT was analyzed by real-time RT-PCR, western blotting and immunohistochemistry. Results: In Pax6-Norrin/DBA/2J mice, a moderate expression of Norrin and an activation of the Wnt/(beta)-catenin pathway were detected in the retina. In DBA/2J mice, IOP increased at the age of 8 months. In contrast, in Pax6-Norrin/DBA/2J littermates, IOP was not changed. Moreover, by light microscopy of the trabecular meshwork, a less severe damage of the aqueous humor outflow tissues was observed in Pax6-Norrin/DBA/2J mice than in DBA/2J littermates. The quantification of RGC axons in the optic nerves showed significantly more axons in Pax6-Norrin/DBA/2J mice compared to DBA/2J littermates. In addition, levels of IGF-1 mRNA and pAKT were significantly increased in retinae of Pax6-Norrin/DBA/2J mice compared to DBA/2J controls. Conclusions: Transgenic overexpression of Norrin reduces glaucomatous damage in DBA/2J mice most likely by IOP reduction and an increased expression of IGF-1 which in turn enhances AKT phosphorylation.

Kategorie: Lecture