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29.09.2010 bis 01.10.2010

Vorträge des Satellitensymposiums „Anatomie im Nationalsozialismus“

Vortrag 1

Rubrik: 12. Anatomie im Nationalsozialismus
Abstract Nr.:

Titel: "Anatomy in the Third Reich: A review of the current status of research"

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Abstract:
Although it is known that anatomists working in Germany during the Third Reich used bodies of victims of the National Socialist (NS) regime for dissection and research, a comprehensive history of the anatomy in the Third Reich has not yet been written. Such a history has to address questions about the anatomists' political activities and ideological support of NS policies as well as about the sources of the body supply for anatomical institutions. This review of the currently available literature gives a first outline of the field. The main conclusions are:
1. German anatomists were both, supporters and victims of NS policies. In a survey of 190 anatomists, political data were available for 128. Of those, 51 were dismissed, emigrated or suffered other career interruptions for racial and political reasons. Of the 77 remaining anatomists, 67 were members of at least one of the NS organizations, with varying political activity.
2. Without exception, all anatomy departments used the bodies of NS victims for dissection and research, including those of executed prisoners. This practice was independent of the political affiliation of individual anatomists.
3. Third, German anatomists under the leadership of Eugen Fischer were instrumental in developing the biological foundation of racial hygiene, which became the scientific justification of NS policies such as sterilization, extermination and mass murder. Anatomists were involved in the research, practice and teaching of racial hygiene.
This first overview shows that the interactions between German anatomists and the NS regime were complex and need further exploration.

Kategorie: Vortrag

Vortrag 2

Rubrik: 12. Anatomie im Nationalsozialismus
Abstract Nr.:

Titel: "„... a corpse can be put at your disposal“ – Judicial and administrative basis for the supply with bodies from executions in the Third Reich"

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For more than 15 years, the memorial site „Roter Ochse“ in Halle/Saale, Germany, has studied documents of special and military courts in Mitteldeutschland (central Germany). Hundreds of death sentences have been executed during the last years of the war in the former state prison of Halle. The resulting corpses were used in biomedical research and teaching. The number of executions was marginal before 1933 but increased steadily after the Nazis seized power. The judiciary delivered an increasing number of death sentences against political opponents and persons who were to be eradicated from the “Volksgemeinschaft” (national community) according to racist ideology. However, the corpses were not distributed evenly to each of the anatomical institutes. The distribution depended on factors such the distance of the institute to an execution place, the court responsible for the sentence, and whether the state or relatives had the right to dispose of the bodies. At the beginning of the year 1939, the Reichsjustizministerium (department of justice) issued a decree that changed the distribution process of corpses. As a rule, after the responsible ministry informed the anatomical institute of a pending execution, the institute confirmed the pick-up day of the body. Details of the actual delivery of bodies can be found in execution protocols, reports by execution overseers, receipts of body deliveries, body registers of the institutes, etc.. This lecture will review the historical progression of ministerial decisions and demonstrate how administrative documents can be used as a point of departure for current research projects.

Kategorie: Vortrag

Vortrag 3

Rubrik: 12. Anatomie im Nationalsozialismus
Abstract Nr.:

Titel: „Corpses of executed victims in the Anatomical Institute of the University of Giessen“

Authoren: Sigrid Oehler-Klein, Volker Roelcke
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Abstract:
Ferdinand Wagenseil, the director of the Anatomical Institute of the University of Giessen since 1940, converted the Institute into an asylum for racially or politically persecuted medical students whereas his predecessors had acted in tune with national socialist policies. But Wagenseil had to face the effects of radicalisation in the course of World War II: In 1942, the delivery of corpses of victims, executed because of political resistance or even marginal breaches of law, extremely increased, parallel to the number of medical students due to the demand for physicians in the war: Up to 600% more students had to be instructed. Although Wagenseil was shocked by the praxis of executions, the relative lack of corpses induced him to complain about the insufficiencies of anatomical teaching. In consequence, the „Reichsstatthalter” asked the General State Prosecutor for a proportional increase in the number of corpses to the nearby universities.
On the basis of the anatomical registration book in Giessen, which shows specifically marked entries, the places of delivery (and sometimes the exact causes of death) the number of delivered executed victims can be reconstructed. Following a comparison with a list of executions performed in the prison of Frankfurt-Preungesheim the names and the sentences of over 50 % of the victims are known today. Historical inquiries disclosed individual fates, like that of a slave-labourer from Poland, who had been hanged because of a “violation of racial law”, or the decapitation of a couple who had distributed flyers against national socialist policies.

Kategorie: Vortrag

Vortrag 4

Rubrik: 12. Anatomie im Nationalsozialismus
Abstract Nr.:

Titel: „Hermann Stieve and the use of execution victims for research“

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Abstract:
Berlin anatomist Hermann Stieve (1886–1952) was one of Germany's leading researchers into female reproduction. Some of his scientific insights derived from histological investigations on the genital organs of executed women. These investgations benefited from the extreme increase in executions during the Nazi regime. While Stieve's research made a relevant contribution to contemporary scientific debates, his use of the organs of execution victims, some of them resistance fighters, meant that he participated in the Nazi justice system, which was a system of injustice. Moreover, he used the terror of death row as a sober scientific variable for his research on the nervous influences on genital morphology. Even if Stieve did not interfere with the execution victims during their lifetime, his use of their bodies meant a critical interference with their biographies. If Stieve's contemporaries criticised this interference at all, it was not primarily because the bodies stemmed from executed women but because certain victims were thus treated like "common criminals".
Hermann Stieve never joined the NSDAP, and the case of his research remains multifaceted. It can therefore serve as a good starting point for a discussion about how we want to judge anatomical research during the "Third Reich". This discussion should produce clear ethical criteria for such a judgement, acknowledge possible historical change of these criteria, and formulate lessons for today's researchers at a time when body donation programs have relieved anatomists of the stigma of being part of a punishment process but still confront them with ethical challenges.

Kategorie: Vortrag

Vortrag 5

Rubrik: 12. Anatomie im Nationalsozialismus
Abstract Nr.:

Titel: „Bavarian anatomies and the executed (1933-1948)“

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Abstract:
During National Socialism the bodies of the executed were used in Bavaria on a large scale for anatomical research and teaching as in other regions of Germany. Since 1933 the institutes of Munich, Wurzburg and Erlangen had played a decisive role in the usage of the dead. They started a macabre competition for the bodies. In very rare cases, when relatives were courageous enough, it was possible however to get the dead out of the institutes for a personal funeral and grave as the example of the resistance fighters of the White Rose shows.
What happened to the dead, who had been executed in the Third Reich, in the anatomical institutes after the liberation in 1945? The situation was determined by several conflicting factors. On the one hand anatomists complained about an extensive deficit of bodies for dissection courses, so that they tended to use the dead for medical purposes. Their position was supported by the popular view on resistance fighters who were seen as traitors to the fatherland and not as honorable political victims. Of course, on the other hand this opinion was contradicted in particular by the survivors of the Nazi regime and the allies. Furthermore, relatives and aid organizations had still been searching for the dead victims of German terror. These factors created a situation full of tension, culminating in 1947/48. In this context the crucial role of Philipp Auerbach, state commissioner for religious, political and racial victims of the Nazis in Bavaria, is pointed out and dignified.

Kategorie: Vortrag

Vortrag 6

Rubrik: 12. Anatomie im Nationalsozialismus
Abstract Nr.:

Titel: „Corpses of executed victims in the Anatomical Institute of the University of Giessen“

Rubrik: 12. Anatomie im Nationalsozialismus
Abstract Nr.:

Titel: „A *“Nacht und Nebel” Aktion*: The Removal of Specimens from German Anatomical and Medical Collections in the early 1990s“

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Abstract:
In the late 1980s German medical institutions came under pressure to dispose of body parts that derived from executed victims under National Socialism. These specimens were primarily brains from “euthanasia” killings. There were also accusations that collections included specimens from concentration camps. After a phase of refusing to respond to the accusations of social critics, pressure from American medical circles resulted in a change to rapid but uncoordinated disposal. Each institution disposed of the body parts, as it thought best. Rarely was there any investigation of provenance and attempt to identify the specimens. Relatives or the public were not invited to any burial and commemoration. The whole “disposal” appears to have been deliberately done with as little documentation as possible and in a manner that was only co-ordinated at an informal level. The University of Tübingen was exceptional in having a full commission; after a ten year delay the University of Vienna organised the Pernkopf commission and memorial, and the universities of Graz and Innsbruck have been even slower to respond. Although the removal of specimens belongs to recent history, documenting the concerted disposals, let alone the provenance of the specimens is now difficult, because of the lack of any systematic survey of collection contents. Fuller documentation remains necessary.

Kategorie: Vortrag

Vortrag 7

Rubrik: 12. Anatomie im Nationalsozialismus
Abstract Nr.:

Titel: "Dissecting the history of anatomy in the Third Reich: A personal account"

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Abstract:
In 1989 it was revealed that anatomical and research institutes in Germany had in their collections specimens from the N-S period which had been derived from victims of N-S terror. The revelation resulted in an effort to hold a commemoration that acknowledged Germany's role as the birthplace of modern medical science and addressed the moral implications of the exploitation of victims of state terror by medical science during the Hitler regime. The resulting *Call for an International Commemoration* was not received positively by the responsible parties in the Federal Republic.
One institution, the University of Tübingen, held an investigation with a published report and commemoration (1990). Over the ensuing two decades there have been further allegations, revelations and investigations concerning the exploitation of victims of state terror by anatomical institutes and research organizations such as the Institute of Anatomy of the University of Vienna, the Vienna Psychiatric Hospital, the Vienna Museum of Natural History and the Anatomical Institute of the Charité Hospital in Berlin. Allegations against other institutions remain outstanding without any investigation or accounting. It is to be assumed that every collection of human specimens in countries or territories that had once been part of the Third Reich may include such misbegotten specimens.
The author will provide a personal account of the responses of various government and academic/research organizations to attempts to have a proper accounting of the role played by German and Austrian academic medical and research establishment in the exploitation of victims of Nazi terror.

Kategorie: Vortrag

Vortrag 8

Rubrik: 12.Anatomie im Nationalsozialismus
Abstract Nr.:

Titel:„"To collect and Process material". August Hirt and the “exceptional opportunities to purvey corpses” for anatomical institutes during National Socialism“

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Abstract:
From the beginning anatomical institutes always suffered from a shortage of corpses for research and teaching purposes. This was to change in the Nazi period. In a letter to the rector of his university, for example, the director of the Tuebingen anatomical institute Robert Wetzes confirms that “today in the war and perhaps further on during a certain postwar period exceptional opportunities to purvey corpses could be counted on”.
On December 13th, 1942, August Hirt, the director of the anatomical institute at the “Reichsuniversity” of Strassburg, wrote to the managing director of the SS research community “Ahnenerbe” (forefathers’ heritage) Wolfram Sievers: A meeting of anatomical scholars in Tuebingen had left him with a “further workload”, meaning: “In that meeting the suggestion came up that anatomists should collect and process material, just as we had already determined in the Beger assignment. Gradually it is dawning on others as well, that something can be done here.” In a handwritten note he adds that he had been commissioned “to set up the terms for the collection of material for all German anatomists”.
With the “Beger assignment” Hirt connected the intention to continue the collection of sculls which had been initiated by the anatomist Gustav Schwalbe at the end of the 19th century in Strassburg “according to modern criteria”. Which meant: according to criteria of NS race policy. Commissioned by Hirt and the SS-“Ahnenerbe” the anthropologists Bruno Beger and Hans Fleischhacker selected 86 Jewish prisoners in Auschwitz in June 1943. They were deported to the concentration camp Struthof near Natzweiler and murdered there. Their corpses were taken to the basement of the anatomical institute in order to dissect them for the collection of the university. For decades nobody cared about the identity of these victims. It took 60 years until they were finally identified through private research initiative.

Kategorie: Vortrag

Vorträge der 27. Arbeitstagung der Anatomischen Gesellschaft

Vortrag 1

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

Titel: The endocannabinoid palmitoylethanolamide (pea) protects dentate gyrus granule cells by acting upon peroxisome proliferator-activated receptor (ppar)-alpha

Autoren: Koch M.(1),Kreutz S.(2),Böttger C.(2),Benz A.(3),Maronde E.(4),Ghadban C.(1),Korf H.(5),Dehghani F.(1),

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Abstract:
Classical endocannabinoids modulate the machinery of secondary neuronal damage and are known to improve neuronal survival after excitotoxic lesion. Palmitoylethanolamide (PEA) is an endogenous lipid that mimics effects of endocannabinoids even without binding to cannabinoid receptors. In the present study the effects of PEA on neuronal damage was proven and the molecular and cellular mechanisms behind its action were investigated. PEA (0.001µM-1µM) and the synthetic peroxisome proliferator-activated receptor (PPAR)-alpha agonist Wy-14,643 (0.1µM-1µM) reduced the number of microglial cells and protected dentate gyrus granule cells in excitotoxically lesioned organotypic hippocampal slice cultures. Treatment with the PPAR-alpha antagonist GW6471 (0.05µM-5µM) blocked PEA-mediated neuroprotection and reduction of microglial cell numbers whereas the PPAR-gamma antagonist GW9662 (0.01µM-1µM) showed no effects. A strong PPAR-alpha immunoreaction was detectable in BV-2 microglial cells and HT22 hippocampal cells. In both cell types, intensity and location of PPAR-alpha immunoreaction remained constant during PEA application. In conclusion our data provide evidence that 1) PEA counteracted excitotoxically induced secondary neuronal damage of dentate gyrus granule cells, 2) PPAR-alpha but not PPAR-gamma is the endogenous binding site for PEA-mediated neuroprotection and 3) PEA may activate PPAR-alpha in microglial cells and hippocampal neurons to exert its neuroprotective effetcs. In addition to classical endocannabinoids acting upon cannabinoid receptors, PEA-mediated PPAR-alpha activation may represent a possible novel target mechanism for therapeutic interventions to mitigate symptoms of secondary neuronal damage.

Kategorie: Vortrag

Vortrag 2

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

Titel: Non-enzymatic down regulation of rhoa by clostridial c3 proteins: beneficial effects on neuronal growth and connectivity

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Abstract:
We have recently reported on beneficial effects of a 29-amino acid peptide derived from Clostridium botulinum C3 protein on axonal outgrowth in vivo and recovery from spinal cord injury in mice. We further truncated down this peptide to pinpoint the minimal essential region of C3bot still capable to exert non-enzymatic growth promoting effects. Using primary hippocampal culture as well as organotypical brain slice culture we aimed to detect effects elicited by nanomolar concentrations of short C3bot fragments (26 and 15 amino acids) on morphology and synaptic connectivity. According to these assays the 26mer exhibited a higher potency than the 15mer to increase the number of synaptic inputs as indicated by synaptophysin/VGLUT staining. To gain insight into the unknown cell uptake mechanism and further signaling cascades by which C3bot proteins mediate their neurotrophic effects we studied potential uptake pathways and the activation pattern of RhoA, the main regulator of the actin cytoskeleton, after application of C3bot peptides. Application of the three C3bot peptides (29mer, 26mer and 15mer) resulted in a differential down-regulation of active RhoA as shown by pull-down experiments. Blockade of main cell trafficking pathways by bafilomycinA1 or brefeldin did not prevent internalization of C3bot. In conclusion, C3bot peptides foster neuronal process growth by a RhoA-dependent mechanism, further signaling elements remain to be elucidated.

Kategorie: Vortrag

Vortrag 3

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

Titel: Fibrinogen triggers astrocyte scar formation by promoting the availability of active tgf-beta after vascular damage.

Autoren: Schachtrup C.(1),Ryu J.(2),Helmrick M.(2),Vagena E.(2),Galanakis D.(3),Degen J.(4),Margolis R.(5),Akassoglou K.(6),

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Abstract:
Scar formation in the nervous system begins within hours after traumatic injury and is characterized primarily by reactive astrocytes depositing proteoglycans that inhibit regeneration. A fundamental question in CNS repair has been the identity of the initial molecular mediator that triggers glial scar formation. Here we show that the blood protein fibrinogen, which leaks into the CNS immediately after blood-brain barrier (BBB) disruption or vascular damage, serves as an early signal for the induction of glial scar formation via the TGF-beta/Smad signaling pathway. Our studies revealed that fibrinogen is a carrier of latent TGF-beta and induces phosphorylation of Smad2 in astrocytes that leads to inhibition of neurite outgrowth. Consistent with these findings, genetic or pharmacologic depletion of fibrinogen in mice reduces active TGF-beta, Smad2 phosphorylation, glial cell activation, and neurocan deposition after cortical injury. Furthermore, stereotactic injection of fibrinogen into the mouse cortex is sufficient to induce astrogliosis. Inhibition of the TGF-beta receptor pathway abolishes the fibrinogen-induced effects on glial scar formation in vivo and in vitro. These results identify fibrinogen as a primary astrocyte activation signal, provide evidence that deposition of inhibitory proteoglycans is induced by a blood protein that leaks in the CNS after vasculature rupture, and point to TGF-beta as a molecular link between vascular permeability and scar formation.

Kategorie: Vortrag

Vortrag 4

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

Titel: Interleukin-4-mediated alternative activation of microglia promotes survival of midbrain dopaminergic neurons

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Abstract:
Microglia are the resident immune cells of the central nervous system and are thought to be involved in a variety of neurodegenerative diseases. Stimulation with the bacterial lipopolysaccharide (LPS) is known to induce a microglia phenotype that promotes neurodegeneration of several distinct neuron populations including midbrain dopaminergic (mDA) neurons. This classical activation of microglia is characterised by the upregulation of inducible nitric oxide synthase (iNOS) and neurotoxic factors, such as TNF-alpha. However, recent studies suggest that microglia, like peripheral macrophages, can also be alternatively activated to induce tissue repair and cellular regeneration. Here we report that treatment of primary microglia with interleukin-4 (IL-4) results in upregulation of the alternative activation markers Arginase-1, Mannose receptor-1 and YM1. IL-4-mediated induction of this markers is further enhanced after co-treatment with TGF-beta1. Moreover, treatment with IL-4 increases the secretion of TGF-beta from primary microglia, suggesting an involvement of endogenous TGF-beta in IL-4-mediated effects. Using a survival assay for primary mDA neurons, we demonstrated that conditioned medium from IL-4 treated microglia promotes neuron survival in vitro. Taken together, these data indicate that alternatively activated microglia have neuroprotective effects on mDA neurons in vitro and that TGF-beta1 enhances the induction of this neuroprotective microglia phenotype.

Kategorie: Vortrag

Vortrag 5

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

Titel: Norrin mediates neuroprotective effects on retinal ganglion cells via the induction of neuroprotective growth factors in Müller cells

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Abstract:
Norrin is a secreted protein that activates the classical Wnt/(beta)-catenin pathway via a specific binding to frizzled 4 receptors. In Norrin-deficient mice a continuous loss of retinal ganglion cells has been observed. To analyze if Norrin has neuroprotective properties, we investigated the effects of Norrin in mice after excitotoxic retinal ganglion cell (RGC) damage following intravitreal injection of NMDA, and in cultured Müller glia. Intravitreal injection of Norrin significantly increased the number of surviving RGC axons in the optic nerve and decreased apoptotic death of retinal neurons following NMDA-mediated damage. This effect could be blocked by adding dickkopf (DKK)-1, an inhibitor of the Wnt/(beta)-catenin signaling pathway. Treatment of eyes with combined Norrin/NMDA activated Wnt/(beta)-catenin signaling and increased the retinal expression of leukemia inhibitory factor and endothelin-2, as well as that of neurotrophic growth factors such as fibroblast growth factor-2, brain-derived neurotrophic factor, lens epithelium-derived growth factor, and ciliary neurotrophic factor. A similar activation of Wnt/(beta)-catenin signaling and an increased expression of neurotrophic factors was observed in cultured Müller cells after treatment with Norrin, effects that again could be blocked by adding DKK-1.
We conclude that Norrin has pronounced neuroprotective properties on retinal neurons with the distinct potential to decrease the damaging effects of NMDA-induced RGC loss. The effects of Norrin involve activation of Wnt/(beta)-catenin signaling and subsequent induction of neurotrophic growth factors in Müller cells.

Kategorie: Vortrag

Vortrag 6

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

Titel: Induction of small heat shock proteins in the retina after optic nerve injury

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Abstract:
Glaucoma is a common cause of blindness which is due to damage of the optic nerve induced by high intraocular pressure. Normally after optic nerve injury (ONI) retinal ganglion cells (RGCs) degenerate. However, inflammatory stimulation in the eye such as intravitreal application of the toll-like receptor agonist Pam3Cys activates astrocytes and Müller cells in the retina leading to a delay of axotomy-induced death of RGCs. In general cells react to cellular stress with the so called stress response which includes upregulation of heat shock proteins leading to increased cellular survival. There is experimental evidence that the group of small heat shock protein (sHsps) exert neuroprotective effects probably via their chaperone-like activity. However, sHsps consisting of 11 family members are poorly characterized in the retina.
To study if sHsps play a role in degeneration and axonal regeneration of RGCs we investigated expression of sHsps in the retina at control conditions, after ONI and after ONI and Pam3Cys treatment in rats by qRT-PCR, Western blotting and immunocytochemistry. We found that four out of 11 family members were expressed in the retina, namely HspB1 (Hsp25), HspB4 (alphaA-crystallin), HspB5 (alphaB-crystallin) and HspB6. ONI led to an increase of HspB1 mRNA, ONI plus Pam3Cys treatment to an increase of HspB1, HspB4 and HspB5 mRNA. Changes in mRNA levels were accompanied by the respective changes in the protein content. HspB6 protein as well as mRNA levels were not altered by ONI with or without Pam3Cys treatment. Interestingly the phosphorylated forms of HspB1 and B5 were confined to different cell types. Whereas p(Ser15)HspB1 and p(Ser59)HspB5 were found in Müller cells p(Ser86)HspB1, p(Ser19)HspB5 and p(Ser45)HspB5 were restricted to RGCs.
Our data indicate that HspB1, HspB4 and HspB5 may play a role in the retina after ONI. They may protect RGCs after ONI and promote axonal regeneration.

Kategorie: Vortrag

Vortrag 7

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

Titel: Ctgf overexpression in the mouse eye causes an increase in intraocular pressure and glaucomatous damage of the optic nerve

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Abstract:
Purpose: The acto-myosin system in the human trabecular meshwork (HTM) plays an important role in modulating intraocular pressure (IOP). The information on factors that modulate the HTM actin cytoskeleton is incomplete. Since CTGF is expressed in high amounts in HTM cells, we analyzed if CTGF-signaling affects HTM actin cytoskeleton and IOP.
Methods: Cultured HTM cells were treated with rCTGF. CTGF overexpression in the TM of mouse eyes was achieved via adenoviral-mediated gene transfer (Ad5-CTGF) and via generation of transgenic mice with ocular overexpression of CTGF. Cells and eyes were analyzed by RT-PCR, immunoblotting, immunohistochemistry and microscopy. The IOP of the mouse eyes were measured by tonometry.
Results:
HTM cells treated with CTGF formed more actin stress fibers than control cells. CTGF overexpression via Ad5-CTGF or via transgenic overexpression caused a substantial increase in CTGF in the anterior eye, and a significant increase in IOP. By immunohistochemistry, a substantial increase of fibronectin and of alpha-smooth muscle-actin in the chamber angle was detected. The increase of IOP in CTGF overexpression models occurred in parallel to a loss of axons in the optic nerve.
Discussion: Our results strongly indicate that CTGF is a key modulator of the HTM actin cytoskeleton and of ECM synthesis, and thereby causes an increase in IOP. The parallel loss of optic nerve axon indicates that a CTGF overexpression system in mice could be a model for POAG. Modification of CTGF signaling appears to be a promising strategy to treat high IOP and glaucoma.

Support: DFG FOR 1075

Kategorie: Vortrag

Vortrag 8

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

Titel: The role of changes in astroglia-capillary connections in glaucomatous optic nerve neuropathy

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Abstract:
Purpose of the study was to further analyze the pathogenesis of the optic nerve neuropathy in glaucomatous eyes and the possible role of astroglia for this process.
Human glaucoma eyes and eyes from monkeys with experimentally induced glaucoma (EG) were investigated qualitatively and quantitatively using immunohistochemical and ultrastructural methods. In the EG eyes axon loss was determined with Heidelberg Retina Tomography and functional loss with electroretinography. The clinical results were correlated with quantitative morphological data.
In normal primate eyes neither in the prelaminar region (PreLR) nor in the postlaminar region (PostLR) of the nerve the astroglia is in direct contact with the capillary endothelium but is separated by a layer of connective tissue (CT).
In glaucomatous eyes in the PreLR the CT-layer significantly increases in thickness as does the CT layer separating the nerve fiber bundles from vessels in the PostLR, thereby significantly increasing the distance between astroglia and endothelium. The nerve fiber degeneration especially in eyes with axon losses up to 10^5 is characterized by myelin detritus partly without any glial reactions. Both axon degeneration and CT thickening show significant regional differences. These changes show highly significant correlations with the functional data obtained in the same living EG eyes.
These correlations now allow studying the sequence of events in eyes with exactly determined stages of the neuropathy and will help to further clarify the pathogenesis of the disease.

Kategorie: Vortrag

Vortrag 9

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

Titel: Functional interaction between formyl peptide receptor like 1 (fprl1) and receptor for advanced glycation end products (rage) in the amyloid-ß1-42-induced signal transduction in glial cells

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Abstract:
Recent studies suggest that the chemotactic G-protein-coupled-receptor (GPCR) formyl-peptide-receptor-like-1 (FPRL1) or the receptor-for-advanced-glycation-end-products (RAGE) play an essential role in the inflammatory response of host defence mechanisms and neurodegenerative disorders such as Alzheimer's disease (AD).
Therefore, we analyzed the involvement of FPRL1 and RAGE in amyloid-beta 1-42 Abeta 1-42 induced signalling by extracellular signal regulated kinase 1/2 (ERK1/2) phosphorylation and cAMP level measurement in glial cells (microglia and astrocytes) and transfected HEK 293 cells. FPRL1 was inhibited by a small synthetic antagonist WRW4 and by an inactive receptor variant delta-RAGE, lacking the transmembrane and intracytoplasmatic domains. Receptor deactivation by antagonists or the use of delta-RAGE verified the importance of FPRL1 for Abeta 1-42 mediated signal transduction by extracellular-signal regulated kinases 1/2 phoshorylation and cAMP level measurement in glial cells. In addition a possible physical interaction between FPRL1 and RAGE can be shown with co-immunoprecipitation and fluorescence microscopy measurements.
These results suggest that FPRL1 plays a pivotal role for Abeta 1-42 induced signal transduction in glial cells and the interaction with RAGE could explain broad ligand spectrum of formyl peptide receptors.

Kategorie: Vortrag

Vortrag 10

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

Titel: Genomic analysis of remyelination and non-remyelination lesions reveals the importance of brain lipid binding protein in activated astrocytes

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Abstract:
The protection of damaged axons from degeneration is a major challenge in myelin disorders such as multiple sclerosis (MS). Several lines of evidence indicate that remyelination represents one of the most effective demands to achieve axonal protection. For reasons, however that have not yet been understood, this process is often incomplete or fails in MS. In this study, we used the well-established toxic demyelination cuprizone mouse model. Affymetrix GeneChip® arrays demonstrated a number of factors related to remyelination failure in chronically demyelinated experimental white matter lesions. Inflammation appears to be important for endogenous remyelination. Besides microglia cells/macrophages, astrocytes are likely to participate in the regulation of remyelination. We could now demonstrate that local quiescent astrocytes re-differentiate into a radial glia cell phenotype. Within the remyelinating corpus callosum, GFAP+ astrocytes express the radial glia marker brain lipid binding protein. Lesions where remyelination fails show weak brain lipid binding protein expression. Nuclear brain lipid binding protein expression correlates with proliferation of astrocytes. Brain lipid binding protein expressing astrocytes are also found in experimental autoimmune encephalomyelitis and human MS lesions. Our data suggest that the re-activation of astroglial developmental properties might contribute to tissue repair in an experimental toxic demyelination model and human MS.

Kategorie: Vortrag

Vortrag 11

Rubrik: 9.Peripheres und vegetatives Nervensystem
Abstract Nr.:9

Titel: Tracheal brush cells are neuronally connected cholinergic sensory cells

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Abstract:
Brush cells are suspected to serve a chemosensory function. Here, we investigated the possibility that brush cells in the mouse trachea produce acetylcholine (ACh). Using mice expressing eGFP under the control of the promoter of the ACh synthesizing enzyme, choline acetyltransferase (ChAT), we identified solitary cholinergic cells in the mouse tracheal epithelium as brush cells by their immunoreactivity for villin and their characteristic ultrastructure. They also expressed the vesicular ACh transporter and proteins of the taste transduction pathway (alpha-gustducin and phospholipase Cbeta2). Messenger RNA for taste receptor 105 involved in perception of the bitter substance cycloheximide was detected in ChAT-eGFP cells isolated by FACS. CLSM-analyses revealed direct contacts with CGRP-immunoreactive nerve fibres. Using another transgenic mouse model that expresses eGFP under the control of the promotor for the alpha3-subunit of the nicotinic ACh receptor, we identified a subpopulation of C-fibres as cholinoceptive. Retrograde neuronal tracing identified airway-projecting sensory neurons with this chemical coding in the jugular-nodose-complex and in cervico-thoracic DRG. Respiratory pattern was measured after tracheal stimulation in a newly established model in spontaneously breathing anesthetized mice. DMPP, a nicotinic agonist, caused a drop in respiratoty rate which was augmented by inhibition of nicotinic receptors with mecamylamine, a nicotinic receptor antagonist. Cycloheximide elicited an epithelium-dependent drop in respiratory rate which was abolished by pretreatment with mecamylamine ($p=0.019$).
We conclude that tracheal brush cells are chemosensory, cholinergic cells that transmit changes in the luminal microenvironment of the airways to the CNS via ACh release and nicotinic stimulation of sensory neurons.

Kategorie: Vortrag

Vortrag 12

Rubrik:
Abstract Nr.:

Titel: Glial cell-derived neurotrophic factor (gdnf) regulates airway sensory nerves in allergic asthma

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Abstract:
Bronchopulmonary C-fibers play an important role in transducing airway inflammation into symptoms of asthma such as reflex bronchospasm, mucus secretion and cough. The mechanisms by which C-fibers are activated have not yet been worked out. Virtually all bronchopulmonary C-fibers express both TRPV1 (the capsaicin receptor) and TRPA1, another ligand-gated cation TRP-channel. TRPA1 is activated by several endogenous and exogenous agents that cause bronchoconstriction and cough in patients with asthma (e.g. cold, ozone and reactive oxygen species). Glial-Cell-Derived-Neurotrophic-Factor (GDNF) has been shown to upregulate TRPV1 and TRPA1 in somatosensory nerves.
To investigate if GDNF may regulate the excitability of bronchopulmonary TRPV1+/TRPA1+ C-fibers in asthma, we first investigated GDNF expression in the airways. GDNF was constitutively expressed in airway smooth muscle cells. After OVA-challenge in OVA-sensitized mice, and in lung sections from patients who died of fatal asthma, the number of GDNF+ cells within the inflammatory infiltrate was increased. To assess whether bronchopulmonary C-fibers express RET and GFRalpha1, forming the GDNF-receptor complex, single cell RT-PCR was performed in DiI-labelled TRPV1+/TRPA1+ neurons. Our data revealed that GFRalpha1 was expressed in the vast majority of these cells, all of which also expressed RET. Neuronal RET mRNA was upregulated following OVA-challenge in OVA-sensitized mice as determined by single-cell qRT-PCR.
Our data show that the pulmonary expression of GDNF is increased in allergic asthma. GDNF may increase the excitability of bronchopulmonary vagal C-fibers by acting via the RET/GFRalpha1 receptor complex and therefore contribute to the symptoms observed in patients with asthma.

Kategorie: Vortrag

Vortrag 13

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

Titel: Defective nf-kappab signaling leads to altered spine morphology and reduced synapse number in excitatory cns neurons.

Autoren: Schmeisser M.(1),Baumann B.(2),Johannsen S.(1),Seither J.(1),Wirth T.(2),Boeckers T.(1),

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Abstract:
NF-kappaB signaling has emerged to play a diverse role in the central nervous system having an impact on several processes including formation of synapses and synaptic plasticity. As the IkappaB (IKK) complex plays a crucial role within this molecular pathway, we analyzed a conditional mouse model expressing a dominant negative allele of the IKK2 gene (IKK2-DN) only in glutamatergic CNS neurons. In our studies, we found that inhibition of NF-kappaB signaling leads to a substantial reduction of synaptic contacts in primary hippocampal cultures from transgene animals while this effect could be completely rescued by switching off IKK2-DN expression in a doxycycline-dependent manner. Compared to control animals, mutant mice further exhibit a significant reduction of morphologically altered dendritic spines. Interestingly, spine number and morphology could both be restored to normal after downregulating IKK2-DN expression also in vivo. Taken together, our results demonstrate a novel role of the IKK/NF-kappaB signaling pathway in neurons with respect to synapse formation. Further studies should focus on IkappaB downstream target genes that might be responsible for synaptogenesis and synaptic maturation.

Kategorie: Vortrag

Vortrag 14

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

Titel: Homeostatic synaptic scaling regulates spine stability of denervated hippocampal neurons

Autoren: Vlachos A.(1),Becker D.(1),Bas Orth C.(1),Helias M.(2),Jedlicka P.(1),Neuwirth M.(1),Winkels R.(1),Diesmann M.(2),Röper J.(3),Schneider G.(4),Deller T.(1),

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Abstract:
Denervation-induced spine reorganization was studied following entorhinal deafferentation of hippocampal granule cells in organotypic slice cultures of Thy1-GFP mice. The highly laminar organization of entorhinal afferents to the dentate gyrus made it possible to selectively denervate distal dendritic segments of granule cells without deafferenting their proximal dendritic segments. Thus, we could study how spines located on denervated and non-denervated dendritic segments of the same neuron react to the loss of innervation. Time-lapse imaging revealed alterations in spine loss and in the stability of newly formed spines but not in spine formation rate in the denervated layer. Patch-clamp analysis revealed homeostatic scaling of excitatory synapses within the same layer, demonstrating that denervated neurons locally adapt their synapses to maintain their afferent drive. The layer-specific functional and structural adaptations observed after denervation required the layer-specific upregulation of tumor necrosis factor-alpha (TNFa). Since induction of homeostatic scaling in non-denervated control cultures also resulted in a destabilization of newly formed spines, we propose that TNFa-mediated destabilization of spines could be a general mechanism by which neuronal networks homeostatically adapt spine and thus excitatory synapse numbers to the level of network activity. Supported by DFG and Helmholtz Alliance on Systems Biology.

Kategorie: Vortrag

Vortrag 15

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

Titel: Subcellular distribution of prg3 is associated with neurite consolidation

Autoren: Bräuer A.(1),

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Abstract:
Neuritogenesis, as a crucial event in the differentiation of neurons during development, is closely followed by neuronal polarization. Subsequently, just defined dendrites and axons grow and synapses form to establish a functional neuronal network. PRG3, a member of the recently identified plasticity-related-gene family, has been suggested as a novel molecule in neuritogenesis.
We show that PRG3 induces filopodia formation depending on the N-glycosylation status of PRG3. The protein was strongly expressed during important stages of the mouse brain development in vivo (i.e. E16 – P5) and expression declined thereafter. Furthermore, in early, not yet polarized hippocampal cultured neurons, PRG3 was expressed in all neurites, whereas after polarization, PRG3 expression shifted to mainly axonal expression, where it resides in the plasma membrane along the neurite shaft. Although the PRG3 distribution is temporally and spatially related to the ongoing synaptogenesis, our recordings of miniature excitatory postsynaptic currents (EPSC) render a connection of these events unlikely: an influence of synatogenesis on PRG3 pattern was excluded by unchanged PRG3 dynamics despite the block of incoming signals; a PRG3 dependence of synaptogenesis, on the other hand, was ruled out by an similar EPSC pattern despite overexpressed PRG3, which stays in the dendrites.
Our data suggest that PRG3 plays a role in the control of active neurite consolidation and therefore could regulate cytoskeleton stabilization during neurite elongation.

Kategorie: Vortrag

Vortrag 16

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

Titel: A mechanism for aromatase-dependent maintenance of synaptic plasticity

Autoren: Fester L.(1),Zhou L.(2),Brandt N.(2),Behem C.(2),Jarry H.(3),Rune G.(4),

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Abstract:
In hippocampal neurons, maintenance of dendritic spine and spine synapse density depends on aromatase activity, which catalyzes the final step of estradiol synthesis in these neurons. In this study, we show that aromatase activity preferentially maintains mushroom-shaped, mature spines, as defined by spine volume and by the presence of a spine apparatus, the Ca2+ store typical of mushroom-shaped spines. After inhibition of aromatase activity, disassembly of F-actin filaments, as indicated by dephosphorylation of cofilin, and reduced synaptopodin expression, an actin-binding protein enriched in mature spines, which is required for the formation of a spine apparatus, may trigger spine synapse loss. These effects are mediated by estrogen receptor beta. Consistently, spine synapse density is reduced in the synaptopodin knock-out mouse, similar to synapse loss in animals treated with letrozole animals. These data suggest that aromatase activity in neurons is essential for memory function, which is presumed to require mushroom-shaped, mature spines. Most importantly, our data show that Ca2+ release from internal stores and reuptake of Ca2+ into the stores control aromatase activity, and this process results in varying intracellular estradiol levels that in turn regulate protein expression. The regulation of cofilin activity and SYNPO expression downstream of the regulation of aromatase activity by Ca2+ transients from internal stores introduces aromatase activity as a hitherto unknown factor in Ca2+-induced signalling cascades with regard to the homeostasis of synaptic plasticity.

Kategorie: Vortrag

Vortrag 17

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

Titel: Go2alpha regulates neurite outgrowth and branching by modulating rap1 activity via interaction with rap1 gtpase-activating protein

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Abstract:
Goalpha, the most abundant G-protein in brain, comprises two splice variants Go1alpha and Go2alpha. Go2alpha regulates vesicular monoamine and glutamate storage, but direct Go2alpha interaction partners are still unkown. Former research on Goalpha interacting proteins focussed on Go1alpha. In the present study we applied a yeast two hybrid screen using constitutively active Go2alpha as a bait which identified several potential downstream effectors. Some of the identified proteins preferentially interacting with Go2alpha, Girdin and Rap1GAP, were further analysed. Their interactions with Go2alpha were confirmed by co-immunoprecipitation. In order to detect effects of Go2alpha absence cerebral expression levels and patterns of Girdin and Rap1GAP were compared in wild type and Go2alpha-/- mice revealing no differences between the genotypes. Rap1GAP regulates activity of Rap1 by stimulating its GTPase activity. The amount of active Rap1-GTP is higher in brains of Go2alpha deletion mutants compared to wild type animals indicating an influence of Go2alpha via Rap1GAP on the Rap1 activating/deactivating cycle. Rap1 has been implicated in neurite outgrowth and cortical dendrite development. Therefore we compared axon and dendrite length and branching in embryonic cortical and hippocampal neuronal cultures of wild type and Go2alpha-/- mice. Axons in cultures from deletion mutants were significantly longer and showed significantly more branching nodes than axons in cultures from wild type embryos. Taken together we could provide evidence that Go2alpha regulates neurite outgrowth and branching probably by modulating Rap1 activity.

Kategorie: Vortrag

Vortrag 18

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

Titel: Neuroligin1 induces structural alterations of the active zone

Autoren: Wittenmayer N.[a], Schrof S.[b], Staudt T.[b], Hell S. W.[b], Dresbach T.[a]

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Abstract:
Synaptogenesis involves the development of an initial contact between axons and dendrites, the induction of the formation of presynaptic active zones and postsynaptic densities, synaptic differentiation and finally the maturation to attain the final protein composition, stability and function. Postsynaptic adhesion molecules called Neuroligins play important roles during these processes. Recently we showed that Neuroligin1, which is exclusively located at excitatory synapses, regulates presynaptic maturation. Overexpression of Neuroligin1 in immature neurons results in a structural maturation of the active zone followed by a functional maturation of the presynaptic compartment. To shed more light on the structural maturation of the active zone we used STED microscopy. Overexpression of Neuroligin1 in immature hippocampal neurons leads to an increase in active zone size which is more densly packed.

Kategorie: Vortrag

Vortrag 19

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

Titel: A new map of motoneuronal subgroups in the human oculomotor nucleus

Autoren: Horn-Bochtler A.(1),Che-Ngwa E.(1),Zeeh C.(1),

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Abstract:
The oculomotor nucleus (nIII) contains the motoneurons of 4 extraocular muscles. In contrast to monkey their motoneuronal distribution in human is not well known.
We compared the histochemical profile in neighbouring nIII sections from monkey and human applying antibodies against choline acetyltransferase (ChAT) combined with immunostaining for either glutamate decarboxylase (GAD), glycine transporter-2 (Gly-T2), urocortin (UCN) or calretinin (CR).
Based on cytoarchitecture and histochemistry, 7 subgroups were delineated in human nIII: a dorsolateral, dorsomedial, central, and ventral group, the central caudal nucleus (CCN), the nucleus of Perlia (NP) and the non-preganglionic Edinger-Westphal nucleus (EWu). The dorsolateral and ventral groups, the NP and EWu receive a strong supply of GAD-positive terminals. The dorsolateral, ventral groups and CCN, but not NP, receive a GLY-T2-positive input, whereas CR-positive terminals were confined to the central group, CCN and NP. Based on the monkey data, the central group is considered to contain superior rectus (SR) and inferior oblique (IO) motoneurons, the dorsolateral and ventral group the B- and A-group of medial rectus (MR) motoneurons, respectively, and the dorsomedial group the inferior rectus (IR) motoneurons.
As in monkey the glycinergic input is confined to MR motoneurons and the CCN. Unlike the monkey the human MR motoneurons receive a much stronger GABAergic input, which may indicate a functional oculomotor specialization in human. A CR-positive input is restricted to eye muscle motoneurons participating in upgaze, e.g. SR, IO and the upper eyelid. The NP neurons share the properties of upgaze motoneurons and may represent SR motoneurons.
DFG (Ho 1639/4-3)

Kategorie: Vortrag

Vortrag 20

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

Titel: The influence of experience on brain lateralisation and hippocampus size using homing pigeons as a model

Autoren: Mehlhorn J.(1),Rehkämper G.(1),

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Abstract:
The brain of homing pigeons seems to be functionally adapted to homing with e.g. larger hippocampi and olfactory bulbs. Furthermore, functional lateralisation occurs as well in homing pigeons.
To show in what way brain structure volume and lateralisation is caused by experience rather than genetically determined, 20 homing pigeons were raised under identical constraints. After fledging, 10 of them were allowed to fly around the loft and participated successfully in races. The other 10 stayed permanently in the loft and did not share the experiences made by the first group. After reaching sexual maturity, all individuals were sacrificed and morphometric analyses were carried out to measure the volumes of 12 brain structures.
Individuals with experience of navigation had an 11.2% larger hippocampus relative to the telencephalon compared to unexperienced individuals. Additionally, the comparison of left/right quotients of both groups reveal that experienced homing pigeons show a smaller left mesopallium in comparison to the right one and homing pigeons without navigational experience a larger left mesopallium in comparison to the right one. There are as well significant differences between left and right brain subdivisions within the two groups namely a larger left hyperpallium apicale in both groups and a larger right nidopallium, left hippocampus and right optic tectum in pigeons with navigational experience.
Our data indicate that plasticity and lateralization are correlated with individual life history and not exclusively based on heritable traits.

Kategorie: Vortrag

Vortrag 21

Rubrik: 5.Entwicklungsbiologie
Abstract Nr.:5

Titel: A role for primary cilia in forebrain development: involvement of ift88 and kif3a

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Abstract:
Primary cilia are important sites of signal transduction involved in many developmental functions. Defects in intraflagellar transport (IFT), which is crucial for the maintenance of primary cilia, can lead to severe developmental defects and diseases. We have previously reported an essential role of primary cilia in forebrain development by investigation of the ENU-induced cobblestone mouse mutant, a hypomorphic allele of the IFT gene Ift88 (Willaredt et al., J. Neurosci., 2008). cobblestone mutants are distinguished by subpial heterotopias in the forebrain and severe defects in the formation of dorsomedial telencephalic structures, such as the choroid plexus, cortical hem, and hippocampus. They also demonstrate a relaxation of both dorsal-ventral and rostral-caudal compartmental boundaries. These defects phenocopy many of the abnormalities seen in the Gli3 mutant forebrain, and the defects in IFT lead to an accumulation of the full-length isoform of Gli3, which is proteolytically processed in a cilia-dependent fashion. Interestingly, the ultrastructure and morphology of ventricular cilia in the cobblestone mutants remains intact.
We report a further investigation of the forebrain phenotype in the cobblestone mutant, in which severe disturbances in the ventricular zone of the cortex are observed. We also describe the phenotypes of two conditional mouse mutants that should replicate the forebrain phenotypes seen in the cobblestone mutants. We have used inducible mutants in Ift88 and Kif3a, both of which are involved in IFT. These genes were specifically eliminated in the forebrain using a variety of different CRE-recombinase expressing lines, including nestin::CRE, EIIa::CRE, Emx1::CRE, and Emx1::CRE-ERT2.

Kategorie: Vortrag

Vortrag 22

Rubrik: 5.Entwicklungsbiologie
Abstract Nr.:5

Titel: New data for a new model of left-right symmetry breaking in the vertebrate embryo.

Autoren: Tsikolia N.(1),Schwartz P.(1),Viebahn C.(1),

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Abstract:
Left-right (LR) symmetry breaking is a fixed feature in vertebrate development and occurs only after the dorso-ventral and anterior-posterior axes are formed. In most model vertebrates LR-determination has been shown to be associated with a unidirectional fluid flow due to cilia rotation in early embryos. Additionally, molecular genetic evidence in human and mouse led to the proposal of universality of a cilia-based mechanism of LR-determination. However, this proposal is still highly controversial due to various functional and morphological data. Recent investigations now lead to a new concept based on asymmetric leftwards cell movements anterior to the node of mid-gastrulation stages of the chick (Gross et al. 2009) which is a potentially applicable also to mammalians. In order to investigate the role and causal context of these movements we studied the perinodal area in defined substages of chick gastrulation. Analysis of semithin sections reveals early morphological asymmetry which manifests itself in leftward bending of the node and division of the node into a left-sided smaller and right-sided larger “shoulder”; this is associated with a rapid change of gene expression within node and primitive streak: Shh is confined to the left shoulder and the notochord whereas nodal expression in primitive streak ceases in a matter of hours and then reappears immediately anterior to the tip of the bent node. To find a functional link between cellular dynamics, early asymmetry and these gene expression domains we started microsurgical explantation of perinodal tissue and fate mapping analysis of the asymmetrical node area using DiI.

Kategorie: Vortrag

Vortrag 23

Rubrik: 5.Entwicklungsbiologie
Abstract Nr.:5

Titel: Gene expression in the developping glomerulus of the zebrafish pronephros

Autoren: Müller T.(1),Mostertz J.(2),Blumenthal A.(1),Chilikoti R.(2),Warsow G.(3),Rumpel L.(1),Englert C.(4),Fuellen G.(3),Homuth G.(2),Endlich K.(1),Endlich N.(1),

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Abstract:
The zebrafish pronephric glomerulus gains functionality at 48-56 hours post-fertilization (hpf), enabling regular blood filtration. Being very similar to mammalian glomeruli, it represents a complex arrangement of highly specialized cell types, most notably the podocytes, which cover glomerular capillary loops with their interdigitating foot processes. Whereas its morphological development is well documented, little is known about how this process is regulated on the molecular level. Given the high speed of development, gene expression has to be regulated differentially and fast, following a complex, chronologically orchestrated pattern.
To elucidate this pattern, glomeruli from transgenic zebrafish expressing wt1a::GFP in podocytes were isolated at 44 hpf, 48 hpf, 54 hpf, 72 hpf and 96 hpf. To this end, larvae were homogenized and intact, fluorescent glomeruli were picked manually and stored in liquid nitrogen. From these, total mRNA was extracted, followed by an expression analysis via an Affymetrix gene chip zebrafish genome array. Of the 6787 genes analyzed, 3825 (56%) were found to change significantly in expression at least once after, and in comparison to, 44 hpf. The most dramatic changes occurred between 54 hpf and 72 hpf. 1168 genes were significantly downregulated during that time, 1142 genes were upregulated. To correlate changes in expression levels to the morphological development of the glomerulus, cryosections and ultrathin sections of were prepared at each timepoint and imaged by confocal fluorescent microscopy and transmission electron microscopy, respectively. In summary, we demonstrate that development of the zebrafish pronephric glomerulus is accompanied by a massive regulation of gene expression.

Kategorie: Vortrag

Vortrag 24

Rubrik: 5.Entwicklungsbiologie
Abstract Nr.:5

Titel: Expression of molecular factors controlling primordial germ cell differentiation in the mammalian embryo

Autoren: Hopf C.(1),Viebahn C.(1),Püschel B.(1),

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Abstract:
Several key factors known to be involved in the epigenetic repression of a somatic cell fate in primordial germ cells of the mammalian embryo have been described in the mouse only. To find mechanisms applicable to all mammals we analysed the spatial and temporal expression patterns of the transcriptional repressor Blimp1 and of the signalling factors Bmp2 and Bmp4 in finely staged perigastrulation rabbit embryos using whole-mount in situ hybridisation and high-resolution light microscopy of semithin sections: Similar to what has been found in the mouse, Blimp1 expression begins in the hypoblast at stage 1 and localises additionally to probable primordial germ cell (PGC) precursors in the posterior epiblast at stage 2. Following the onset of gastrulation Blimp1 is found in the mesoderm at positions similar to those identified by PGC specific antibodies. Bmp2 – initiating at stage 1 in the hypoblast - and Bmp4 – commencing between stage 2 and 3 in all three embryonic layers - are expressed in anular expression domains at the boundary of the embryonic disc, which - in contrast to mouse data, belong to intraembryonic tissue-layers and show the strongest Bmp4 expression at the posterior pole of the embryo. The expression patterns observed here suggest that function and chronology of factors involved in germ cell differentiation are similar in mouse and rabbit, whereas the early polarized expression recognized in the rabbit points to the requirements of different pregastrulation morphologies amongst mammals.

Kategorie: Vortrag

Vortrag 25

Rubrik: 5.Entwicklungsbiologie
Abstract Nr.:5

Titel: Divergent regulation of wnt-mediated maintenance of the dorsomedial and ventrolateral dermomyotomal lips

Autoren: Krück S.(1),Scaal M.(1),

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Abstract:
The dermomyotome is the dorsal compartment of the somite which gives rise to multiple cell fates including skeletal muscle, connective tissue, and endothelia. It consists of a pseudostratified, roughly rectangular epithelial sheet, the margins of which are called the dermomyotomal lips. The dermomyotomal lips are blastema-like epithelial growth zones, which continuously give rise to resident dermomyotomal cells and emigrating muscle precursor cells, which populate the subjacent myotomal compartment.
Wnt signaling has been shown to regulate both dermomyotome formation and maintenance of the dermomyotomal lips. Whereas the epithelialization of the dermomyotome is regulated via canonical, beta-catenin-dependent Wnt signaling, the signaling mechanisms regulating EMT in the mature dermomyotomal lips have been unknown.
Here, we present evidence that dermomyotomal lip sustainment is differentially regulated. Whereas the dorsomedial dermomyotomal lip is maintained by canonical Wnt signaling, maintenance of the ventrolateral dermomyotomal lip is regulated by non-canonical Wnt signaling, likely via the PCP signaling pathway.

Kategorie: Vortrag

Vortrag 26

Rubrik: 5.Entwicklungsbiologie
Abstract Nr.:5

Titel: Planar cell movements and premitotic cellular behaviour at the onset of gastrulation in the mammalian embryo

Autoren: Halacheva V.(1),Fuchs M.(2),Dönitz J.(2),Viebahn C.(1),

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Abstract:
Cell proliferation and cell migration are important prerequisites for the formation of the body axes. During gastrulation a combination of both processes form the primitive streak, which is the first irreversible structure in establishing the longitudinal body axis. However, the balance between proliferation and migration and the mechanisms that regulate both processes are still incompletely understood, particularly in the mammalian embryo whose topography at the pre-gastrulation stages is principally different as compared to the chick. In this study cell movement and behaviour of dividing cells immediately prior to gastrulation were investigated in time lapse recordings of rabbit blastocysts by means of differential interference contrast, two-photon laser microscopy and subsequent statistical analysis. During the observation period of two hours epiblast cells in the posterior half of the embryonic disc move towards the future region of the primitive streak, whereby the cells in the area of the presumptive primitive streak show complex movements such as U-turns, for example. Metaphase plates in the future primitive streak forming area were preferentially oriented parallel to the anteroposterior axis of the embryo. Within a time period of 10-25 minutes prior to anaphase rapid rotations of the metaphase plates up to 90° were observed; however, close to the onset of anaphase, the rotation was less than 20° and there is no marked deviation from the final orientation of the metaphase plates. Our study reveals new insights into the cell movements and the premitotic cellular behaviour at the early stages of the mammalian gastrulation.

Kategorie: Vortrag

Vortrag 27

Rubrik: 6.Reproduktionsbiologie
Abstract Nr.:6

Titel: Expression of steroid hormone receptors in human testis

Autoren: Fietz D.(1),Beck C.(1),Lang D.(1),Siebert S.(2),Konrad L.(3),Geyer J.(4),Gromoll J.(5),Kliesch S.(5),Bergmann M.(1),

Adressen:(1)Justus-Liebig-Universität Gießen|Institut für Veterinär-Anatomie, -Histologie und -Embryologie|Gießen|Deutschland; email:daniela_fietz@arcor.de; (2)Ludwig-Maximilian Universität|Institut für Anatomie und Zellbiologie|München|Deutschland; (3)Justus-Liebig-Universität Gießen|Zentrum für Frauenheilkunde und Geburtshilfe|Gießen|Deutschland; (4)Justus-Liebig-Universität Gießen|Institut für Veterinär-Pharmakologie und -Toxikologie|Gießen|Deutschland; (5)Universitätsklinikum Münster|Centrum für Reproduktionsmedizin und Andrologie|Münster|Deutschland

Abstract:
CAG repeat length (9-36 repeats) in the transactivation domain of the human androgen receptor (AR) is suggested to be responsible for testosterone sensitivity. Increasing CAG repeats are related to the risk of infertility. However, there is evidence that high levels of estrogens are responsible for spermatogenic impairment in patients showing long CAG repeats.
We examined CAG repeat length and AR expression in blood lymphocytes, testis homogenate DNA and mRNA and in separated tubules and interstitium.
Additionally, AR and clusterin expression were examined in a rat Sertoli cell line transfected with human AR containing 17 or 33 CAGs, via RT-qPCR. ER alpha localization and expression was examined by immunohistochemistry and RT-PCR/RT-qPCR using separated seminiferous tubules and interstitial tissue.
We found no connection between CAG repeat length and AR expression related to spermatogenic impairment. Functional analysis showed a slight down-regulation of clusterin expression, but there was no difference between different CAG repeat lengths. ER alpha is localized within germ cells and single interstitial Leydig cells. It is significantly down-regulated in germ cells related to spermatogenic defects, and reduced mitotic activity of spermatogonia. Our data show, that AR expression and function is not mainly affected by CAG repeat length being within the physiological range and suggest estrogens and ER alpha expression to play an important role for normal spermatogenesis.

Kategorie: Vortrag

Vortrag 28

Rubrik: 4.Zellbiologie
Abstract Nr.:4

Titel: Modulation of calcium activated potassium channels induces cardiogenesis of pluripotent stem cells and enrichment of pacemaker-like cells

Autoren: Liebau S.(1),Kleger A.(2),Böckers T.(1),Fleischmann B.(3),Wobus A.(4),

Adressen:(1)Ulm|Institut für Anatomie & Zellbiologie|Ulm|D; email:stefan.liebau@uni-ulm.de; (2)Ulm|Innere Medizin I|ulm|D; (3)Bonn|Institut für Physiologie I, Life and Brain Center|Bonn|D; (4)Leibniz Institute of Plant Genetics and Crop Plant Research|Ag In vitro-Differenzierung|Gatersleben|D

Abstract:
Ion channels are key determinants for the function of excitable cells but little is known about their role and involvement during cellular and embryological development. Earlier work identified Ca2+-activated potassium channels of small and intermediate conductance (SKCas) as important regulators of neural stem cell fate. Here, we have investigated their impact on the differentiation of pluripotent cells. We have applied the SKCa-activator EBIO on embryonic stem cells and identified this particular ion channel family as a new critical target involved in the generation of cardiac pacemaker-like cells: Similar to the neural stem cell system, SKCa-activation led to rapid remodeling of the actin cytoskeleton. But interestingly in ES cells it led to strong inhibition of proliferation, induction of differentiation and diminished teratoma formation. Time-restricted SKCa-activation induced cardiac mesoderm and commitment to the cardiac lineage as shown by gene regulation, protein and functional electrophysiological studies. In addition, the differentiation into cardiomyocytes was modulated in a qualitative fashion, resulting in a strong enrichment of pacemaker-like cells. This was accompanied by induction of the sino-atrial gene program and in parallel by a loss of the chamber-specific myocardium.

Kategorie: Vortrag

Vortrag 29

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

Titel: Structural left-right asymmetries in the rodent auditory cortex – morphology of pyramidal cells and minicolumns

Autoren: Budinger E.(1),

Adressen:(1)Leibniz Institute for Neurobiology Magdeburg|Dept. Auditory Learning and Speech|Magdeburg|Germany; email:budinger@ifn-magdeburg.de

Abstract:
There are several evidences for functional specializations of the left and right auditory cortex in humans as well as in animals.
Here, we investigated potentially underlying anatomical asymmetries at the level of single cortical neurons and of cortical minicolumns in Mongolian gerbils.
By quasi-intracellular recordings and subsequent stainings with biocytin we could demonstrate that the morphology of pyramidal cells is closely related to their best frequency (BF) and to their left- or right-hemispheric affiliation. For example, layer II/III pyramidal cells display a more condensed local termination pattern of their axons as lower their BF. At a given BF this condensation is more robustly expressed on the left side.
By means of their specific zinc staining pattern (TIMM), which is particularly strong for the zinc-containing terminals of the above described layer II/III pyramidal cells, we found that the cortical columns in the left hemisphere are smaller (~50µm) and more densely packed (~400 columns per mm2) than in the right hemisphere (~60µm diameter, ~300 columns per mm2).
Taken together, these results indicate that there are more but smaller functional units in the left and less but larger functional units in the right auditory cortex of gerbils. This hemispheric asymmetry may possibly underlie the lateralized processing of tone-sequences (left) and tone-sweeps (right) in the two hemispheres of this species and may be an evolutionary precursor for similar processes in higher developed species like humans.

Kategorie: Vortrag

Vortrag 30

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

Titel: Connecting the entorhinal cortex with the hippocampus: does actvity matters?

Autoren: Vogt J.(1),Schlüter L.(2),Strauss U.(3),Nitsch R.(1),

Adressen:(1)Gutenberg-Universität Mainz|Institut für Mikroskopische Anatomie und Neurobiologie|Mainz|Deutschland; email:johannes.vogt@unimedizin-mainz.de; (2)Gutenberg Universität Mainz|Institut für Mikroskopische Anatomie und Neurobiologie|Mainz|Deutschland; (3)Charité- Universitätsmedizin Berlin|Institut für Zell- und Neurobiologie|Berlin|Deutschland

Abstract:
Axon outgrowth in the central nervous system is conducted by specific guiding cues. However the role of early neuronal activity on axonal outgrowth as well as the underlying intracellular signaling are still mostly unsolved. Genetical models leading to ablation of neuronal activity lead to perinatal death thereby preventing further analysis of fiber tract development. Moreover, to date there are no genetical or pharmacological models which allow to analyze the effect of enhanced spontaneous neuronal activity, which is believed to be a prerequisite for the development of axon fiber tracts. Analysis of PRG-1 deficient mice, which display an enhanced spontaneous glutamate transmission, revealed that axon fiber tracts were significantly diminished in adult mice. We have analysed this effect during development of the entorhinal-hippocampal fiber tract in an organotypic co-culture model and performed live-imaging on a single axon level showing that axons originating from PRG-1 deficient neurons had a slower outgrowth speed than wild-type neurons. Electrophysiologic recordings revealed that PRG-1-deficiency lead to hyperexcitability already at early developmental stages (P5). Accordingly, application of glutamate to WT cultures inhibited axonal outgrowth to a similar extent like PRG-1 deficiency and blockade of neuronal activity rescued the decreased outgrowth of PRG-1-deficient neurons to wild-type levels. Moreover, deletion of the LPA2-R, the reported functional antagonist of PRG-1, rescued the outgrowth deficit observed in PRG-1-/- neurons. We propose bioactive phospholipids as modulators of axonal outgrowth by altering early neuronal activity and PRG-1 as molecule which facilitates axonal outgrowth by its modulatory control of neuronal excitability.

Kategorie: Vortrag

Vortrag 31

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

Titel: A potential role of estrogen in schizophrenia via regulation of erbb3 expression

Autoren: Brandt N.(1),Wehrenberg U.(2),Fester L.(2),Rune G.(2),

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Abstract:
Fading estrogen secretion at menopause in vulnerable women leads to relapse of schizophrenic symptoms or to new late-onset schizophrenia, which gave rise to the estradiol protection hypothesis for schizophrenia. ErbB3, has been shown to be reduced in brains of 50% of all chronically schizophrenic patients (Hakak et al 2001, Tkachev et al. 2003; Aston et al., 2004). Impaired maturation of dendritic spines and behavioral deficits, that have been associated with schizophrenia-like symptoms were found in mice lacking NRG1/ErbB signalling in the CNS. We studied estrogen responsiveness of ErbB3 expression in hippocampal cultures, ErbB3 was significantly upregulated in response to estradiol, as shown by image analysis of immunoblots, quantitative immunhistochemistry of ErbB3, and quantification of ErbB3 transcripts by TaqMan PCR. Inhibition of hippocampal estrogen synthesis in the cultures, similar to the situation of women at menopause when estradiol synthesis is downregulated due to elevated levels of GnRH, resulted in a downregulation of ErbB3 expression and spine synapse loss. Our data suggest that the decline in brain-derived estrogen in women at menopause may contribute to the relapse of schizophrenia symptoms and they are in favour of the estrogen protection hypothesis in schizophrenia.

Kategorie: Vortrag

Vortrag 32

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

Titel: The role of mutations in postsynaptic proteins associated with autism spectrum disorders

Autoren: Schön M.(1),Grabrucker A.(1),Schmeisser M.(1),Pröpper C.(1),Bockmann J.(1),Böckers T.(1),

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Abstract:
Recent studies have strongly implicated several postsynaptic proteins, notably ProSAP2/SHANK3 (ProSAP2) and Neuroligins (NLs), as causal actors in the pathogenesis of autism. ProSAP2 is a master scaffolding protein of the postsynaptic density (PSD). This large multi-domain molecule serves as a platform molecule at excitatory PSDs as it interconnects glutamatergic receptors of the postsynaptic membrane with cytoskeletal elements of the dendritic spine. NLs are adhesion molecules that, along with Neurexins, bridge the synaptic cleft. ProSAP2 and NLs play a critical role during synaptogenesis and the maintenance of synaptic function.
In our work we investigated the role of ProSAP2 on the normal function of synapses and neuronal networks. Further on, we transfected cultured rat hippocampal neurons with vector constructs of wildtype and mutated ProSAP2, which have been found in autistic individuals. We are analyzing several mutations and their effects on synapse density with a focus on the balance of excitatory and inhibitory synapses, non-synaptic clustering and dendritic morphology resulting in alterations of synapse formation. Besides, some of the mutations affect the Golgi apparatus, pointing towards an impaired folding of mutated ProSAP2. The observation of similar effects of the mutations on synapse numbers, affecting both excitatory and inhibitory synapses, reinforces the hypothesis of altered synaptic networks as a major factor in autism pathogenesis.

Kategorie: Vortrag

Vortrag 33

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

Titel: Ontogeny of two-way active avoidance behavior: learning capacity in infant and adolescent rats may be determined by differential metabolic recruitment of cognitive, emotional and modulatory brain regions.

Autoren: Riedel A.(1),Bock J.(1),Gruss M.(1),Braun K.(1),

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Abstract:
Despite repeated training, infant rats are not able to generate two-way active avoidance (TWA) behavior. However, these animals obviously deposit a certain “memory trace” during early training as they learn much faster than their naive littermates when re-trained in adulthood. We hypothesize that the TWA learning differences between infant and adolescent rats are mirrored by differential metabolic recruitment of brain regions involved in the task.
Mapping 2-Fluoro-deoxy-glucose utilization, we compared the metabolic activity in 39 brain regions in infant (P17-P21) and adolescent (P38-P42) rats during acquisition and retrieval of a TWA task.
Principal component analysis revealed 1) a cognitive/sensory-motor, 2) an emotional-autonomic and 3) a modulatory component contributing to the variance of the metabolic activity. Inter-regional correlation analysis of the metabolic activity revealed an increased degree of correlation with repeated training. During retrieval, the infant rats displayed a correlation pattern resembling that of adolescent animals during acquisition implying that their brains are still “under acquisition” after repeated training.
During adolescence, the avoidance performance was negatively correlated with the metabolic activity of cortico-limbic, hippocampal, modulatory and sensory-motor brain regions implying that learned tasks require less energy. During infancy, only the total shock exposure was negatively correlated with the metabolic activity of most regions, except hippocampus, extended amygdala and PAG indicating that the aversive stimulus broadly suppresses activation. We conclude that especially the mature functional connectivity of the extended amygdala with prefrontal areas and modulatory brain stem regions is essential for the translation of acquired information into behavioral output.

Kategorie: Vortrag

Vortrag 34

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

Titel: Differential expression of mover, a novel phospho protein of synaptic vesicles

Autoren: Dresbach T.(1),Islinger M.(1),Wittenmayer N.(1),Körber C.(1),Kuner T.(1),Kirsch J.(1),

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Abstract:
Neurotransmitter release relies on a highly organized structural arrangement and functional interplay of presynaptic components, including synaptic vesicles, active zone scaffolding proteins and associated proteins. These components work at all synapses, but our understanding of how the functional architecture of these key components is modified to account for the specialization of neurotransmission across synapses and for activity-dependent plasticity is limited. Here we report on the identification and characterization of Mover, a novel presynaptic protein. We find that Mover is a peripheral membrane protein of synaptic vesicles that also binds the scaffolding protein Bassoon. Using a phospho-specific antibody we show that phosphorylated Mover is highly enriched in the synaptic vesicle fraction. Blocking neuronal activity in cultured neurons using tetrodotoxin leads to down-regulation of Mover, implicating Mover in events of presynaptic homeostatic plasticity. In the brain, Mover is differentially expressed among subsets of synapses. In the auditory system, Mover is differentially expressed among the highly specialized calyceal synapses termed Calyces of Held and Endbulbs of Held. We propose that Mover may bridge synaptic vesicles to the active zone cytomatrix and mediate aspects of activity-dependent presynaptic regulation.

Kategorie: Vortrag

Vortrag 35

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

Titel: Adult neurogenesis of the dentate gyrus requires the zinc finger transcription factor bcl11b

Autoren: Baumann L.(1),Brylka H.(1),Jenkins N.(2),Copeland N.(2),Simon R.(1),Britsch S.(1),

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Abstract:
Adult neurogenesis occurs only in two regions of the brain, the dentate gyrus and the olfactory bulbs. The dentate gyrus is the major afferent part of the hippocampus, the center for spatial navigation, learning and memory. Previously we have shown that the zinc finger transcription factor Bcl11b plays an essential role during the postnatal development of the hippocampus, in particular of the development of the dentate gyrus.
Here we report that Bcl11b is not only required during postnatal development of the dentate gyrus, but also in adult neurogenesis. Adult mice harboring a forebrain specific deletion of Bcl11b exhibit a persistently smaller size of the dentate gyrus, and a significantly reduced number of granule cells. While numbers of BrdU-incorporating granule cell precursors are significantly reduced in Bcl11b mutant animals, survival of existing neurons is not affected by the mutation. The phenotype of Bcl11b mutants could be caused directly or indirectly, either by a reduced proliferation rate of progenitor cells or a depletion of the stem cell compartment. To distinguish between these hypotheses we determined numbers of cells expressing stem cell markers within the dentate gyrus of conditional Bcl11b mutants generated either by intermating with Emx1-Cre (mitotic recombination) or Nex-Cre (postmitotic recombination) mice. Finally, we demonstrate that Bcl11b is required for cell-type specific differentiation of neurons generated during adult neurogenesis.

Kategorie: Vortrag

Vortrag 36

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

Titel: Estradiol regulates aromatase activity via calcium-induced calcium release (cicr) in hippocampal neurons

Autoren: Zhou L.(1),Fester L.(1),Jarry H.(2),Rune G.(1),

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Abstract:
In hippocampal neurons, we have previously shown that both, inhibition of aromatase and estradiol downregulate SYNPO expression, a postsynaptic protein enriched in mushroom shaped spines. Since SYNPO is closely associated with internal Ca2+ stores, we tested whether Ca2+ transients would contribute to these discrepant findings. Blocking of Ca2+ channels on internal stores and depletion of internal Ca2+ stores increase estradiol synthesis and SYNPO immunoreactivity dose-dependently. The effect of estradiol regarding SYNPO expression was abolished, when we treated the cultures with estradiol together with Ca2+ channel blockers. Estradiol treatment was also ineffective in the presence of thapsigargin, which depletes internal Ca2+ stores. Estradiol-induced downregulation of SYNPO expression was mediated by estrogen receptor (ER) beta and was achieved using BSA-estradiol. Since estradiol induces Ca2+ influx into neurons, our data indicate that exogenously applied estradiol to hippocampal cultures downregulates SYNPO expression via Calcium release from internal stores, which in turn inhibits aromatase by phosphorylation. As a consequence, downregulation of SYNPO after aromatase inhibition was enhanced by additional application of estradiol, whereas the downregulation of SYNPO in Aromatase-deficient mice was rescued by treatment of the mutants with estradiol. Our findings show that treatment of hippocampal neurons with estradiol concomitantly inhibits aromatase activity within the neurons.

Kategorie: Vortrag

Vortrag 37

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

Titel: Increased astroglial differentiation of precursor cells in the developing reeler dentate gyrus

Autoren: Bock H.(1),Zhao S.(2),Frotscher M.(2),Brunne B.(2),

Adressen:(1)Universität Freiburg|Zentrum für Neurowissenschaften|Freiburg|Deutschland; (2)Freiburg|Institut für Anatomie und Zellbiologie|Freiburg|Deutschland

Abstract:
Secondary radial glial cells of the developing dentate gyrus can function as precursor cells. In reeler mice, the secondary radial glial scaffold is morphologically severely altered. However, it is unknown to which extent the lack of Reelin affects the maturation and astroglial vs. neuronal differentiation of precursor cells in the developing postnatal dentate gyrus. To address these questions, we established an immunohistochemical marker profile to delineate the maturation of secondary radial glial cells in wild type and reeler mice. Our results indicate that the maturation of secondary radial glia is virtually unaffected, whereas differentiation studies of BrdU-birthdated cells demonstrated a tendency toward increased astrogliogenesis in reeler mice. Future studies are required to establish whether this effect is a direct consequence of disrupted Reelin signaling in radial precursor cells or whether this is secondary to the altered radial glial morphology.

Kategorie: Vortrag

Vortrag 38

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

Titel: Adam12 is expressed by astrocytes during experimental demyelination

Autoren: Baertling F.(1),Beyer C.(1),Kipp M.(1),

Adressen:(1)RWTH Aachen|Institut für Neuroanatomie|Aachen|Deutschland; email:mkipp@ukaachen.de

Abstract:
“A disintegrin and metalloproteinase 12” (ADAM12) is a member of a large family of multi-domain proteins. Within the CNS, ADAM12 has been described as a valid marker for oligodendrocytes. During experimental autoimmune encephalomyelitis (EAE), a commonly used animal model for multiple sclerosis (MS), ADAM12 expression is up-regulated in demyelinated lesions due to infiltrating T-cells. We investigated the cell-specific ADAM12 expression in the cuprizone mouse model, another MS model where T-cell independent demyelination and oligodendrocyte loss occurs.
Double-labelings in healthy C57BL6 mice revealed that ADAM12 is not only expressed by numerous oligodendrocytes but also by many neurons and very few astrocytes. Microglia cells neither expressed ADAM12 under resting conditions nor upon activation.
After the induction of acute demyelination, the expression of ADAM12 mRNA remained unchanged in brain regions affected by loss of ADAM12-positive oligodendrocytes. These regions in turn revealed a remarkable increase of ADAM12 expressing astrocytes. Astrocytes were further investigated in vitro with regard to their capacity of ADAM12 expression. Exposure to distinct neuropathological agents revealed a highly stimulus-specific regulation of ADAM12 gene expression in primary astrocytes.
These results show that ADAM12 is not a valid oligodendrocyte marker. The loss of ADAM12 expressing oligodendrocytes in the cuprizone model seems to be compensated by an increase of ADAM12 expression by astrocytes. Therefore, astrocytic ADAM12 expression might be implicated in the course of distinct CNS diseases such as demyelinating disorders.

Kategorie: Vortrag

Vortrag 39

Rubrik: 6.Reproduktionsbiologie
Abstract Nr.:6

Titel: Obesity, infertility and oxidative stress

Autoren: Serke H.(1),Hirrlinger J.(2),Nowicki M.(1),Blumenauer V.(3),Hmeidan F.(3),Jogschies P.(3),Spanel-Borowski K.(1),

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Abstract:
The response of granulosa cells of different origins to oxidative stress in preovulatoric follicle is still ill-define. We have recently investigated the oxLDL-dependent cell death of granulosa cell subtypes to specific lipoprotein receptors and antioxidativen enzymes (Serke et al. Autophagy 2009, JCEM 2010). In the present study we hypothesized that obesity leads to infertility via oxidative stress. Cultures of cytokeratin (CK)-positive, CK-negative granulosa cells and of cumulus cells taken from women undergoing in vitro fertilization (IVF) therapy were treated with 150 µg/ml oxLDL or native LDL under serum-free conditions for 36 h. Protein extracts were studied by Western blotting for LOX-1, toll-like receptor 4 (TLR4), CD36, microtubule-associated-light-chain-protein 3 (LC3, as autophagy marker) and cleaved caspase-3 (as marker for apoptosis). The activities of catalase, superoxide dismutase (SOD) and glutathione peroxidase (GPx) were determined in their lysates and supernatants. CK-- and cumulus cells underwent reparative autophagy, whereas non-apoptotic cell death was noted in CK+ cells. The oxLDL-dependent increase in lipoprotein receptors and in antioxidant activities was cell type-specific. TLR4/SOD was elevated in CK+ cells; LOX-1/GPx was increased in CK- cells and CD36/catalase in cumulus cells. The inhibition of the lipoprotein receptors prevented both the up-regulation of their own receptors and of cell death markers. In summary our results show that catalase and GPx levels are likely to reflect the oxidative state of the CK+ population. Thus, CK+ cells acts as a functional sensor of oxidative stress thus providing a predictive and diagnostic parameter in infertility (supported by the DFG Sp232/12-1).

Kategorie: Vortrag

Vortrag 40

Rubrik: 7.Immunbiologie
Abstract Nr.:7

Titel: Comparison of a cd3 antibody therapy alone or in combination with the immunomodulatory agent fty720 for protection of beta cell function in a model for type 1 diabetes

Autoren: Jörns A.(1),Akin M.(1),Meyer zu Vilsendorf A.(2),Arndt T.(1),Lenzen S.(1),

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Abstract:
The IDDM (LEW.1AR1-iddm) rat is a very suitable model of type 1 diabetes mellitus (T1DM) to prove new prevention strategies with immunomodulatory agents such as CD3-antibody (AB) and FTY720 for protection of beta cells from autoimmune destruction.
Therefore IDDM rats were treated with CD3-AB (0.25 mg/kg body weight) consecutively over 5 days before and after diabetes manifestation and additionally in a combined therapy over 40 days with FTY720 (1 mg/kg body weight). Metabolic parameters and islet morphology were analysed in pancreatic biopsies.
Prevention therapy with CD3-AB initiated before diabetes manifestation suppressed clinical diabetes manifestation. However, 60 days thereafter pancreatic islets of 60 % of the treated animals were infiltrated with macrophages and T-lymphocytes without pro-inflammatory cytokine expression. These silent immune cells were never found during spontaneous diabetes development. In contrast starting the therapy after overt diabetes manifestation, only 25% of the treated animals returned to normoglycaemia with remaining beta cells in infiltrated islets 60 days after the end of therapy. The combined treatment of CD3-AB and FTY720 induced a permanent normoglycaemia over 60 days after therapy without signs of immune cell infiltration in the islets. The beta cells showed a strongly reduced apoptosis rate and well preserved cell organelles.
The remaining infiltration in the pancreas of IDDM rats after CD3-AB treatment explained the limited success in humans with T1DM. In the combined therapy, however, the second compound retains proliferating lymphocytes in the pancreas draining lymph nodes and thereby leads to a permanent therapy success by islet immune cell infiltration prevention.

Kategorie: Vortrag

Vortrag 41

Rubrik: 4.Zellbiologie
Abstract Nr.:4

Titel: Identification of transport related structural changes in the organic cation transporter 1

Autoren: Egenberger B.(1),Koepsell H.(1),Gorboulev V.(1),

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Abstract:
The organic cation transporter 1 (OCT1) is critically involved in pharmacokinetics of cationic drugs. OCT1 translocates organic cations with diverse structures such as choline, 1-methyl-4-phenylpyridinium (MPP) and tetraethylammonium (TEA) and is inhibited by various compounds that are nottransported like tetrabutyammonium (TBuA). Functional characterization of rat OCT1 (rOCT1), mutagenesis and homology modeling of two conformations of the tertiary structure provided evidence that organic cations bind to overlapping domains in the cleft formed by transmembrane helices (TMHs) 2, 4, 5, 7, 8, 10 and 11. Subsequently, the cleft opens to the intracellular side and releases the substrates. Asp475 in the middle of TMH11 and Trp218 in the middle of TMH4 are critical for the substrate induced translocation. Employing voltage-clamp-fluorometry after labeling of Phe483 one turn above Asp475 in TMH11 with the fluorescent dye tetramethylrhodamine-6-maleinamide (TMRM) voltage induced movements were observed that were blocked by choline, MPP and TBuA. To determine whether these movements are part of a large conformational change during transport we tried to detect voltage and cation dependent movements after TMRM labeling of amino acids in extracellular parts of additional TMHs. We observed voltage dependent fluorescent changes at Pro260(TMH5) and Phe380 (TMH8) that were differentially modulated by choline, MPP and TBuA. When the transport function was blocked after covalent modification of Gly478 in TMH11 of rOCT, the voltage and substrate dependent movements of Phe380 and Phe483 were blocked. The data indicates that organic cation transport by rOCT1 encompasses a major conformational change including TMHs 5 and 11.

Kategorie: Vortrag

Vortrag 42

Rubrik: 4.Zellbiologie
Abstract Nr.:4

Titel: Genetic renal epithelial deletion of von Hippel Lindau does not augment the progression of a rapid progressive kidney disease

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Abstract:
Renal tubulointerstitial hypoxia is thought to account for the progression of chronic kidney disease. Additionally, the epithelial induction of hypoxia-inducible factors (HIF) are hypothesized to induce renal fibrosis although, protective effects of HIF expression was demonstrated as well.
Using inducible von Hippel Lindau (VHL)-knock out mice leading to increased epithelial stability of HIF □-subunits we aimed to analyze HIF-functions on the tubulointerstitial changes during the progression of chronic kidney disease.
Therefore, VHL knock out was induced by administration of doxycyclin (+DOX) and a rapid progressive glomerulonephritis (GN) was achieved by injection of anti-glomerular basement membrane antibodies. After 3 weeks, animals were perfusion-fixed for histochemical analysis or kidneys were removed for biochemical assays.
Administration of DOX lead to the epithelial expression of HIF-1□ and HIF-2□. Morphological analysis of glomerular and tubulointerstitial damage revealed a strongly reduced disease development in +DOX/ GN compared to –DOX/ GN. Vascular endothelial growth factor was strongly increased in kidneys and plasma of +DOX and +DOX/ GN mice. This increase was associated with augmented endothelial cell proliferation and capillary growth. Doxycyclin further induced the formation of transforming growth factor-□1, □-smooth muscle actin, collagen 1 and the expression of myofibroblasts which was strongly pronounced after GN. Capillary growth of the tubulointerstitium and therefore tubular oxygen supply did not prevent tubular degeneration.
Renal damage of +DOX/ GN was reduced compared to –DOX/ GN, suggesting that increased HIF-□ subunit expression is not harmful. VHL knock out indeed lead to increased interstitial matrix production through myofibroblast formation, but renal functions and morphology remained normal.

Kategorie: Vortrag

Vortrag 43

Rubrik: 4.Zellbiologie
Abstract Nr.:4

Titel: Phospholipase A2beta is expressed in mammalian distal tubule and regulates the urine concentrating mechanism

Autoren: Paliege A.(1),Röschel T.(1),Seidel S.(1),Kahl T.(1),Daigler A.(1),Mutig K.(1),Mrowka R.(2),Ferreri N.(3),Wilson B.(4),Raymond-Stintz M.(4),Bleich M.(5),Himmerkus N.(5),Bachmann S.(1),

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Abstract:
Na+2Cl-K+ cotransporter (NKCC2)-mediated NaCl reabsorption in the thick ascending limb (TAL) is effectively activated by vasopressin (AVP) via V2 receptor/PKA/cAMP signalling. This is counteracted by locally produced eicosanoids such as 20-HETE or prostaglandin E2 which are generated from arachidonic acid released by phospholipase A2 (PLA2). A Ca++-insensitive PLA2 isoform, whose identity was unkown, has been shown to regulate TAL transepithelial transport. We have used PCR, Western blot, and immunohistochemistry to identify this isoform as iPLA2beta. iPLA2beta expression was studied in kidneys of rodents and man. Effects of the iPLA2 inhibitor, bromoenol lactone (BEL, 5µM, 1h), on NKCC2 activity were studied in cultured TAL cells. The effect of AVP on iPLA2beta expression was studied in rats with central diabetes insipidus (DI) supplemented with the V2R agonist, desmopressin (dDAVP, 5ng/h; 3d). Immunohistochemistry showed strong iPLA2beta expression in TAL and distal convolutions and weaker expression in podocytes and inner medullary collecting ducts. In TAL cells, inhibition of iPLA2beta substantially increased surface expression, and phosphorylation of NKCC2. Supplementation of dDAVP in DI rats markedly reduced outer medullary iPLA2beta abundance (-65%; p<.05). Concomitantly, NKCC2 was activated. These results suggest that iPLA2beta acts as an inhibitory modulator of NKCC2 activity. Downregulation of iPLA2beta may therefore be an important mechanism of AVP-mediated urine concentration.

Kategorie: Vortrag

Vortrag 44

Rubrik: 4.Zellbiologie
Abstract Nr.:4

Titel: Vasopressin v2 receptor signalling in kidney distal tubule – links to nephrogenic diabetes insipidus

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Abstract:
Vasopressin promotes urinary concentration by activating the V2 vasopressin receptors (V2R). Major expression of V2R was localized to the thick ascending limb (TAL), distal convoluted tubule (DCT), and collecting duct. Inactivating mutations in the V2R gene result in X-linked form of nephrogenic diabetes insipidus (NDI) with loss of renal urine concentrating ability.
To distinguish between the pathogenetic mechanisms originating in collecting duct and distal tubule we generated a transgenic rat model (Ni3-V2R) with selective overexpression of a dominant-negative mutant of V2R (Glu242 stop; identified in human NDI) in TAL and early DCT under the control of the Tamm-Horsfall protein promoter. Overexpression of the mutated V2R was confirmed by immunohistochemistry and Western blot. Ni3-V2R rats displayed polyuria (1.5 fold in steady state and 3.5 fold under water deprivation for 18h, p<0.05) and altered electrolyte handling. Urine osmolality was 50% lower at steady state and under water deprivation than in control littermates (p<0.05). Morphological evaluation of adult transgenic kidneys demonstrated a size reduction of the renal medulla and fibrotic areas within the medullary rays. Biochemical profiling revealed decreased mRNA and protein abundance of the major distal Na,(K),Cl-co-transporters, NKCC2 (-55% and -64%, respectively; p<0.05) and NCC (-76% and -48%; p<0.05).
These data indicate that suppression of the V2R signalling in the distal tubule results in impaired urinary concentration and may be significantly involved in the pathogenesis of human X-linked NDI.

Kategorie: Vortrag

Vortrag 45

Rubrik: 4.Zellbiologie
Abstract Nr.:4

Titel: Blebbing confers resistance against cell lysis

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Abstract:
Plasmalemmal injury is a common event in the life of cells and often leads to their premature, necrotic death. Blebbing - a display of plasmalemmal protrusions - is a characteristic feature of injured cells. We have disclosed a previously unknown role for blebbing in furnishing resistance to plasmalemmal injury. Blebs serve as precursors for injury-induced intracellular compartments that trap damaged segments of the plasma membrane. Hence, loss of cytosol and the detrimental influx of extracellular constituents are confined to blebs that are sealed off from the cell body by plugs of annexin A1 - a Ca(2+)- and membrane-binding protein. Our findings shed light on a fundamental process that contributes to the survival of injured cells.

Kategorie: Vortrag

Vortrag 46

Rubrik: 4.Zellbiologie
Abstract Nr.:4

Titel: Protective endogenous camp signaling triggered by pemphigus autoantibodies

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Abstract:
Pemphigus vulgaris (PV) is an autoimmune skin disease mediated by autoantibodies directed against the cadherin-type intercellular adhesion molecules desmoglein (Dsg) 3 and Dsg1 and is characterized by loss of keratinocyte cohesion and epidermal blistering. Several intracellular signaling pathways such as p38MAPK activation and RhoA inhibition have been demonstrated to be altered following autoantibody binding and to be causally involved in loss of keratinocyte cohesion. Here, we demonstrate that cAMP-mediated signaling completely prevented blister formation in a neonatal pemphigus mouse model. Furthermore, elevation of cellular cAMP levels by forskolin/rolipram or isoproterenol blocked loss of intercellular adhesion, depletion of cellular Dsg3, and morphologic changes induced by antibody fractions of PV patients (PV-IgG) in cultured keratinocytes. Incubation with PV-IgG alone increased cAMP levels, indicating that cAMP elevation may be a cellular response pathway in order to strengthen intercellular adhesion. Our data indicate an involvement of protein kinase A signaling in this protective pathway which finally inhibits PV-IgG-induced activation of p38MAPK. Taken together, our data provide insights into the cellular response mechanisms following pemphigus autoantibody binding and point to a possible novel and more specific therapeutic approach in pemphigus.

Kategorie: Vortrag

Vortrag 47

Rubrik: 4.Zellbiologie
Abstract Nr.:4

Titel: Role of nf-kb activation in lps-induced endothelial barrier breakdown

Autoren: Schlegel N.(1),Leweke R.(2),Gemer C.(1),Waschke J.(2),

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Abstract:
Endothelial barrier breakdown contributes to organ failure in sepsis. The key mechanism by which the sepsis inductor Lipopolysaccharide (LPS) disrupts the endothelial barrier is controversial. Here we tested the hypothesis that NF-kappaB activation is critically involved in endothelial barrier breakdown.
Application of LPS to monolayers of porcine pulmonary artery endothelial cells (PAEC) and human dermal microvascular endothelial cells (HDMEC) induced a rapid and sustained activation of NF-kappaB as revealed by translocation of NF-kappaB subunit p65 into the nuclei in nuclear extraction assays and by immunostaining. Measurements of transendothelial electrical resistance (TER) and intercellular gap formation demonstrated significant disruption of endothelial barrier properties following LPS treatment after 3h. Interestingly, monolayers recovered spontaneously beginning after 10h. Increased cAMP (treatment with forskolin/rolipram) prevented LPS-induced loss of endothelial barrier properties but did not block NF-kappaB activation. In contrast, application of the cell-permeable NEMO binding domain (NBD) synthetic peptide was effective to prevent NF-kappaB activation but did block neither LPS-induced loss of TER nor intercellular gap formation. Interestingly, in the presence of NBD-peptide the barrier-compromising effects of LPS were enhanced compared to monolayers treated with LPS alone. Known targets of NF-kappaB-driven protein expression such as caveolin1 or vasodilator-stimulated phosphoprotein (VASP) remained unaltered by LPS treatment of endothelial cells.
In summary, our data indicate that NF-kappaB activation by LPS is not critically involved in disruption of endothelial barrier properties. Rather, our data are in line with the hypothesis that NF-kappaB activation may act as a part of a rescue mechanism involved in endothelial barrier restoration.

Kategorie: Vortrag

Vortrag 48

Rubrik: 4.Zellbiologie
Abstract Nr.:4

Titel: Tumour progression switches chemokine receptor expression and functional role in gliomas

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Abstract:
Chemokines and their receptors play a major role in tumour progression. Especially the CXCL12 - CXCR4 pair is known to contribute to tumour invasion, proliferation and metastasis. Recently, CXCR7 was discovered as a novel, alternative receptor for CXCL12 / SDF-1 and CXCL11 / I-TAC. Since both chemokines are expressed abundantly in human astrocytomas and glioblastomas, we investigated expression and function of ligands and receptors in these tumour specimens. In situ, CXCR7 is highly expressed on tumour endothelial, microglial and glioma cells whereas CXCR4 expression is much more restricted. CXCR7-transcription in homogenates increased with malignancy and CXCR7 was also highly expressed in all glioma cell lines investigated whereas CXCR4 was only scarcely transcribed on 1 of 8 lines. In contrast, a glioma stem-like cell line predominantly expressed CXCR4 that diminished upon differentiation whereas CXCR7 rose drastically. CXCR7-positive glioma cells (CXCR4- and CXCR3-negative) were activated by CXCL12 stimulation as shown by transient phosphorylation of extracellular-signal regulated kinases Erk1/2 indicating that the receptor is functionally active. Whereas proliferation and migration was little influenced, CXCL12 stimulation prevented Camptothecin- and Temozolomide-induced apoptosis. The selective CXCR7-antagonist CCX733 reduced this anti-apoptotic effects of CXCL12 as shown by nuclear (Nicoletti) staining, caspase-3 activity assays and cleavage of poly(ADP-ribose) polymerase-1 (PARP). Thus, CXCR7 is a functional receptor for CXCL12 in astrocytomas / gliomas and mediates resistance to apoptosis. Whereas CXCR4 is found on glioma stem-like cells, the receptor expression switches to CXCR7 in more mature glioma cells.

Kategorie: Vortrag

Vortrag 49

Rubrik: 7.Immunbiologie
Abstract Nr.:7

Titel: Toll-like receptor 2/6 stimulation promotes angiogenesis via granulocyte-macrophage colony stimulating factor

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Abstract:
Background: Toll-like receptors (TLRs) are known as pathogen recognition receptors of the innate immunity, initiating inflammatory pathways to organize the immune defense. Since inflammation involves angiogenesis, we here elucidated the effect of a TLR-dependent pathway on angiogenesis using the TLR2/6 agonist MALP-2, a common bacterial lipopeptide.
Methods and Results: MALP-2 induced angiogenesis in vivo (Matrigel implants, hemoglobin content, H+E staining, P=0.01, n=4-8) and tube formation in vitro (Matrigel, P=0.05, n=3-5) which could be inhibited with neutralizing antibodies against TLR2/6. Moreover, endothelial cells responded to MALP-2 with enhanced proliferation and migration (BrdU-incorporation, transwell, P=0.01, n=5-7). Protein array and ELISA revealed a strong secretion of granulocyte-macrophage colony stimulating factor (GM-CSF) from endothelial cells of different origin (venous, arterial, microvascular) which could be inhibited with antibodies against TLR2/6 (P=0.01, n=4). By contrast, smooth muscle cells and fibroblasts did not secrete GM-CSF in response to MALP-2 (ELISA, n=3-6). MALP-2 containing Matrigel implants exhibited vascular structures as well as CD45+ cells (CD45 immunostaining, n=4). Following MALP-2 stimulation CD45+ cells showed likewise enhanced migration (transwell, P=0.05, n=5) and GM-CSF release (ELISA, P=0.01, n=5), particularly from the monocyte population. Finally, inhibition of GM-CSF by siRNA in vitro (Matrigel, P=0.05, n=4) or by antibodies in vivo (Matrigel implants, P=0.01, n=4-8) suppressed MALP-2 induced angiogenesis.
Conclusion: These results clearly identified a TLR2/6-dependent induction of angiogenesis by the bacterial lipopeptide MALP-2, which is mediated by GM-CSF. This might represent a general mechanism to enhance or restore blood flow and recruit immune cells for pathogen defense and tissue regeneration.

Kategorie: Vortrag

Vortrag 50

Rubrik: 4.Zellbiologie
Abstract Nr.:4

Titel: Ultrastructural analysis reveals camp-dependent enhancement of microvascular endothelial barrier functions via rac1-mediated reorganization of intercellular junctions

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Abstract:
Evidence exists that cAMP stabilizes the endothelial barrier in part via activation of the small GTPase Rac1. However, despite the high medical relevance of this signaling pathway the mechanistic effects on intercellular contacts on the ultrastructural level are largely unknown. In microvascular endothelial cell monolayers, in which increased cAMP strengthened barrier properties similar to intact microvessels in vivo, both forskolin/rolipram (F/R) (to increase cAMP) and O-Me-cAMP (to stimulate Epac/Rap 1 signaling) enhanced transendothelial electrical resistance (TER) and induced activation of Rac1. Concurrently, augmented immunofluorescence intensity and linearization of signals at cell borders were observed for intercellular junction proteins VE-cadherin and claudin 5. Ultrastructural analysis of the intercellular contact zone architecture documented that exposure to F/R or O-Me-cAMP led to a significant increase in the proportion of contacts displaying complex interdigitations of cell borders in which membranes of neighboring cells were closely apposed over comparatively long distances and which were stabilized by numerous intercellular junctions. Interference with Rac1 activation by NSC-23766 completely abolished both barrier stabilization and contact zone reorganization in response to O-Me-cAMP whereas F/R-mediated Rac1 activation and barrier enhancement were not affected by NSC-23766. In parallel experiments using macrovascular endothelium, increased cAMP failed to induce Rac1 activation, barrier enhancement and contact zone reorganization. These results indicate that in microvascular endothelium Rac1-mediated reorganization of contact zone architecture contribute to cAMP-induced barrier stabilization.

Kategorie: Vortrag

Vortrag 51

Rubrik: 4.Zellbiologie
Abstract Nr.:4

Titel: Nitric oxide-sensitive guanylyl cyclase is mainly expressed in pericytes in the murine lung

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Abstract:
Nitric oxide (NO) serves important functions in the lung. However, information on the cell type(s) expressing its receptor NO-sensitive guanylyl cyclase (NO-GC) is lacking.
Immunohistochemistry was performed in thick sections of murine lungs on the light and electron microscopic level. Double and triple labeling were performed in combination with appropriate cell type-specific markers as well as with antibodies against downstream components of the NO-GC pathway. The specificity of the antibody against the beta1 subunit of NO-GC was tested in NO-GC beta1-deficient mice.
As expected, vascular smooth muscle cells exhibited NO-GC immunoreactivity. However, the highest intensity of NO-GC-immune reaction was located in cells in the alveolar region. NO-GC immunoreactivity was not located in endothelial cells, as previously reported, but was present in highly branched cells whose processes were in close contact to capillary endothelial cells. Endothelial cells themselves were not immunoreactive for NO-GC. Close to the pleura where capillaries were more elongated as compared to the more central portions of the lung, NO-GC-immunoreactive cells encompassed endothelial cells and had the classical morphology of pericytes. Preembedding immuno-electron microscopy confirmed that NO-GC-immunoreactive cell processes shared the basememt membrane with endothelial cells which is a distinct feature of pericytes. NO-GC-immunoreactive cells were also immunoreactive for other downstream components of the NO-GC pathway such as vasodilator-stimulated phosphoprotein and phosphodiesterase 5.
These results show that NO-GC is predominantly expressed in pericytes in the alveolar region and co-localizes with other parts of the NO-GC signal transduction machinery.

Kategorie: Vortrag

Vortrag 52

Rubrik: 4.Zellbiologie
Abstract Nr.:4

Titel: Nrf2 protects hepatocytes against chronic liver injury and fibrosis progression in a mouse model of sclerosing cholangitis

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Abstract:
Background: Increasing evidence suggests that oxidative stress play a key role in the etiology of sclerosing cholangitis. Aim of this study was to elucidate the hepatic defence mechanisms employed against oxidative stress and, in particular, the specific role of nuclear factor erythroid 2-related factor 2 (Nrf2). The DDC-model leads to chronic cholestatic liver injury and therefore resembles human diseases like sclerosing cholangitis and forms of metabolic liver diseases.
Methods: Mice were treated with 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC) containing diet and analysed over time.
Results: Mice deficient in Nrf2 showed an increased and earlier mortality compared to wild type mice. Over time significantly more necrosis, apoptosis and cholestasis became evident in Nrf2-knockout mice. This was associated with stronger periportal oval cell activation. Long term analysis unravelled the development of severe liver fibrosis in Nrf2-knockout animals evidenced by increased collagen accumulation. Moreover, we show evidence that Nrf2 is the transcription factor that activates IL-6 expression in this cholestatic hepatitis mouse model. In contrast, mice with hepatic deletion of Keap1, the inhibitor of Nrf2, has a higher survival rate and significant less liver damage compared to wild type mice.
Conclusion: Here we demonstrate that Nrf2 signaling in hepatocytes provides protection in a sclerosing cholangitis mouse model. Nrf2-dependent signaling pathways in hepatocytes protect from liver necrosis and tissue injury which subsequently prevents from fibrosis progression.

Kategorie: Vortrag

Vortrag 53

Rubrik: 4.Zellbiologie
Abstract Nr.:4

Titel: Altered fracture repair in the absence of nrf2

Autoren: Beckmann R.(1),Lipross S.(2),Varoga D.(3),Glüer C.(4),Tohidnezhad M.(5),Brüggemann S.(6),Özdogru F.(7),Pufe T.(5),Wruck C.(5),

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Abstract:
The transcription factor nuclear factor E2p45-related factor 2 (Nrf2) is involved in the response to oxidative stress. Nrf2 forms heterodimers with small Maf proteins for selective recognition of the antioxidant response element to promote the expression of phaseII detoxifying enzymes as well as oxidative stress-inducible proteins in different tissues. In recent studies we could detect microfractures in Nrf2 knockout mice after antibody induced arthritis. Based on this observation, we investigate the influence of Nrf2 in fracture healing and bone structure in vivo.
To study the Nrf2-activation during fracture healing we used the transgenic ARE-luc mice, in which the ARE-sequence is linked to a luciferase reporter gene. Using a standard femur fracture model we evaluated callus formation of the Nrf2 knockout mouse via microCT and histology technique (HE, Toluidine blue, Ladewig staining).
In our ARE-luc mouse model of fracture healing we could demonstrate an initial increase of ARE-activity and a time dependent decrease of ARE-activity during 21 days of fracture healing by Xenogen-analysis. MicroCT-analysis of fractured femora revealed a smaller callus formation in Nrf2 knock out mice compared to wild type. In addition, fractured femora of Nrf2-knockout mice showed a lower number of bone trabecules and decreased cortical bone volume compared to the wild type mice. Ladewig staining revealed a decreased remodelling in Nrf2 knock out mice compared to wild type.
The results demonstrate the importance of oxidative stress in fracture healing and suggest that Nrf2 plays an essential role in bone regeneration.
Keywords: Nrf2, Fracture healing,

Kategorie: Vortrag

Vortrag 54

Rubrik: 4.Zellbiologie
Abstract Nr.:4

Titel: Biomaterial-associated tissue-engineered tenocytes constructs for tendon repair

Autoren: Schulze-Tanzil G.(1),Stoll C.(1),Conrad C.(1),Hondke S.(1),Kohl B.(1),Endres M.(2),Kaps C.(2),Rosen C.(1),Ertel W.(1),John T.(1),

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Abstract:
Tendon healing is a time consuming process generally leading to tendon scar formation and hence, impaired or altered function. Tendon tissue engineering might provide novel approaches to optimize tendon healing. Hence, the aim of this study was to establish and characterize tissue engineered tenocytes PLGA (poly[lactic-co-glycolic-acid]) and PGA (poly-glycolic-acid) constructs for the use in a tendon healing model in the rabbit.
Human and rabbit derived primary tenocytes were isolated from native tendon and sufficiently expanded in monolayer culture before seeded on PLGA and PGA scaffolds. Tenocytes vitality on the PLGA scaffolds and their gene expression profile for extracellular tendon matrix components and scleraxis were assessed using RTD-PCR. Rabbit tenocytes PGA constructs or cell-free scaffolds were implanted into partial Achilles tendon defects in the rabbit whereby non-treated defects served as a control. Tendon healing was assessed after 6 - 12 weeks using clinical, macroscopical and histological scoring systems.
Tenocytes revealed a high vitality and tendon matrix expression on the PLGA and PGA scaffolds. Compared with tenocytes gene expression in native tendon, monolayer-cultured tenocytes revealed distinct features of dedifferentiation. However, tenocytes cultured on the PLGA scaffolds exhibited an expression profile which showed some approximation to native tendon. Implantation of PGA-constructs seeded with tenocytes into rabbit tendon defects led to a reduced tendon swelling compared with the outcome of the untreated defects. However, some aspects of inflammation could be observed.
Tenocyte PGA constructs could serve as a basis for the combination with anabolic growth factors or anti-inflammatory agents to improve tendon healing.

Kategorie: Vortrag

Vortrag 55

Rubrik: 4.Zellbiologie
Abstract Nr.:4

Titel: Assessment of satellite cell and myonuclear number in human vastus lateralis muscle in response to different endurance exercise regimens

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Abstract:
Satellite cells (SC) are mononuclear stem cells of skeletal muscle which play an important role in the muscular adaptive response to exercise. The exact extent of SC involvement in structural und functional remodelling processes in response to endurance exercise induced stimuli has received much less attention.
The aim of this study was to analyze the effect of load-dependent endurance exercise on the number of SC and myonuclei in skeletal muscle of 10 young male cyclists (17.3±0.5yr).
We investigated the training period (TP1, Nov-Feb), the competitive season (CS, Feb-Oct) and the following TP (TP2). After each period muscle biopsies were obtained from the vastus lateralis muscle. SC were labelled with a monoclonal Pax-7 antibody and myonuclear number was analysed from HE-cross-sections (7µm). The muscle fiber diameter was measured. The SC number per muscle fiber at the end of the CS (0.56±0.059) was significantly decreased towards TP1 (0.94±0.269) and TP2 (0.82±0.092; $p<0.05$). The analysis of myonuclear number illustrated an inverse pattern to the SC number. After CS the number of nuclei/fiber was significantly increased (4.00±0.933) towards TP1 (1.75±0.727) and TP2 (1.75±0.184), while no changes of fiber diameter were observed.
SC content is affected strongly in response to load-dependent endurance exercise. A change of exercise regimen from TP to CS by a reduction of volume and increase of intensity leads to a significant decrease of SC and a massive addition of the myonuclear number. According to the unaltered fiber diameter this indicates a non-hypertrophic hyperplasia.

Kategorie: Vortrag

Vortrag 56

Rubrik: 4.Zellbiologie
Abstract Nr.:4

Titel: Estrogen-mediated regulation of claudin-5 in vascular endothelium: lessons from estrogen receptor beta knockout mice

Autoren: Burek M.(1),Roewer N.(1),Foerster C.(1),

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Abstract:
Estrogens have multiple effects on vascular physiology and function. The biological actions of estrogens are mediated by two different estrogen receptor (ER) subtypes, ER alpha and ER beta. Estrogens have beneficial effects in several vascular disorders. Claudin-5 is an integral membrane protein that plays an important role in the structure and function of tight junctions. Claudin-5 is strongly expressed in the vascular endothelium. Recently, we have demonstrated an increase in claudin-5 mRNA and protein in brain and heart endothelial cell lines after treatment with 17 beta-estradiol (E2). The aim of the present study was to further characterize estrogen-mediated regulation of claudin-5 in vivo using the ER beta knockout mice and ovariectomized mice model. The treatment of endothelial cell lines with selective estrogen receptor agonists revealed that E2 regulation of claudin-5 is most probably mediated by ER beta. To confirm these results in vivo we used first an ovariectomized mice model. We demonstrated an increase in claudin-5 mRNA and protein level in ovariectomized mice that were treated with E2. Next, we examined the ER beta knockout mice for the expression of claudin-5 in the brain tissue. We detected a lower level of claudin-5 mRNA and protein in ER beta knockout mice in comparison to wild type mice. Further experiments are needed to examine which role estrogen-mediated claudin-5 regulation plays in cellular physiology. In conclusion, we describe claudin-5 as a novel estrogen target in the vascular endothelium and give an evidence for the involvement of ER beta in E2-regulation of claudin-5.

Kategorie: Vortrag

Poster des Satellitensymposiums „Anatomie im Nationalsozialismus“

Poster 1

Rubrik: 12. Anatomie im Nationalsozialismus
Abstract Nr.:

Titel: „The Hidden Faces of Hermann Voss“

Autoren: Jerzy Gielecki, Anna Żurada, Paweł Wiereńko, Anna Proba

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Abstract:
Those who are still questioning the evidence of the diary of Hermann Voss (†1987) covering the years 1932 to 1942 do not have to be concerned to search for a long time.
The available documents located in the archives of the “Instytut Zachodni” in Poznan were discovered by the anatomist Stefan Rożycki in 1945, released by the press and also used elsewhere particularly as an action for the prosecution of the Gau-Leader, Arthur Greiser.
Medical historian researchers excluded any falsification of the material and were convinced of its authenticity.
However, it did not lead to any consequences for Hermann Voss, who, at that time, was professionally active as a chief anatomist in Halle, Jenna, and, lastly, Greifswald. What was more, he was even honored by the then East Germany government with the title of a “Brilliant Scientist of the Nation”.
Hermann Voss, one of the founders of the Reich University in Poznan, established the Medical Faculty, and as its Dean, signed a contract with the Gestapo.
Until 1990 the Historical Museum of Nature in Vienna exposed the skulls of Polish and Jewish victims which were dissected and sold personally by Hermann Voss.
The diary gives us plenty of insights about Voss’ family life, his career, political views and his psychological condition, especially during his residence in Poznan. The authors analyzed the multiple personalities of that character as a “brilliant scientist” and a “thoughtful family person” in opposite to an “ambitious business-oriented person” who, as a “racist”, profited from murdered victims.

Kategorie: Poster

Poster 2

Rubrik: 12. Anatomie im Nationalsozialismus
Abstract Nr.:

Titel: „An investigation into the origin of corpses received by the anatomical institute in Jena during the Third Reich“

Autoren: Christoph Redies (1), Michael Viebig (2), Rosemarie Fröber (1) and Susanne Zimmermann (3)

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Abstract:
From 1933 to 1945, the Anatomical Institute of the University of Jena received the corpses of about 200 executed prisoners and an equal number of corpses from Thuringian nursing homes and mental institutions where decentralized "euthanasia" crimes took place. The body register from that time listed the names, origin, cause of death and usage in the institute for most of these corpses. In 2004, a group of anatomists and historians launched an investigation into the institute's association with Nazi crimes and the origin and whereabouts of these corpses, with a particular focus on the question whether any remains of Nazi victims were displayed in the anatomical collections of the institute. The press was informed from the beginning of the investigation. After a year of research, the court sentences for most of the executed and the circumstances of the euthanasia crimes were elucidated. The records of the anatomical collections did not reveal any direct link of specific anatomical specimens to Nazi victims. Nevertheless, following the suggestions by an external expert reviewer, some specimens of uncertain origin were buried in a place of honor. Results were made public at the institute's website, scientific meetings and in medical journals. A plaque installed permanently in the institute's foyer and a service held during the annual remembrance for the body donors commemorated the Nazi victims. First-year medical students are regularly informed about this period of the institute's history. The investigation received a positive response by the scientific community and the general public.

Kategorie: Poster

Poster 3

Rubrik: 12.Anatomie im Nationalsozialismus
Abstract Nr.:

Titel:„Executed People for the Anatomy, Halle 1933-1945“

Autoren: Schultka R. (1), Viebig M. (2)

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Abstract:
From 1933 until 1945 dead bodies of people sentenced to death, among them politically persecuted ones, were given to the Institute of Anatomy in Halle (memorial site ROTER OCHSE Halle/Saale, project lead M. Viebig). We are trying to answer two important questions:
1. What happened to the bodies of those executed, i.e. which anatomical "purposes" did they serve?
2. Were anatomical preparations manufactured that were added to the institutes' anatomical collection (originated by Meckel) and that are still present today? And if so, can they be traced back to the dead bodies of politically persecuted ones?
So far we have discovered that between 1933 and 1936 the institution received 30 dead bodies, among them the bodies of two politically motivated death sentences. From 1937 until the end of 1942 only a few dead bodies arrived at the institute, from November 1942 until the end of the war in 1945 the institute documented the transfer of 64 dead bodies of executed people. The death sentences pronounced during the first years were usually executed because of the severity of the criminal acts committed (e.g. murder). During the war special courts sentenced people to death mostly because of theft, looting, etc.
The dead bodies of those executed were used for anatomical education, anatomical research, and for the manufacture of preparations to be added to the anatomical collection. There are eight macroscopic preparations that can be safely associated with the dead bodies of people executed during the Nazi regime. However, jury courts sentenced those people to the maximum penalty because of the severity of their criminal acts. Up to now we have found no evidence that preparations of the anatomical collection were made of bodies of victims whose execution was politically motivated. However, we are still investigating this matter.

Kategorie: Poster

Poster 4

Rubrik: 12.Anatomie im Nationalsozialismus
Abstract Nr.:

Titel:„… *agreeing to provide 500 RM for the transfer of an increased number of corpses from Wolfenbüttel*."

Autoren: Ude-Koeller S.(1), Viebahn C.(2),

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Abstract:
Since Albrecht von Haller introduced - as the first the German speaking anatomist - the dissection of human corpses to the medical curriculum at the Georg-August-University of Göttingen in the middle of the 18th century, it was mainly places of disciplining, detention and punishment which supplied the necessary corpses to anatomical institutes: poorhouses, asylums and prisons. Nevertheless, many anatomical institutes complained about the scarcity of corpses. Still in the early 1930s, the Göttingen Anatomists were in doubt about the feasibility of conducting their regular dissection courses if the annual requirement of about 100 corpses could not be met. With the implementation of the NS terror regime this uncertainty came to a halt for many anatomical institutes, also for the Anatomical Institute in Göttingen. In 1937, a place for execution was opened in Wolfenbüttel, about 120 km from Göttingen. Until 1945 about 700 people were executed there as victims of the NS regime. The director of the Anatomical Institute personally contacted the deputy director of the prison and asked the curator of the University for additional funding to help with the procurement of corpses arising now in Wolfenbüttel. By collecting information on the number, origin and destiny of the corpses delivered to the Göttinger Anatomical Institute during the Nazi regime this paper describes how the Göttingen Anatomists made use of the increase of executions under NS law – in continuity with the historical "procurement policy" of the 18th and 19th century.

Kategorie: Poster

Poster 5

Rubrik: 12.Anatomie im Nationalsozialismus
Abstract Nr.:

Titel: Die Karriere des Anatomen August Hirt (1896-1945)

Autoren: Uhlmann A.

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Abstract:
Der Anatomie-Professor der Reichsuniversität Straßburg SS-Hauptsturmführer August Hirt (1898-1945) erreichte durch seine Greueltaten im Rahmen des „Ahnenerbes“ eine traurige Berühmtheit. Die Menschenversuche an Häftlingen im nahe gelegenen Konzentrationslager Natzweiler-Struthof und sein Projekt der jüdischen Skelettsammlung lagen in seiner Verantwortung. Allerdings ist wenig bekannt über seinen beruflichen und wissenschaftlichen Werdegang vor seiner Straßburger Zeit.
Hirt begann sein Medizinstudium 1917 an der Universität Heidelberg und verfolgte seine berufliche Laufbahn im Fachgebiet der Anatomie unter Erich Kallius. Von 1936 bis 1938 folgte er Karl Peter als planmäßiger Professor an der Greifswalder Universität. Anschließend wechselte Hirt im Rahmen eines Ordinarientausches mit Wilhelm Pfuhl nach Frankfurt, wo er bis zu seiner Berufung an die Reichsuniversität Straßburg im Jahr 1941 tätig war. Die Berufungsverhandlungen waren immer geprägt von Zuschreibungen Hirts wie „menschlich schwierig“, „er versteht es, Geld flüssig zu machen“, „er gehört nicht zu den großen schöpferischen Naturen, aber er hat niemals Wertloses veröffentlicht.“ Hirt war ein international anerkannter Wissenschaftler auf dem Gebiet der Mikroanatomie und Fluoreszensmikroskopie. Zusammen mit dem jüdischen Pharmakologie-Professor Philip Ellinger entwickelte er das erste praktisch einsetzbare Fluoreszenz-Mikroskop. Die Anatomen Anton Kiesselbach und Karl Wimmer begleiteten Hirt ebenso wie der Laborant August Mayer als Mitarbeiter von Greifswald bis Straßburg.
Die Karriere Hirts von der Universität Heidelberg bis zur nationalsozialistischen Vorzeigeuniversität in Straßburg kann als typisch für die Berufungspolitik des Nationalsozialismus gesehen werden. Die weitgehende Autonomie der Universitäten, die vor 1933 für die Besetzung der Lehrstühle Usus war, galt nicht mehr.

Kategorie: Poster

Poster 6

Rubrik: 12. Anatomie im Nationalsozialismus
Abstract Nr.:

Title: „Prof. Dr. med. Curt Elze (1885-1972), Professor for Anatomy in Würzburg (1940-1945, 1947-1951)“

Autor: Anna Wegener

Adresse: Institut für Geschichte der Medizin der Universität Würzburg, Oberer Neubergweg 10a, D-97074 Würzburg; e-mail: kerani_3@hotmail.com

Abstract:
Prof. Dr. med. Curt Elze (1885-1972) was a German Professor for Anatomy. During the time of the National Socialism he worked in Rostock (1921-1936), Gießen (1936-1940) and in Würzburg (1940 – 1945).
On the one hand he enjoyed high reputation. He was known for being a particularly experienced anatomist and was popular with students for being a great Professor and lecturer. On the other hand for a long time he was exposed to constant threat and spying by the Nazis because of his obvious dislike for National Socialism, which stopped in 1940 when he became a member of the NSDAP.
At the End of Second World War he was dismissed from his position as a Professor. He was able to prove his aversion against National Socialism and he was given back his position as a Professor for Anatomy in Würzburg in 1947.
Resources: Spruchkammerakte, Berufungsakten

Kategorie: Poster

Poster 7

Rubrik: 12.Anatomie im Nationalsozialismus
Abstract Nr.:1

Titel: "Too much honour for Max Clara? The Clara cell and the "Third Reich"

Autoren: Winkelmann A.(1),Noack T.(2),

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Abstract:
PURPOSE: Since its original description by Clara in 1937, the club-shaped secretory cells of the bronchiolar epithelium are known as Clara cells. This contribution asks whether Max Clara's support for National Socialism and the context of his histological research justify the honour conferred by naming an anatomical structure after him.
METHODS: Search in historical archives and analysis of publications by Clara and about Clara.
RESULTS: Following work in Innsbruck and Padua, Max Clara (1899-1967) held the chairs of anatomy in Leipzig (1935-1942) and Munich (until 1945). In his inaugural speech in Leipzig, he asked his colleagues to 'line up with the marching columns of our Führer'. He also proposed a "new" functional anatomy, which he labelled 'holistic'. After 1939, he explicitly saw a parallel between this holism and the '[emerging] totality in the life of the peoples'.
Clara was politically active as NS-Dozentenbundsführer in Leipzig and as chairman of the Anatomische Gesellschaft. After the war, he was officially cleared by the "denazification" process, a decision partly based on wrong assumptions.
Clara's broad histological research in Leipzig benefited from the rising number of executions after 1933, giving him 'perfectly fixed material'. His original description of the bronchial epithelium is based on such human tissue preserved 'immediately after death'. For investigations on brain tissue, Clara obviously asked prison staff to give one of the doomed prisoners vitamin C tablets for research purposes. Apparently, he was ready to see those sentenced to death as "guinea pigs".
CONCLUSION: The Clara cell should be renamed.

Kategorie: Poster

Poster des Programms der 27. Arbeitstagung der Anatomischen Gesellschaft

1. Methoden/Unterricht

Poster 1

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

Titel: From the laboratory book to data mining: implementation of an elec-tronic science documentation system

Autoren: Strohmaier C.(1),Runge C.(2),Bogner B.(3),Trost A.(4),Schrödl F.(5),Grabner G.(3),Reitsamer H.(3),

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Abstract:
Purpose: Modern laboratories use various techniques in concert to address scientific questions, usually organized in multiple projects running in parallel. Traditional, written documentation results in time consuming and inflexible analysis of the data as each aspect in the analysis process requires a complete walk-through of all written labora-tory books. Here, we present an electronic database system for scientific documenta-tion to combine a tissue bank with immunohistochemical, physiological and molecular-biological applications and enabling the cross-link of all applications in all directions.
Methods: For the documentation of scientific methods and results a relational data model was designed. The data model was implemented using Microsoft Access and Microsoft Visual Basic for Applications.
Results: The relational data model enables the documentation of all techniques used. Furthermore, it allows the documentation of the cross links between all steps of a pro-ject. An analysis can be performed retrospectively under various aspects, i.e. not only project-wise, but also for certain aspects of all collected lab data.
Conclusion: The system presented demonstrates the advantages of electronic lab-documentation. Automated post-hoc analysis by variable parameters are an improve-ment over traditional documentation systems. Furthermore, the normalized data model and the system architecture allows for an easy adaptation to an increasing method portfolio and growing lab infrastructure.

Kategorie: Poster

Poster 2

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

Titel: My Microscope – Development of a novel e-learning platform for microscopic anatomy

Autoren: Schmidt C. *[,1], Reinehr M. *[,1], Leucht O.[2], Betz T.[3], Behrendt N.[2], Rödling M.[2], Britsch S.[1]

Adressen: [1] Institute of Molecular and Cellular Anatomy, Ulm University, Albert-Einstein-Allee 11, 89081 Ulm [2] Net-Base, Computer- und Netzwerktechnik e.K. Zinkmattenstrasse 6, 79108 Freiburg i. Br. [3] Carl Zeiss Micro Imaging GmbH, Königsallee 9-21, 37081 Göttingen
* equal contribution

Abstract:
During the past decades the learning opportunities for medical students at university have changed. Computer-based learning media offering spatiotemporal independence and self-directed learning have become increasingly important.
In addition to the classical features such as dissection and histology courses, anatomical education nowadays demands e-learning tools to strengthen the position of anatomy as profession but also to consolidate anatomical knowledge as basis for the clinical practice of the future physician.
In a multidisciplinary approach, we have developed the novel computer-assisted virtual microscope „MyMicroscope“ (MyMi). Previously, the term „virtual microscope“ implied static pictures with a legend or scalable pictures without any text information. Both systems neglected the fact that the unexperienced user demands guided support regarding a given histological picture. MyMi combines zoomable, high-resolution pictures with systematic annotations. The latter can be opened via a text link or via scrolling through the picture. By this means even the unexperienced is able to find specialised structures at different magnifications and get appropriate information about them.
A survey among medical students (n=369) using MyMi as a test version during the regular histology course at Ulm University showed that there is a very high acceptance: 97% of the students would recommend MyMi and 94% want to use it as self-directed e-learning tool for the first oral exam.
Future perspectives of MyMi will not only include a complete atlas of histology but also „teaching trails“ guiding the student through different chapters of microscopic anatomy.

Kategorie: Poster

2. Klinische Anatomie/Makroskopie

Poster 3

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

Titel: Can the plantaris muscle nerve be used as a direct donor to restore the function of the deep fibular nerve?

Autoren: Karahan S.(1),Esmer A.(1),Sen T.(1),Armangil M.(2),Basarir K.(2),Tuccar E.(1),

Adressen:(1)Ankara Universitaet, Medizinische Fakultaet|Institut für Anatomie|Ankara|Türkei; email:karahan@medicine.ankara.edu.tr; (2)Ankara Universitaet, Medizinische Fakultaet|Klinik für Orthopaedie und Traumatologie|Ankara|Türkei

Abstract:
OBJECTIVE: In cases of high sciatic nerve injuries, there is usually a pattern of complete or incomplete functional recovery of the tibial division with no recovery of its fibular division. The plantaris muscle (PM) is often dismissed as a small, vestigial muscle. The PM has been given little attention in the reviewed literature. Because of the lack of information regarding the PM, the anatomical and functional understanding of this muscle is limited. The purpose of this study was to obtain a detailed anatomical data about the PM and its innervation pattern to hold a view that whether it is suitable for the reinnervation of the deep fibular nerve or not.
MATERIAL and METHOD: 18 cadaveric lower limbs (14 formaline-fixed and 4 fresh frozen) were investigated in this study.
RESULTS: The morphometry of the PM was investigated. The nerve of the PM was originated either from the nerve of the soleus muscle or directly from the tibial nerve in all cases. The diameter of these nerves and the diameter of the deep fibular nerves were measured to evaluate the anatomical feasibility for reinnervation.
CONCLUSION: In the light of our results, we think that the nerve of the PM is convenient for neurotization of the deep fibular nerve in anatomical aspect.

Kategorie: Poster

Poster 4

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

Titel: A comparative sem study of the equine and feline uterotubal junction (utj)

Autoren: Feyl F.(1),Schweiger M.(1),Steffl M.(1),Amselgruber W.(1),

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Abstract:
Referring to the physiology of reproduction in female domestic animals, the ostium of the oviduct into the uterine horn is one of the most important structures. Its function as a tight transition area to control the transport and the survival of gametes leads to intense discussions and further studies of anatomical variations between different species. The purpose of this study was to investigate the spatial arrangement as well as structural differences of the UTJ between two mammalian species using scanning electron microscopy (SEM) techniques. A total of 14 UTJs (4 mares and 10 cats) with adjacent isthmus and uterine horn were collected and prepared for scanning electron microscopy.
The results clearly show that in both species a special embossment as entrance into the oviduct exists. Contrary to cats, in which the entrance into the UTJ has the appearance of a low mucosal mound, in mare there is a prominent protruding papilla. The orifice at the papillary apex is thereby exceptionally wrinkled leading into longitudinal folds through the intramural portion of the papilla. Cushion-shaped processes, arranged in a row is a special feature of the isthmus portion in the feline UTJ. Regarding the distribution of luminal ciliary (CC) and secretory (NCC) cells, marked differences between both species exists and in the feline mound region almost exclusively NCC can be found. The results demonstrate considerably morphological differences of the UTJ between cats and horses, which reflect different barrier-mechanisms.

Kategorie: Poster

Poster 5

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

Titel: Morphological changes in magnum structures of domestic fowl during the egg-laying period and molt

Autoren: Dorner I.(1),Steffl M.(1),Schweiger M.(1),Amselgruber W.(1),

Adressen:(1)University of Hohenheim|Anatomy and Physiology of Domestic Animals|Stuttgart|Germany; email:Isabelle.Dorner@gmx.de

Abstract:
Purpose: Previous studies have focused on ultrastructural details of uterine epithelial cells because of its role in egg shell formation, but little is known about the morphological changes of the magnum (albumen-producing region) in egg-laying and molting hens. The purpose of this study therefore was to investigate changes in the cellular composition and surface architecture in dependence of reproductive activity by using scanning electron microscopy (SEM) and light microscopy.
Methods: Tissue samples of 5 hens being in the egg-laying period and 11 hens being in different quiescent phases were isolated and prepared for scanning electron and light microscopic techniques by conventional procedures.
Results: During the egg-laying period the size and the arrangement of the mucosal folds and the epithelial cells lining the luminal surface structures are well developed. The same is shown for the underlying glandular tissue which is densely packed in this region.
In different quiescent stages the regression especially regards the arrangement of mucosal folds as well as the underlaying glands. In egg-laying periods the folds are highly and well developed whereas during molting periods regression goes rapidly during the first 8 days. The luminal folds are reduced in number and height. The alterations in glands are mainly seen in size and activity of secretory cells.
Conclusions: The results clearly show that the redraw of stimulating hormones during quiescent phases has tremendous influence on the size and complexity of luminal folds as well as the configuration of tubular glands.

Kategorie: Poster

Poster 6

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

Titel: Two variant locations of the fork of the median nerve

Autoren: Claassen H.(1),Schmitt O.(2),Schulze M.(3),Wree A.(2),

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Abstract:
The classic description of the brachial plexus, with the C5 to TH1 nerve roots followed by three trunks, six divisions, three cords, and five main motor/sensory branches to the upper extremity, is a simplification. In actuality, many variant forms of the brachial plexus exist without preferences. Recently, we found two variant locations of the fork of the median nerve among a sample of 32 upper extremities (6%).
In the right arm of a 76-year-old male cadaver, circumflex scapular artery, anterior and posterior circumflex humeral artery and profunda brachii artery derived from a common trunk which has its origin at the axillary artery. Surprisingly, fork of median nerve covered the common trunk instead of axillary artery. In addition, musculocutaneous nerve and lateral root of median nerve came from lateral cord of brachial plexus while ulnar nerve, medial root of median nerve, axillary nerve and radial nerve derived from posterior cord of brachial plexus. Medial cord of brachial plexus was missed. Furthermore, in the right arm of a 91-year-old female cadaver, the fork of the median nerve has moved from axillary artery to the upper third of brachial artery.
When compared with general anesthesia for surgery of the upper extremity, brachial plexus blockade has many potential benefits, including reduced requirement of systemic opioids. The above described variation that fork of median nerve was covering a deep branch of axillary artery is clinically important as this knowledge may help the anesthesiologists to avoid any inadvertent damage to nerves during blocks and surgical interventions.

Kategorie: Poster

Poster 7

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

Titel: Minimally invasive plate osteosynthesis of the tibia – is the deep peroneal nerve at risk?

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

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Medical University of Graz,, Department of Traumatology, Graz 8036, Auenbruggerplatz 7a, Austria, wolfgang.pichler@medunigraz.at (4)Royal London Hospital, Specialist Registrar Royal London Rotation, London, E1 1BB, Whitechapel, United Kingdom, n.heidari@gmail.com (5)Medical University of Graz, Department of Paediatric Surgery, Graz 8036, Auenbruggerplatz 34, Austria, anneliemartina.weinberg@medunigraz.at

Abstract:
Background: Percutaneous stabilization of tibial fractures with locking plates has become a standard procedure. The main disadvantage of this technique is the risk of damage to the neurovascular bundles in the anterior compartment. Purpose of this anatomical study is to examine the relation of the deep peroneal nerve to a percutaneously inserted tibial LISS plate.
Methods: Eighteen cadaveric lower limbs were examined. Percutaneous LISS plate insertion was performed. A five centimetres longitudinal skin incision was made from Gerdy's tubercle running distally and a 13-holes LISS plate was inserted just superficial to the tibial periosteum. The plate was checked by fluoroscopy in two different planes and the screws were inserted according to the standard technique. Then the deep peroneal nerve was dissected out in order to demonstrate its relation to the plate
Results: In all cases the nerve was in direct contact with the distal portion of the plate. In 9 cases the nerve crossed the plate at the level of the 13th hole. In three cases the nerve crossed the plates at the level of the 11th hole and in a further three cases at the level of the 12th hole. In 3 cases the nerve skirted the distal edge of the plate. In 10 cases the nerve crossed superficial to the plate and in 6 cases the nerve was trapped in-between the plate and the bone. In 2 cases, the nerve did not cross the plate, but skirted the distal edge.
Conclusion: Percutaneous insertion of plates longer than ten holes is not to recommend. By extending the distal approach, the neurovascular bundle may be displayed and protected, avoiding injury to these structures.

Kategorie: Poster

Poster 8

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

Titel: Computerassisted 3D-analysis of the anatomy of the radial head

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

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Abstract:
A few years ago the industry started to produce surgical plates and prostheses for the surgical treatment of simple or comminute radial head fractures. However, those implants do not always fulfil the requirements of the complex anatomical structure of the radial head. The aim of this anatomical study of the proximal end of the radius was to comprehend its 3-dimensional morphology as good as possible and to analyse it in order to be able to construct preformed surgical plates as well as prostheses of the radial head that are well suited for the purpose. With a 64 CT scanner the elbow joints of 30 extremities of cadavers were scanned and then measured with special computer software. The analysis of the result of the measuring process showed the following situation:
(A) The average maximal diameter of the radial head was 22, 79 mm (ranging from 19, 55 mm to 25, 76mm)
(B) The mean diameter of the proximal articular facet between the lips of the joint was 18, 81 mm (ranging from 15, 90 mm to 22, 01 mm)
(C) The diameter of the verge of head and neck of the radius was 15,08mm (ranging from 12, 30 mm to 17,88 mm) (D9 the mean length of the radial head was 11, 80 mm (ranging from 10,14mm to 15, 18 mm) Gender specific differences were found concerning the values (A), (C) and (D). There were no differences in the values concerning right or left side of the body.
These results show that it is not advisable to use the same size of implants for men and women. The examination and measurement of the non-injured elbow however is a valid means of determining the size of the fractures radius head, and therefore offers the possibility to chose the right implant

Kategorie: Poster

Poster 9

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

Titel: The Neurovascular Relationships of the Oculomotor Nerve

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Abstract:
In this study, the arterial supply of the cisternal (initial) and the subcavernous parts of the oculomotor nerve (ON) and the relationship between the nerve and adjacent vascular structures like posterior cerebral artery (PCA) and superior cerebellar artery (SCA) were investigated. 140 formalin fixed hemispheres from 70 human cadaveric brains were examined. The nutrient branches which reached the cisternal and subcavernous parts of the ON and penetrating branches of adjacent vascular structures which penetrated the nerve and passed through it were investigated. After arising mesencephalon, the ON continued laterally between PCA and SCA or between PCA and rostral trunk of SCA, mostly. But in three hemispheres of specimens the ON run between the rostral and caudal trunks of SCA. We observed that the branches of P1 segment of PCA supplied the cisternal part of the ON in all specimens. In one specimen, the cisternal part of the ON was supplied by a branch which arose from rostral trunk of SCA in which we also observed that the rostral trunk of SCA originated from PCA. In four hemispheres, branches which arose from PCA or SCA perforated the cisternal part of the ON and passed through it differently. We also observed a tortuous caudal trunk of duplicated SCA in one of our specimens as a rare variation. The anatomy of the ON and its vascular relationships is significant for understanding the compression syndromes and vascular dysfunctions of the ON and it's necessary for the exact diagnosis and treatment.

Kategorie: Poster

Poster 10

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

Titel:Vagal paraesophageal plexus- morphological variability

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Abstract:
In diaphragmatic portion of the esophagus the vague nerve fibers are dissociated and being anastomosed between them, realize a complete vagal paraesophageal plexus. Our study was based on macroscopic dissection of a number of 50 cases. We have seen that in four cases (8%) there was an early branch of left vague, whose fibers are dissociated before reaching the diaphragm opening of the esophagus; three cases from these (6%) showed a bifurcation and one case (2%) presented trifurcation. The other 92% (46) of cases branching is the same hole. Right vagus did not have any early branching in any case (0%), separation being always performed at the diaphragm aperture. We observed two types of esophageal plexus: high extension and low extension. The large extension was found in 22 (44%) cases, of which in eight cases (16%) network was extensive and in 14 cases (28%) network was poor. Vagal paraesophageal plexus with large extension was found in 28 cases (56%), of which 11 cases (22%) there was an extensive network and in 17 cases (34%) network was poor. In conclusion we can say that more often plexus has a low extension (34%) with a poor network, and only in 16% of the cases network was of large extension and high.
Keywords: vagus nerve, esophagus, plexus.

Kategorie: Poster

Poster 11

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

Titel:Biomechanical investigation of the iliotibial tract related to anatomic fixation

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Abstract:
The iliotibial tract (IT) plays an important role in knee and pelvis biomechanics with a strong power transmission. The IT is broad but thin and thus easy to handle in material testing. Its parallel fibres allow obtaining Young's modulus of elasticity (YM). 13 probes of fresh IT were gained from donators. Because obtaining the YM in a fresh condition was unsatisfactory, the ends of the probes were plastinated with resin. The YM of the fresh probes averaged 397.3 N/mm² with a standard deviation (SD) of 151.5 N/mm². Then, the probes were fixed with ethanol and respectively dehydrated. Here the YM was significantly higher (673.2 N/mm², SD 328.5 N/mm²). After rinsing with tap water, the YM again decreased to 377.4 N/mm² (SD 144.5 N/mm²). This was 0.95 of the fresh condition and not a significant change from the fresh state. After formaldehyde fixation, the YM reached 490.3 N/mm² (SD 143.0 N/mm², significant change). When the formaldehyde-fixed specimens were rinsed, the YM was 452.6 N/mm² (SD 115.1 N/mm²). This was a non significant change of 1.14 from the fresh condition. Alcohol fixation (dehydration) is suggested for obtaining statistically significant data of the YM, based on a small number of probes. This can be accomplished in combination with plastination of the ends of the probes, creating a sharp interface between the clamp and the probe. The YM can then be used as a parameter for integration of the ligaments into Finite Elements models.

Kategorie: Poster

Poster 12

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

Titel: P75ntr acts as a negative differentiation regulator in the adult dentate gyrus

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Abstract:
The p75NTR receptor binds all neurotrophins with low affinity and, depending on co-signalling with the high affinity receptors (trks), can mediate survival and differentiation, but also cell death. The p75NTR receptor is highly expressed in the dentate gyrus (DG) of the adult hippocampus, a structures capable of adult neurogenesis. Neurogenesis, together with plastic changes in dendritic spines is thought to play fundamental roles in hippocampal neuronal plasticity. The process of neurogenesis can be divided into several distinct phases, which can be identified by a number of markers that recognize specific proteins indicative of respective stages.
The present study addresses the possible role of p75NTR in adult neurogenesis and spinogenesis within the DG. For that purpose, we compared p75NTR-deficient mice and control littermates. Using different stage-specific neurogenic markers, we could demonstrate that lack of p75NTR affects the steps of differentiation, but not of proliferation of adult neurogenesis within the DG. In addition, Scholl-analysis revealed that DG granule cells of p75NTR-deficient mice display a more complex branching pattern than those of control littermates. Moreover, by using Golgi-impregnated material, we could demonstrate that p75NTR deficient mice display altered spine densities on dendrites of DG granule cells. Based on these data, it could be speculated that p75NTR acts as a negative differentiation factor at least in the hippocampus.

Acknowledgment: supported by the DFG (BO1971/5-1).

Kategorie: Poster

Poster 13

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

Titel: Activity-dependent intracellular chloride accumulation and diffusion control gabaa receptor-mediated synaptic transmission

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Abstract:
In the CNS, prolonged activation of GABAA receptors (GABAARs) evokes biphasic postsynaptic responses, consisting of an initial hyperpolarization followed by a depolarization. A potential mechanism underlying the depolarization is an acute chloride (Cl-) accumulation resulting in a shift of the GABAA reversal potential (EGABA). The amount of GABA-evoked Cl- accumulation and accompanying depolarization depends on presynaptic and postsynaptic properties of GABAergic transmission, as well as on cellular morphology and regulation of Cl- intracellular concentration ([Cl-]i). To analyze the influence of these factors on the Cl- and voltage behavior, we studied spatiotemporal dynamics of activity-dependent [Cl-]i changes in multicompartmental models of hippocampal cells based on realistic morphological data. Simulated Cl- influx through GABAARs was able to exceed physiological Cl- extrusion rates thereby evoking HCO3- - dependent EGABA shift and depolarizing responses. Depolarizations were observed in spite of GABAA receptor desensitization. Changes in the dendritic diameter and in the speed of GABA clearance in the synaptic cleft were significant sources of depolarization variability. In morphologically reconstructed granule cells subjected to an intense GABAergic background activity, dendritic inhibition was more affected by accumulation of intracellular Cl- than somatic inhibition. Interestingly, EGABA changes induced by activation of a single dendritic synapse propagated beyond the site of Cl- influx and affected neighbouring synapses (heterosynaptic ionic plasticity). The simulations suggest that EGABA may differ even along a single dendrite supporting the idea that it is necessary to assign EGABA to a given GABAergic input and not to a given neuron.

Kategorie: Poster

Poster 14

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

Titel: The gtpase rhog regulates axonal and dendritic branching in a microrna-dependent manner

Autoren: Schumacher S.(1),Otto W.(1),

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Abstract:
RhoG is a member of the Rho/Rac/Cdc42 family of small GTPases which are essential for cytoskeletal reorganizations within cells. The Elmo-Dock180 signaling pathway has been shown to be important for RhoG-mediated effects on the cytoskeleton. The physiological relevance of RhoG for neuronal differentiation processes is still unclear. Here we show that RhoG is involved in the regulation of axonal and dendritic branching in primary neurons. The expression of RhoG is inhibited by miR-124, a microRNA which is specifically expressed in neurons. The regulation of RhoG expression by miR-124 has a functional impact on RhoG-mediated effects of axonal and dendritic branching. Interestingly, the RhoG-stimulated signal transduction in primary neurons leading to the shaping of axons and dendrites is not dependent on the classical Elmo-Dock180 pathway. We describe here a new isoform of the Elmo protein, which seems to be important for RhoG-dependent regulation of neuronal process development.

Kategorie: Poster

Poster 15

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

Titel: Existence of the neuropeptide alarin in the eye of various species

Autoren: Schrödl F.(1),Eberhard N.(2),Santic R.(2),Trost A.(3),Strohmaier C.(3),Runge C.(3),Bogner B.(3),Kofler B.(2),Grabner G.(3),Reitsamer H.(3),

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Abstract:
Purpose: Alarin is a lately discovered neuropeptide of the galanin peptide family with vasoconstrictor acitivity in murine skin. In the eye, ocular blood flow and aqueous humor production are essential for ocular homeostasis and a pool of intrinsic and extrinsic neuropeptides is involved to fulfill this task. Alarin might be an additional regulatory peptide in these processes.
Methods: Eyes of human (meeting the Declaration of Helsinki), mouse, and posterior globes of rat were processed for immunohistochemistry against alarin using affinity purified antibodies. Confocal laser-scanning microscopy was used for documentation and quantitative RT-PCR was performed to detect alarin mRNA expression in human eyes.
Results: Alarin-like immunoreactivity (alarin-LI) was detected in corneal epi- and endothelium of mouse and human and in the conjunctiva of mouse and rat. Alarin-LI was found in the iris of mouse and human, in the latter species concentrated around blood vessels. All three species showed distinctive alarin-LI in the non-pigmented epithelium of the ciliary body. In the retina of mouse and rat, maximum signals were detected in the outer nulear and opticus ganglion cell layer, whereas in humans strong alarin-LI was found around retinal blood vessels and in intrinsic choroidal neurons (ICN). Quantitative RT-PCR in human revealed higher alarin mRNA expression in the choroid than retina.
Conclusion: Alarin is widely distributed in the eyes of species investigated. The existence around blood vessels/ in ICN indicates an involvement in ocular blood flow regulation. Alarin in the non-pigmented epithelium of the ciliary body might be involved in aqueous humor production.

Kategorie: Poster

Poster 16

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

Titel: Vegf and its role in cell communication and cell dynamics

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Abstract:
Vascular Endothelial Growth Factor (VEGF) is a dimere polypeptide, which is synthesized in low concentrations in different regions of the adult brain. There are 4 isoforms of the VEGF-A gene: 121, 165, 189 and 206, with VEGF-165 as the most abundant and biological active one. VEGF is known to bind to two receptors, VEGFR-1 (flt-1) and VEGFR-2 (KDR/flk-1). Many effects of VEGF are mediated by VEGFR-2, e.g. actin polymerization via Rho/ROK-pathway, forced cell migration via SAPK2/p38 (MAPK) and angiogenesis and cell proliferation via Raf-Mek-ERK1-2 pathways.
In the recent years it was shown that VEGF has also a trophic activity on neurons. Besides this, in case of hypoxia, ischemia or injury VEGF is upregulated to stimulate angiogenesis and cell proliferation. But it is not quite clear if these effects, which are postulated for endothelial cells and astrocytes, can also be transferred to other cell types.
The purpose of the present study was to analyze the influence of VEGF on cell communication and cell dynamics in astrocytes, fibroblasts and HeLa cells. Therefore we cultivated these cells in nutrient medium containing mouse VEGF for several days. To investigate the effects of VEGF on gap junctional intercellular communication we injected Neurobiotin into a single cell, and monitored dye-spreading into adjacent cells within a 10 minutes time-period. To study cell-dynamics subsequent VEGF incubation, we additionally transfected different cells with YFP-actin, before motion analysis of these cells was done with aid of confocal laser scanning microscopy.

Kategorie: Poster

Poster 17

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

Titel: Increased dendritic spine density and reduced stress-related neuroplasticity of laterobasal amygdaloid pyramidal neurons in serotonin transporter deficient mice

Autoren: Bonn M.(1),Nietzer S.(2),Lesch K.(2),Schmitt A.(2),Asan E.(1),

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Abstract:
Lower expression of the human serotonin transporter (5-Htt) gene presumably interacts with stressful life events enhancing susceptibility for affective disorders. 5-Htt-knockout (KO) mice display an anxious phenotype compared to wild-type (WT) mice and are therefore a common model for anxiety- and stress-related disorders. Exacerbation of behavioral differences after repeated loser experience in a resident-intruder social stress paradigm was previously documented in these mice. Since it is well-known that different kinds of stress can cause morphological changes in brain areas relevant for emotion processing, we investigated whether genotype-dependent and stress-induced behavioral differences are reflected in characteristics of neuron morphology. For this purpose, we analysed dendritic morphology of pyramidal neurons in the lateral (La) and basolateral (BL) amygdaloid nuclei in Golgi-Cox-stained brains of male WT and 5-Htt-KO control and loser mice. La and BL pyramidal neurons displayed significantly higher spine density in 5-Htt-KO than in WT controls. Differences were most pronounced in medium-sized basal dendrites of La pyramids and indicated a substantially higher synaptic input into this dendritic compartment in 5-HTT-KO animals. Stress exposure in WT animals caused higher spine densities in the same dendritic compartments, and significant differences between WT losers and 5-Htt-KO controls were absent. In contrast, stressed 5-HTT-KO animals displayed spine densities equally high or even lower than 5-Htt-KO controls. Thus, while social stress induces neuronal plasticity in the WT, this response appears considerably dampened in 5-Htt-KO mice. The present findings may represent neuronal substrates underlying stress- and genotype-dependent behavioral responses to environmental challenges.

Kategorie: Poster

Poster 18

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

Titel: Plasticity-related genes: from nogo-rhoa to divergent signaling at the phosphoinositol-bisphosphate kinase

Autoren: Broggini T.(1),Schnell L.(2),Savaskan N.(1),

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Abstract:
To this day we cloned 5 members belonging to the plasticity-related genes (PRG) implicated in axon growth (PRG3) and synaptic plasticity (PRG1). Recently we reported on PRG5, sharing high homologies to PRG3 with solely diverse carboxy terminal domain structure. Indeed, PRG3 and PRG5 are the sole members of the PRG family which induce spontaneous neurite outgrowth. Here, we compared effects of PRG3 and PRG5 on neurons. Both, PRG3 and PRG5 were tested in live cell imaging experiments both showing resistance towards neurite growth inhibitory factors such as LPA or Nogo-A, whereas control neurons collapsed under these conditions. The PRG3 C-terminus was identified as the functional domain responsible for filopodia and axon formation. Moreover, the induced filopodia form potential initiation structures for the development of synapses. However, PRG5 shows further axonal growth activity in different parts of the N-terminus. We continued the analysis by studying down stream effectors of Nogo-A and LPA and found diverse responses between PRG3 and PRG5. PRG3 in contrast to PRG5 overcame even RhoA dependent PIP5K signaling and stabilized neurite structures. To investigate the role of PRG3 in vivo, spinal cord lesions were studied in adult PRG3 transgenic mice. Compared to lesioned wild-type littermates, mice overexpressing PRG3 showed enhanced regenerative axon growth. Thus PRG3 expressing neurons are able to overcome an inhibitory environment and can grow beyond the lesion. All together, these data demonstrate that molecules of the PRG family are functionally diverse and do not execute their effects in an interchangeable manner.

Kategorie: Poster

Poster 19

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

Titel: Characterization of gabaergic hippocampal-amygdalar projection neurons

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Abstract:
GABAergic neurons are neuromodulators of the information flow in and between hippocampus and amygdala. They are suggested to play an important role in different aspects of emotional learning and memory processes. There are different subsets of these neurons in both structures which can be distinguished by their content of neuropeptides, morphology and their location. In the present study we characterized these subpopulations of the hippocampo-amygdalar GABAergic projection neurons. To this end, we injected fluorogold into the amygdala of C57Bl/6 GAD67-GFP knock-in mice to detect GABAergic projection neurons. GABAergic neurons were further characterized in these mice by immunostaining against the peptides NPY, CCK, Somatostatin and the calcium-binding proteins parvalbumin, calbindin and calretinin.
We found retrogradely labeled neurons in the hippocampal formation exclusively in the ventral CA1 (stratum pyramidale and stratum oriens), in the subiculum and in the entorhinal cortex. A small number of these GABAergic projection neurons also contained either somatostatin, NPY, calretinin or parvalbumin. GAD67-GFP-labeled neurons were found in the entire hippocampal formation. These neurons also contained neuropeptides or calcium-binding proteins but were not labeled by the tracer.
From these data we conclude that there is only a small number of GABAergic hippocampo-amygdalar projection neurons. Furthermore, this population is rather heterogenic (heterogenous) as shown by their content of different neuropeptides and calcium-binding proteins.
(Supported by DFG, SFB 779/B5)

Kategorie: Poster

Poster 20

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

Titel: Endocannabinoids in the rodent circadian system

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Abstract:
Introduction: The cannabinoid receptor type 1 (CB1) and its endogenous ligands, the endocannabinoids (EC) play an important role in the regulation of food intake and energy balance. The coordinated daily rhythms in food intake and metabolism are controlled by an endogenous rhythm generator residing in the suprachiasmatic nucleus of the hypothalamus (SCN). However, little is known about the impact of the EC system on the molecular clockwork and, vice versa, the impact of the molecular clockwork on the EC system. Objective: To investigate the interactions between the molecular clockwork and the EC system, we analyzed (1) distribution of CB1 and of key enzymes in EC metabolism (NAPE-PLD, DAGLa and FAAH) in different hypothalamic regions by immunohistochemistry in mice with a disturbed molecular clockwork (PER1-/-) and the corresponding wildtype (WT) and (2) the spontaneous locomotor activity rhythms of CB1-deficient (CB1-/-) mice and the corresponding wildtype. Results: (1) The WT SCN shows circadian rhythms in CB1-, DAGLa, NAPE-PLD- and FAAH-immunoreactivity (Ir). These rhythms are significantly altered in the SCN of PER1-/- mice. In the median eminence (ME) NAPE-PLD-Ir was significantly elevated in PER1-/- mice as compared to WT. (2) Both, WT and CB1-/- mice showed high locomotor activity during the dark phase and low locomotor activity during the dark phase. However, after a 6 h phase delay of the photoperiod, CB1-/- mice entrained significantly faster to the new phase. Conclusion: Our data suggest that the molecular clockwork affects the EC system and the EC system affects the endogenous rhythm generator.

Kategorie: Poster

Poster 21

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

Titel: Mesovascular morphometry of cerebelli. morphological considerations.

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Abstract:
This paper reports an anatomical study about the cerebellar vascularisation of the man and laboratory animals, on the mesoscopic level. Studies have been made on 1436 vessels. The method of the UMF Anatomy Laboratory was used – injecting with China ink and obtaining clearance in Tetraline preparations.
Morphologically, on dissection and clearance-transparency in Tetraline preparations and histological cups, origin, route, territorialization and I-II-III-IV rank ramifications, and also cerebellar microvascular layer have been studied. One of the research purposes was to stand out the territorialization at microcirculation level most discussed morphofunctional zones of Purkinje cells.
The results of morphometry have been exposed in sinoptic mathematic-statistic tables.
Using mathematical-biological statistics of morphometric data (length, diameter, vascular density) we have pointed out significant differences concerning the vascularity of cerebellar segments by computing hemodynamic indexes such as: vascular volume, vascular section surface and total surface of vessels.
Utility of corroboration and comparison between morphological data and functional data, in assessment of cerebellar vascularity has been pointed out. The results contribute to better knowledge of the cerebellar vascularisation.
Keywords: cerebellum, vascularisation, morphometry

Kategorie: Poster

Poster 22

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

Titel: Alteration of c-fos expression in pvn, bnst and locus coeruleus in gad67 transgene mice and wild-type mice after tmt-exposition

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Abstract:
Alteration of c-fos expression in PVN, BNST and Locus coeruleus in GAD67 transgene mice and wild-type mice after TMT-exposition
Stress may have facilitating and impairing effects on learning and memory processes. Here, we investigated the influence of acute exposure to TMT, a relevant stressor of mice. TMT is olfactory stimulus extracted from the feces of fox, a natural predator. Before olfactory treatment, the mice had to perform an eight-arm radial maze task (RAM) for 5 days. On the 6th day mice were exposed to TMT or DEP (control group) before further RAM training . 40-60 min after exposition effects on RAM performance was studied. 20 min after completing the RAM task mice were sacrificed by rapid decapitation. Brains were removed and three brain areas were selected: Bed Nucleus of the stria terminalis (BNST), Nucleus paraventicularis hypothalami (PVN) and Locus coeruleus (LC). From these areas mRNA was isolated using trizol-technique followed by quantitative real time PCR to detect mRNA levels of c-fos and corticotropin-releasing hormon (CRH).
Wildtyp mice made less spatial learning error indipendent from either odor exposure. On the other hand TMT-exposed mice showed poorer learning performance independent from genotype. In addition, we found a strain difference for c-fos and CRH-expression in PVN and LC (wildtype < GAD67-GFP).
In conclusion, stress application under the present conditions lead to impaired learning performance and to a modified gene expression of c-fos and CRH in candidate regions of the brain.
(supported by a stipend of the faculty of medicine of the O.-v.-G. University of Magdeburg).

Kategorie: Poster

Poster 23

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

Titel: Neurod family transcription factors NEX and NDRF-Regulate neocortical remote axogenesis

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Abstract:
Establishment of long-range fiber tracts by neocortical projection neurons is fundamental for higher brain functions. The molecular control of axon tract formation, however, is still poorly understood. We have identified two basic helix-loop-helix (bHLH) transcription factors NEX (Neurod6) and NDRF (Neurod2) as key regulators of fasciculation and targeted axogenesis in the neocortex. In NEX/NDRF double mutants, fiber tracts of neocortical origin are massively reduced or completely absent. Callosal axons, which are most severely affected, lack expression of the cell adhesion molecule contactin 2, defasciculate in the subventricular zone, and follow random trajectories within the ipsilateral cortex instead of crossing the midline. In contrast to long-range axogenesis, generation and maintenance of pyramidal neurons, initial axon outgrowth, dendritogenesis, and glutamatergic synapse assembly are largely unaffected, and thus under distinct transcriptional control. These findings demonstrate that neocortical projection neurons require transcriptional specification by neuronal bHLH proteins to execute an intrinsic program of remote connectivity.

Kategorie: Poster

Poster 24

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

Titel: Conditional knock out of the type 2 tgf- beta receptor and smad7 in neurons of the mouse retina and their influence on ontogenetic cell death

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Abstract:
Purpose: TGF-beta signaling was proposed to have a pro-apoptotic role (Dünker and Krieglstein CTR 2003). Ontogenetic neuronal cell death in the mouse retina has its peak after birth. Therefore we developed an alternative animal model to investigate the role of TGF-beta signaling during ontogenetic cell death in the postnatal mouse retina.
Methods: Floxed Tgfbr2 mice, with LoxP sites flanking exon 2 of the type 2 TGF-beta receptor gene, were crossed with alpha-Cre mice expressing Cre recombinase in retinal neurons under control of the alpha-enhancer of the Pax6 promoter. Accordingly, we used Smad7 floxed mice, with LoxP sites flanking exon 1 of the Smad7 gene. Apoptotic cell death in the retina was analyzed by TUNEL labeling of retinal neurons at P 4, 7 and 10.
Results: Western blot analysis for Tgfbr2 and Smad7 expression levels confirmed the conditional knockout in the retina of both mouse lines. TGF-beta activation was confirmed by immunohistochemistry of phosphorylated Smad2/3 in wild-type littermates compared to TGFBR2flox/flox/alpha-Cre mice. TUNEL analysis showed significant more TUNEL positive cells in the retina of TGFbr2flox/flox/alpha-Cre mice compared to wild-type littermates. Adult TGFBRr2flox/flox/alpha-Cre mice showed significant thinner inner nuclear layer (INL) and a reduced axon number in the N. opticus. As expected, SMAD7flox/flox/alpha-Cre mice showed less apoptotic cell death in the retina compared to wild-type littermates.
Conclusion: TGF-beta signaling seems to protect retinal neurons from apoptotic cell death in postnatal life, rather than promoting it, indicating different roles of TGF-beta signaling during embryonic and postnatal life.

Kategorie: Poster

Poster 25

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

Titel: Electromagnetic field effect or simply stress? Effects of UMTS exposure on morphology and proteinexpression of hippocampal neurons

Autoren: Prochnow N.[1*], Hardan N. [1], Gebing T.[1*], Ladage K. [1], Krause-Finkeldey D.[1], El Ouardi A.[2], Bitz A.[2#], Kränke C. [1], Streckert J.[2], Hansen V.[2], Dermietzel R.[1]

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Abstract:
Harmful effects of electromagnetic fields (EMF) on cognitive and behavioural features of humans and rodents have been controversially discussed, and raised persistent concern about toxic effects of EMF on general brain functions.
In the present we applied Universal Mobile Telecommunication System (UMTS) signals to full head exposed male Wistar rats to elaborate putative influences on stress hormone release (Corticostrone; CORT and Adrenocorticotropic hormone; ACTH) and hippocampal immunoreactivity. Exposure was computer controlled providing double-blind conditions. Specific absorption rates as a measure of tissue specific energy (W/kg) was in the range of 0, 2, and 10W/kg over a period of 120 min.
Animals following the standardized visit of the exposure setup exhibited significantly altered distributions of Parvalbumin positive neurons within all hippocampal regions. In comparison to cage controls, Sham and 2 W/kg exposed groups revealed significant reductions in Parvalbumin positive neurons in the CA1- and CA2 region of the hippocampus. This effect was directly related to significant increases in blood derived stress hormone levels. A radiation strength of 10 W/kg which is above the limit of allowed exposure, lead to additive morphological and morphometrical changes which can be clearly distinguished from the stress derived background.
Along with the outcome of related electrophysiological studies on hippocamal neuronal plasticity in equivalent tissues, our findings suggest that UMTS exposure with specific absorption rates in the range of 2 W/kg is not harmful to critical markers for memory storage and memory consolidation, however, an influence of UMTS at high energy absorption rates (10 W/kg) can not be excluded.

Kategorie: Poster

Poster 26

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

Titel: Cytoarchitectonic probabilistic maps of the human frontal pole in stereotactic space

Autoren: Bludau S.[1], Schleicher A.[2], Mohlberg H.[1], Zilles K.[1,2] , Amunts K.[1,3]

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Abstract:
Brodmann's area (BA) 10, occupying the human frontal pole, is involved in higher cognitive functions such as planning of future actions and the ability to draw analogies. Its localization in stereotaxic space and intersubject variability, however, are still unknown. Aim of the present study was to analyse the cytoarchitecture of human area 10, to evaluate its extent with respect to gross macroscopic landmarks, and to create 3D probability maps in a standard reference space. Therefore, area 10 was mapped in serial histological sections of ten postmortem brains using an observer-independent approach for the definition of areal borders (1). Multivariate statistical analysis was used to analyse cytoarchitectonic differences of area 10 with respect to surrounding areas. Area 10 occupies the frontal pole and rostral parts of the superior and middle frontal gyri and borders BA46 latero-caudally, 9 dorsally, 11 ventrally, and 32 medially. A 3D cytoarchitectonic probability map of area 10 was generated, which quantified the considerable intersubject variability in its localization and extent. The probabilistic map showed a smaller medial extent of area 10 than a previous study (2). A subdivision into a medial and polar area was demonstrated; this finding provides an anatomical correlate for a functional subdivision shown in a recent fMRI study (3) where the medial part was involved in suppressing internally-generated thoughts, whereas the lateral part was maintaining them.

(1)Amunts et al. (1999). J Comp Neurol 412, 319-341, (2)Ongur et al. (2003). J Comp Neurol 460, 425-449, (3)Burgess et al. (2003). Neuropsychologia, 41, 906–918

Kategorie: Poster

Poster 27

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

Titel: $Gaba_B$ receptor activity dynamically regulates surface expression of Kir3 channels

Autoren: Kulik A.[1], Fukazawa Y.[2], Shigemoto R.[2], Frotscher M.[1], Vida I.[3]

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Abstract:
Inhibition mediated by G-protein-coupled inwardly rectifying K^+ channels (Kir3 channels) coupled to metabotropic $GABA_B$ receptors is essential for the control of neuronal activity in the brain. At the subcellular level, these channels and the receptors show co-clustering at the extrasynaptic plasma membrane of dendritic shafts and spines. Here, we used a combination of pharmacological and ultrastructural approaches to test whether the activation of $GABA_B$ receptors can modulate the surface expression of the Kir3.2 subunit. Acute hippocampal slices obtained from 21-day-old rats were treated with either the $GABA_B$ receptor agonist baclofen (20 □M) or the antagonist CGP 55845 (30 □M) to enhance or reduce the activation level of the receptors. After 20 min incubation with the drugs slices were immersion fixed and processed for SDS-digested freeze-fracture replica immunolabeling. Quantitative analysis of immunogold particles for Kir3.2 revealed a reduced density of the channel subunit on dendritic shafts in CGP-treated slices (6.1 particles/□m^2) in comparison to controls (12.4 particles/□$m^{□}$). In contrast, baclofen-treated slices showed an increased density of the protein (23.6 particles/□m^2). These results suggest that surface expression of Kir3 channels is dynamically regulated by $GABA_B$ receptor activation in hippocampal principal cells. (Supported by the DFG: SFB 780, project A2).

Kategorie: Poster

Poster 28

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

Titel:Vegf signaling in the neuronal growth cone – analysis of growth cone morphology and motility

Autoren: Föhring D.(1),Theiss C.(1),

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Abstract:

Axonal outgrowth is of paramount significance for establishing the intricate neuronal network in both embryogenesis and nerve regeneration. The growth cone receives the guiding signals and translates them into new cytoskeletal arrangements to lead the axon to the right direction. Recently, vascular endothelial growth factor (VEGF), which is essential for vascular sprouting and highly involved in cancer development, has also been found to exert a trophic activity on neurons, which leads to an increased axonal outgrowth. Although two receptors, named VEGFR-2 and neuropilin-1 (NRP-1), potentially mediating these effects were identified on neurons, the signaling pathways are not well understood.
In our study, we cultivated chicken dorsal root ganglia in medium containing VEGF, analyzed growth cone size in line with immunostaining and found a positive effect of VEGF on growth cone size. Additionally, live experiments illustrated that VEGF directly attracts the neuronal growth cone and thus influences growth direction and velocity. To get more insights into VEGF signaling in growth cones, we blocked either the extracellular domain of NRP-1 or the tyrosine-residue 1214 (tyr-1214) of VEGFR-2 with specific antibodies. Subsequent to these blocking experiments, growth cone size was significantly diminished.
Based on these data we suggest a positive effect of VEGF on growth cone size. Additionally, we propose that NRP-1 and the tyr-1214 dependent pathway of VEGFR-2 are of importance in VEGF signaling, influencing actin-organization in the neuronal growth cone.

Kategorie: Poster

Poster 29

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

Titel:The role of satb2 and ctip2 in cortical neuronal connectivity

Autoren: Sgourdou P.(1),Parthasarathy S.(1),Tarabykin V.(2),

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Abstract:
Satb2 belongs to a family of transcription regulators that control neuronal differentiation of specific cell types, by influencing the expression of multiple loci over long distances (Britanova et al., 2005). It controls the post mitotic fate of cortical neurons that reside on the upper layers (II-IV) of the developing neocortex, in part by downregulating the expression of Ctip2. Ctip2 is a transcription factor that is primarily expressed in layer V neurons, which are destined to form corticospinal connections (Britanova et al., 2008). In Satb2 null mice, the cortico-cortical connections fail to form and instead there is an ectopic induction of corticospinal connectivity.
Ctip2 mutants loose their normal corticospinal connections of layer V neurons which are instead misrouted into forming callosal projections, similar to those seen in neuronal layers II to IV. Additionally there is a lack of fasciculated bundles that normally perforate the striatum to form the internal capsule and a complete absence of CSMN (corticospinal motor neurons) axons extending pas the pons (Arlotta et al., 2005). These data along with the lack of Ctip2 expression in the ventricular and subventricular zones suggest a role of this gene in controlling the postmitotic differentiation of CSMN neurons.
In order to investigate the genetic interactions between Satb2 and Ctip2 genes and to identify the downstream targets of the above transcription factors we generated compound Satb2-/-;Ctip2-/- mutants and analyzed the resulting phenotype by comparing it to the phenotypes of Satb2 and Ctip2 single mutants respectively.

Kategorie: Poster

Poster 30

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

Titel:Crosstalk of smad- and foxg1-dependent transcription in neuronal differentiation and neurovascular development

Autoren: Vogel T.(1),Vezzali R.(1),Büttner N.(1),Wahane S.(1),

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Abstract:
Transforming Growth Factor beta (Tgfbeta) have versatile functions in different organ systems including delivery of cytostatic signals to neuroepithelial cells. Thereby, Tgfbeta activate Smad proteins that form a complex with FoxO transcription factors. Smad/FoxO complexes promote expression of p21Cip1 that blocks cell cycle progression at the G1/S transition. Through binding of the activating Smad/FoxO complex, FoxG1 inhibits transcription of p21Cip1 and thereby supports the progenitor state.
Tgfbeta not only exert cytostatic effects on cortical progenitors through regulation of p21Cip1, but also induce neuronal differentiation of these progenitors derived from E16.5 mouse forebrain. However, in contrast to E16.5 derived cultures, Tgfbeta does not mediate neurogenesis in cells generated from E13.5 brains or in adult stem cells. This indicates that the competence of progenitors to respond to Tgfbeta signals with differentiation depends on their respective developmental age. Mutation of FoxG1 confers a cellular competence that enables progenitors to respond to Tgfbeta signals with neuronal differentiation at E13.5. Microarray analyses of FoxG1 mutant and wild type E13.5 cortical progenitors show that as expected Tgfbeta treatment increased significantly p21Cip1 expression in mutant cells. Further, Tgfbeta treatment increased expression of genes implicated in neuronal differentiation in FoxG1 mutant cortical progenitors. These genes might therefore also be regulated through competition of Smad/FoxO and FoxG1 complexes. To analyse Smad- and FoxG1-signalling in vivo, we generated mice with reduced FoxG1 and TgfbetaRII expression. These mutants die at E17.5 and suffer from haemorrhages caused by defects in neurovascular development.

Kategorie: Poster

Poster 31

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

Titel:Tumor angiogenesis and brain edema: the old vascular corrosion casting technique revisited

Autoren: Broggini T.(1),Meyer E.(2),Eyupoglu I.(3),Savaskan N.(1),

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Abstract:
Primary brain tumors (gliomas) are one of the most aggressive and lethal human neoplasias and are hallmarked by neuronal impairment and development of brain swelling. To date are the mechanisms by which malignant gliomas cause brain edema still unclear, however, there is strong evidence that tumor angiogenesis plays a central part in these processes. Here, we followed this open query by acquiring the xCT knock down brain tumor model which shows alleviated brain swelling. First, we dissected the effects of the glutamate-cystine transporter xCT and investigated the role of glutamate signaling in brain tumors. We revealed a strong cystine-dependency on proliferation, whereas glutamate itself required the tumor microenvironment for its devastating tumor fostering effects. Second, we investigated glutamate effects on endothelial cells and tumor angiogenesis. For that, we used in vitro tubule formation and migration assays. In addition, we utilized the reestablished old vascular corrosion casting technique with new polymers and combined this with a hierarchical micro-computed tomography. This technique allows the analysis of the smallest angiogenic quantum, a single microvessel as well as micro-networks assembled to higher order vessel architecture. We will present data from wild type tumors as well as gliomas in which xCT was genetically silenced and compare analysis from 2D as well as reconstructed 3D data. With the old vascular corrosion casting technique in combination with MRi we aim to resolve the interrelation of tumor angiogenesis and brain edema.

Kategorie: Poster

Poster 32

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

Titel:The zinc-finger homeodomain factor teashirt1 is essential for the development of olfactory bulb granule cell neurons

Autoren: Garratt A.(1),Rocca E.(1),

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Abstract:
The mouse Teashirt1 (Tshz1) gene is one of three homologues of the Drosophila gene Teashirt, which encodes a zinc-finger protein that functions both in the Wnt signaling pathway and as a homeotic determinant of trunk identity. All vertebrate Teashirt genes encode zinc-finger homeodomain factors, which are expressed throughout the developing and adult nervous system. We present here a detailed characterisation of the cell populations within the granule cell layer of the embryonic mouse olfactory bulb, and assign to Tshz1 an essential role in both the radial migration and the molecular specification of early born, distally generated granule cell neurons. Early-born granule cell neurons arrived within the bulbs of Tshz1-/- mutant mice, but distributed aberrantly within the radial dimension, forming closely-packed cell aggregates. Within these clusters, cells of the Tshz1 lineage failed to express the zinc-finger transcription factors Sp8 and Sall3 and remained in an immature state as defined by the loss of expression of the markers neuN, glutamic acid decarboxylase (GAD)-67, gamma-amino butyric acid (GABA), tyrosine hydroxylase and guanine deaminase (cypin). Our analyses demonstrate that Tshz1 is essential for GABA-ergic and dopaminergic differentiation of olfactory bulb granule cell interneurons. Tshz1 is the first molecule to be characterised, which functionally distinguishes between the differentiation programs of olfactory bulb granule cell and periglomerular cell neurons. Furthermore, our analyses indicate that soluble and/or cell surface factors produced by the Tshz1+ outer granule cell lineage regulate the spatial distribution of other neuronal populations within the olfactory bulb.

Kategorie: Poster

Poster 33

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

Titel:Estrogen regulates mitochondrial respiratory chain enzyme transcription in the mouse spinal cord

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

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Abstract:
Regulation of mitochondrial energy metabolism is important for functioning of neuronal cells but appears also essential during neurodegeneration. This makes mitochondria an interesting regulatory target for therapeutic approaches. We analysed the influence of estrogen (E) on the expression of mitochondria-encoded genes and mitochondrial activity in spinal cord cells in vitro and vivo. Hormone application increased the transcription of mitochondrial respiratory chain enzymes (MRCE). This effect was only observed in spinal cord neurons, where it was inhibited by a nuclear estrogen receptor (ER) antagonist and mainly mediated by the activation of ER beta. No E effect was observed in spinal cord astroglia. In addition, the mitochondrial transcription factor A (Tfam) was up-regulated by E in a similar way as MRCE, and ATP levels were elevated after application of the specific ER beta agonist DPN in cultured spinal cord neurons. Exposure of male mice to E yielded increased levels of MRCE transcripts in the spinal cord. These data clearly show that systemic application of E stimulates MRCE expression in the spinal cord and predominantly in neurons.

Kategorie: Poster

Poster 34

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

Titel:Role of sip1 and cbln cytokine family in regulating feedback signaling during neocorticogenesis

Autoren: Parthasarathy S.(1),Tarabykin V.(2),Nityanandam A.(2),

Adressen:(1)Georg-August-Universität Göttingen|Max Planck Institute for Experimental Medicine|Goettingen|Deutschland; (2)Charité – Universitätsmedizin Berlin|Institute of Cell Biology and Neurobiology|Berlin|Deutschland; email:tarabykin@em.mpg.de

Abstract:
The layers of the neocortex are comprised of functionally diverse neurons. These neurons are generated sequentially from progenitors located in the germinal zone lining the lateral ventricles(VZ). Their fate and final position in the cortical plate is specified at the progenitor stage before they migrate into the cortical plate. However, not much is known about how the VZ learns how many pyramidal neurons of each type to produce and when the switch from producing one cell type to another should be made. One source of such signals is the cortical plate itself, creating a feedback loop. Recently we identified Sip1 (Smad Interacting protein1) as a transcriptional repressor of several cortical feedback signals which control the timing of sequential fate decisions in the VZ.Sip1 mutants show a precocious shift in corticogenesis wherein upper layer neurons are generated earlier at the cost of deeper layers and gliogenesis starts earlier ending neurogenesis precociously. However, little is known about the cortical signals. Our data suggests that Cbln4, a transneuronal cytokine which was significantly upregulated in the Sip1 mutant neocortex could be under the negative control of Sip1 and play a key role in cortical feedback signaling during corticogenesis. Overexpression of Cbln4 in wild type cortices causes a fate switch in progenitors from deep to upper layer neurons. Cbln2, a close family member could also be acting in a similar manner. We have also shown that Fgf9, which is under the negative control of Sip1 can induce precocious gliogenesis in wild type cortices.

Kategorie: Poster

Poster 35

Rubrik: 4.Zellbiologie
Abstract Nr.:4

Titel: 2, 4 Dichlorophenol reduces Cx43 expression and functional gap junction coupling in NTera-2/D1 cells

Autoren: Reuss B.(1),

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Abstract:
2, 4 Dichlorophenoxy acetic acid (2,4D) is a systemic herbicide widely used to control broadleaf weeds. A major soil degradation product of 2,4D is 2,4 Dichlorophenol (2,4DCP) which is already known to act neurotoxic, however, by yet unknown mechanisms. A subcellular system regulating early neuronal differentiation is intercellular communication by gap junctions, which is downregulated during retinoic acid dependent neuronal differentiation of human derived NTera2/D1 cells. We used these cells here to clarify whether 2,4D or 2,4DCP are able to change the gap junction protein Connexin(Cx)43 and whether this affected also functional gap junction coupling. NTera2/D1 cells were treated for 2, 4, 6 and 8 days with up to 10 µmol/l of either 2,4D or 2,4DCP, and Cx43 protein was detected using immunocytochemistry and Western blot analysis. A significant time and concentration dependent downregulation of Cx43 immunoreactivity was detectable at 10µmol/l 2,4DCP but not 2,4D. According to this, the scrape loading technique using Lucifer yellow, revealed a significant reduction in functional coupling by 10 µmol/l 2,4DCP, but not 2,4D. Together these results demonstrate for the first time a time and concentration dependent effect of 2,4DCP on amount and functionality of gap junctions in NTera2/D1 cells. This suggests an effect of this substance on neuronal differentiation the exact nature of which will have to be further clarified in the future. I would like to thank the medical faculty of the University of Göttingen (UMG) for persistent and reliable support of this project.

Kategorie: Poster

Poster 36

Rubrik: 4.Zellbiologie
Abstract Nr.:4

Titel: Inflammation-dependent down-regulation of the alpha1-, beta1- and alpha2-subunit of sgc in human odontoblasts

Autoren: Korkmaz Y.(1),Raab W.(1),Beikler T.(1),Behrends S.(2),Bloch W.(3),Addicks K.(4),

Adressen:(1)Heinrich-Heine-University|Department of Operative Dentistry, Periodontics and Endodontics|Düsseldorf|Germany; email:yueksel.korkmaz@uni-duesseldorf.de; (2)Technical University of Carolo-Wilhelmina at Braunschweig|Institute for Pharmacology, Toxicology and Clinical Pharmacy|Braunschweig|Germany; (3)German Sport University|Department of Molecular and Cellular Sport Medicine|Cologne|Germany; (4)University of Cologne|Department I of Anatomy|Cologne|Germany

Abstract:
The nitric oxide (NO) receptor enzyme soluble guanylate cyclase (sGC) contains one prosthetic heme group as an alphabeta heterodimer and two heterodimer isoforms (alpha1beta1, alpha2beta1) were characterized to have enzyme activity. In heterodimer forms, sGC is activated by NO to convert guanosine triphosphate (GTP) to cyclic guanosine 3',5'-monophosphate (cGMP) regulating vasodilatation, neurotransmission, cell differentiation and matrix biomineralization. To test inflammation-dependent regulation of sGC in human odontoblasts, decalcified frozen sections of healthy and inflamed human third molars were incubated with antibodies against alpha1-, beta1-, and alpha2-subunits of sGC. In odontoblasts of the consecutive healthy and inflamed human molar sections, different staining intensities for alpha1-, beta1- and alpha2-subunit of sGC were detected by quantitative immunohistochemical analysis. The staining intensities of the alpha1-, beta1- and alpha2-subunits were higher in healthy odontoblasts than in inflamed odontoblasts. Inflammation-dependent production of reactive oxygen and nitrogen species and inflammatory mediators may impair the expression of the alpha1-, beta1- and alpha2-subunits of the sGC in odontoblasts. The down-regulation of sGC in inflamed human odontoblasts indicates critical role/s for sGC in odontoblast survival producing dentin matrix within NO-cGMP signaling in health.

Kategorie: Poster

Poster 37

Rubrik: 4.Zellbiologie
Abstract Nr.:4

Titel: Prion protein prpc is localized within the nucleus and associated with a subset of microtubuli in cultured glioblastoma cells

Autoren: Klinz F.(1),Telentschak S.(1),Bloch W.(2),Addicks K.(1),

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Abstract:
Spongiform transmissible encephalopathies are characterized by the accumulation of the pathologic form (PrPsc) of the normal host prion protein (PrPc). PrPc is a GPI-anchored cell surface protein and has attracted recent interest because of its essential role in signalling between neurons and Schwann cells within the PNS. Although a cryptic nuclear localization signal has been described for PrPc, only PrPsc and C-terminally truncated prion proteins associated with familial prion disorders have been shown to be localized within the nucleus of infected cells.
Using a new commercially available rabbit monoclonal antibody against PrPc we can demonstrate by confocal immunofluorescence analysis that PrPc is strongly enriched within the nucleus of cultured glioblastoma cells. Glioblastoma cells often contain pleomorphic/multiple nuclei. Remarkably, PrPc immunofluorescence intensity did not correlate with intensity of DNA staining, because within single cells “micronuclei” with low content of DNA showed higher levels of PrPc than normally sized nuclei. Enrichment of PrPc was also often seen in the perinuclear region. Confocal double immunofluorescence analysis with a mouse monoclonal antibody against alpha-tubulin revealed that PrPc is associated with a subpopulation of microtubuli that are often but not exclusively localized near the nucleus.
Our results demonstrate that PrPc is highly expressed in cultured glioblastoma cells. The nuclear enrichment of PrPc implies an important role for the normal cellular prion protein in nuclear function of glioblastoma cells.

Kategorie: Poster

Poster 38

Rubrik: 4.Zellbiologie
Abstract Nr.:4

Titel: Mechanotransduction in rat skeletal muscle – effects of treadmill running on extracellular matrix constituents

Autoren: Suhr F.(1),Niehoff A.(2),Hamann N.(2),Bloch W.(1),

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Abstract:
Mechanotransduction, as induced by physical activity, has a significant impact on cellular systems, such as skeletal muscle. However, severity of mechanical stimuli depends on mode and intensity of exercise. Therefore, these tissues have developed strategies to register and to process mechanical stimuli by protein classes sensing mechanical stress, such as collagens (Col I,III) and integrins (Itga5,7). It still remains largely unknown how exercise regulates these proteins in skeletal muscle and thus cellular integrity.
48 female Sprague-Dawley rats were assigned to the following groups to study influence of different modes of running exercise: BC (basic control), AC (age-matched control, sedentary), Conc (running at 0° 5d/wk, 6wks), Ecc (see Conc, but –20°). Real-time RT-PCR and WB were used to determine mRNA and protein contents of targets in Vastus lateralis muscles, respectively.
Real-time RT-PCR (normalized to BC) revealed downregulations of all targets in AC. However, the downregulation of targets was less severe in CONC and ECC groups compared to AC. WB analysis (normalized to BC) showed an increase in Itga5,7 and Col I in exercise groups CONC and ECC. In contrast, Col III seems to be downregulated in skeletal muscle tissues of exercise groups.
The present data suggest that mechanotransduction has an effect on skeletal muscle ECM protein regulation. These findings might be important, because physical exercise seems to have a positive influence on collagens and integrins in skeletal muscle. As it was demonstrated that collagens and integrins play crucial roles in muscular dystrophies, the present findings shed further light on molecular mechanisms.

Kategorie: Poster

Poster 39

Rubrik: 4.Zellbiologie
Abstract Nr.:4

Titel: Putative motor proteins for synapto-nuclear transport mechanism

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

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Abstract:
Intercellular communication is mainly accomplished via chemical synapses that form unidirectional, functional connections between neurons and other cell types. These synapses are highly dynamic and undergo fast morphological changes, which seems to be essential for learning and memory formation. Abelson interacting protein 1 (Abi-1), a component of the postsynaptic density (PSD), was found to be important for dendrite branching, spine morphology and synapse formation. Abi-1 is targeted to PSDs via its direct interaction partner ProSAP2/SHANK3 that functions as a master scaffolding protein. Upon NMDA receptor stimulation Abi-1 translocates from the postsynapse to the nucleus. We are interested in the molecular mechanisms that underlay these transport processes. Therefore, we performed a yeast two-hybrid screen of a fetal human brain library using a full length cDNA of Abi-1 as bait. Among several candidate genes we found the partial cDNA of a N-kinesin of the kinesin superfamily, namely Kif26b and a Myosin called Myo16. Both proteins are motor proteins that move along microtubuli and actin filaments, respectively. First experiments show that Abi-1 specifically interacts with Kif26b and Myo16. Further experiments are set up to elucidate the importance of Kif26b and Myo16 in synapto-nuclear transport mechanisms.

Kategorie: Poster

Poster 40

Rubrik: 4.Zellbiologie
Abstract Nr.:4

Titel: Serotonin- but not muscarine-induced airway constriction is region-specific and dependent on caveolin-1.

Autoren: Schlenz H.(1),Hartmann P.(1),Kummer W.(1),Krasteva G.(1),

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Abstract:
Asthma is associated with hyperreactivity of the airway smooth muscle induced by acetylcholine release acting on muscarinic receptor subtypes 2 (M2R) and 3 (M3R). Previously, we have shown that these MR pathways are functionally coupled to caveolae and that M2R is associated with caveolin-3 in bronchial smooth muscle while Gosens and coworkers (2007) linked M3R to caveolin-1. Here, we investigated whether deletion of caveolin-1 has an impact on airway reactivity to muscarine or serotonin on different segments of the lower airway tree.
In organ bath experiments, the dose-dependent reactivity to muscarine (in percent of KCl-induced constriction) was equal in cranial, middle, and caudal tracheal segments and main bronchi of Cav-1+/+ mice. Muscarine-induced constriction of intrapulmonary bronchi determined by videomicroscopy in lung slices was identical in Cav-1-/- and Cav-1+/+ mice. These strains, however, differed in airway reactivity to muscarine in the middle (5x10-8 M, 10-7 M), and caudal tracheal segment (10-5 M, 5x10-5 M) and in the main bronchi (5x10-8 M). In Cav-1+/+ airways, the response to serotonin increased from cranial to caudal airway segments. Cumulative stimulation with serotonin caused a decrease in reactivity at concentrations of 10-6 M and higher, probably due to receptor desensitization. In Cav-1-/- mice, serotonin-induced constriction was absent in extrapulmonary airways while intrapulmonary airways of Cav-1-/- and Cav-1+/+ mice reacted equally strong.
In conclusion, serotonin- but not muscarine-induced airway constriction is region-specific and dependent on caveolin-1. Therefore, addressing caveolin-1-dependent signalling pathways may target allergic serotonin-induced but not cholinergic bronchoconstriction.

Kategorie: Poster

Poster 41

Rubrik: 4.Zellbiologie
Abstract Nr.:4

Titel: Cross talk between blood-derived leukocytes and autologous tenocytes

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Abstract:
Tendon ruptures are still a major orthopaedic challenge because tendon healing leads to scar formation and inferior biomechanical characteristics of tendon. These injuries result in tendon bleeding whereby blood-derived leukocytes immigrate into the damaged tissue. The impact of the immunoregulatory cross-talk between tenocytes as resident cells in tendon and extrinsic blood-derived leukocytes on the healing process is rudimentary investigated. Hence, the intention of this study was to elucidate the interaction between tenocytes and leukocytes.
Primary tenocytes, peripheral blood derived mononuclear cells (PBMCs) and neutrophils were isolated from Achilles tendons and peripheral blood of the same rabbits. Co-cultures of primary tenocytes with autologous PBMCs and neutrophils were established by using a transwell system in order to avoid direct cell-to-cell contacts. Tenocyte gene expression was studied by using RTD-PCR for ECM components involved in tendon healing such as type III collagen, decorin, fibronectin, immunoregulatory cytokines (IL-1beta, TNFalpha, IL-6) and matrix degrading matrix-metalloproteinase (MMP)-1. Caspase 3/7 and TUNEL assays were applied to detect leukocyte-mediated tenocyte apoptosis or cell death.
This study showed no significant effects of leukocytes on tenocytes ECM gene expression. Tenocytes gene expression of IL-1beta, TNFalpha, IL-6 as well as MMP1 was significantly increased by PBMCs. The caspase-3/7 activity remained mainly unaltered, whereby enhanced DNA fragmentation could be found in tenocytes co-cultured with PBMCs.
PBMCs might be a source of pro-inflammatory factors during the inflammatory phase of tendon healing activating the tenocytes to upregulate their pro-inflammatory cytokine and MMP expression. Whether these factors contribute to tendon scarring, remains unclear.

Kategorie: Poster

Poster 42

Rubrik: 4.Zellbiologie
Abstract Nr.:4

Titel: Establishment of an in vitro cell culture model for studying transendothelial migration of different cell types

Autoren: Kaiser T.(1),Foerster C.(1),Burek M.(1),

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Abstract:
Migration of different cell types takes place in many physiological processes as well as in pathological processes. Circulatory cells after adhering to capillaries extravasate into surrounding tissue. Although extravasation is crucial in many physiological processes, mechanisms regulating this complex process remain to be fully elucidated. The aim of this study was to establish a reliable in vitro model for studying the transmigration of different cell types through endothelial monolayer. For this purpose we used a human brain microvasucular endothelial cell line hCMEC/D3 and a murine brain microvascular endothelial cell line cEND. Both cell lines are well established and extensively characterized blood-brain-barrier models. HCMEC/D3 as well as cEND cells express endothelial and blood-brain-barrier markers and form in vitro a tight barrier, which could be demonstrated in transendothelial electrical resistance measurements. cEND cells form a tighter monolayer than the hCMEC/D3 cells. For the transmigration experiments we used different cell culture transwells. Different growth conditions of endothelial cells on the transwell membrane and different staining methods have been used. This established cell culture model will be used in studying of 17beta-estradiol effects on transendothelial migration.

Kategorie: Poster

Poster 43

Rubrik: 4.Zellbiologie
Abstract Nr.:4

Titel: Expression and immunolocalization of gpr91 and gpr99 in murine organs

Autoren: Paddenberg R.(1),Diehl J.(1),Goldenberg A.(1),Faulhammer P.(1),Gries B.(1),Pfeil U.(1),Kummer W.(1),

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Abstract:
GPR91 and GPR99 are G-protein-coupled receptors, which are activated by the citric acid cycle intermediates succinate and α-ketoglutarate, respectively. Among others binding of succinate to GPR91 stimulates the release of renin in the kidney and regulates lipolysis in white adipose tissue. GPR99 mRNA is predominantly detectable in the kidney with limited expression in testis and smooth muscle.
Here, homogenates of 30 murine organs were analysed for GPR91 by western blotting. Highest amount of GPR91 was observed in white adipose tissue whereas it was undetectable in heart, aorta, and sciatic nerve. Clear signals were also obtained in kidney, adrenal and submandibular glands. In the kidney, GPR91 was localized mainly in the juxtaglomerular apparatus whereas GPR99 was found in intercalated cells of the collecting ducts and the pelvic epithelium. Immunohistochemistry of adrenal glands revealed strong GPR91 immunoreactivity of the zonae fasciculate and reticularis, but not of the zona glomerulosa and the medulla. Interestingly, submandibular glands from female mice exhibited the twofold amount of GPR91 as compared to glands of males. It was localized in the acini which are more densely packed in organs of females than of males. In contrast, GPR99 was mainly detectable in the duct system which is more pronounced in males.
In conclusion, we have demonstrated a widespread expression and localization of GPR91 and GPR99 protein in mouse including endo- and exocrine glands suggesting involvement in regulation of secretory function.

Kategorie: Poster

Poster 44

Rubrik: 4.Zellbiologie
Abstract Nr.:4

Titel: Connective tissue growth factor is involved in the regulation of basal cell functions in human trabecular meshwork cells

Autoren: Junglas B.(1),Struller T.(1),Tamm E.(1),Fuchshofer R.(1),

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Abstract:
Purpose: A pathological increase in intraocular pressure (IOP) is the major risk factor for glaucoma. The increase in IOP is due to an increase in outflow resistance to aqueous humor, in the trabecular meshwork, which is associated with an increase in extracellular matrix (ECM) deposition. In a previous study we could show that the Connective Tissue Growth Factor (CTGF) is a potent modulator of the ECM in trabecular meshwork cells. The aim of this study was to analyze the morphological, physiological and molecular effects of a stable knockdown of CTGF in trabecular meshwork cells.
Methods: Human trabecular meshwork cells (HTM5) were transfected with a pSilencer vector coding for a CTGF specific short-hairpin RNA. Cells were analyzed by phase contrast and fluorescence microscopy, attachment, wound healing and BrdU assays, quantitative real-time RT-PCR and western blotting.
Results: Knockdown cells showed reduced formation of stress fibres and protrusions but an increased number of focal adhesions. Expression of ECM and cytoskeleton components as well as RhoA activity was reduced. In addition, we could observe a reduced migratory and proliferatory activity. In contrast, the ability to attach to surfaces was increased. Treatment with recombinant CTGF led to a rescue of the phenotype regarding proliferation and adhesion.
Discussion: CTGF is involved in the basal expression of ECM and cytoskeleton components, in the regulation of cytoskeleton activity and therefore in establishing correct cell morphology.

Supported by DFG grant FOR1075

Kategorie: Poster

Poster 45

Rubrik: 4.Zellbiologie
Abstract Nr.:4

Titel: Spak-dependent activation of thiazide-sensitive na,cl-cotransporter by vasopressin

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Abstract:
The Na,Cl-cotransporter (NCC) of distal convoluted tubule (DCT) effectively contributes to the fine regulation of urine electrolyte composition. Acute activation of NCC by angiotensin II involves luminal trafficking as well as phosphorylation by WNK/SPAK kinases. Vasopressin (AVP) activates NCC by binding to type 2 receptors (V2R), but the kinases involved have not been elucidated. We propose a significant role for SPAK in the AVP-induced activation of NCC.
SPAK-knockout (SPAK-/-, Behav. Brain Res. 2010; 208:377-82.) and wild-type mice (WT) and AVP-deficient Brattleboro rats (DI) were treated with the V2R agonist, dDAVP, or vehicle for 30 min. Kidneys were studied by immunohistochemistry and Western blot.
SPAK and phospho-SPAK were co-expressed with NCC in DCT. dDAVP treatment increased NCC phosphorylation at S71 in WT mice (+87%, p<0.05) but less so in SPAK-/- mice (+45%, p<0.05). Phosphorylation of NCC at T53 was also significantly increased in WT (+153%, p<0.05) whereas SPAK-/- mice showed no change. In DI rats, dDAVP produced increases of SPAK and NCC phosphorylation in 11ß-hydroxysteroid dehydrogenase 2-negative DCT1 (pS373-SPAK: +52%; pS71-NCC: +42%; pT53-NCC: +147%; p<0.05) but not in DCT2 of DI rats.
We conclude that V2R stimulation enhances NCC activity via phosphorylation events that depend on SPAK, although other kinases are also involved. Activation of NCC by V2R occurs predominantly along the aldosterone-insensitive DCT1, suggesting that NCC activation by aldosterone is mechanistically distinct.

Kategorie: Poster

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Rubrik: 4.Zellbiologie
Abstract Nr.:4

Titel: Compromised nuclear accumulation of laminopathy-causing lamin a mutants

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Abstract:
Lamins, constituents of the nuclear lamina, exhibit a tripartite domain structure (head, rod and tail), with the tail domain containing a nuclear localization sequence (amino acids 417 to 421). Mutations of A-type lamins (lamin A and lamin C) have been shown to cause distinct human diseases (laminopathies), including lipodystrophies, myopathies and multisystem diseases characterized by progeroid syndromes. We have previously documented that nuclear import is compromised in progeria-derived fibroblasts. Investigating nuclear import of selected laminopathy-inducing lamin A mutants using lamina incorporation incompetent but nuclear import competent lamin A molecules, deleted for the head and the rod domain (referred to as tail mutants), we additionally demonstrated that nuclear accumulation of tail mutants carrying progeria-causing deletions was severely reduced. As lamin A has been shown to be the target of PKC and Akt kinases at sites close to the NLS (S403 and S404) together with the observation of reduced nuclear import of a lamin A S403/S404 mutant, we wanted to study nuclear import efficiencies of lamin A tail mutants carrying the following laminopathy-inducing mutations: R401C (myopathy), R399C (lipodystrophy/cardiomyopathy) and R399H (metabolic syndrome). The metabolic syndrome-causing L421P mutation was similarly investigated. We found significantly reduced nuclear import activities for all tail mutants (i.e. R399H, R401C and L421P) except for the mutant R399C. We thus conclude that mutations which negatively interfere with the NLS function of lamin A could possibly contribute to the pathogenesis of certain laminopathies.

Kategorie: Poster

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Rubrik: 4.Zellbiologie
Abstract Nr.:4

Titel: Norrin interacts with tgf- beta1 signaling in vitro and in vivo

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Abstract:
Purpose: Norrin is an angiogenic and neuroprotective growth factor that can activate the canonical Wnt/beta-catenin signaling pathway. The predicted tertiary protein structure of Norrin indicates a cysteine-knot motif comparable to that observed in growth factors of the transforming growth factor (TGF) superfamily. Here we analyzed if Norrin can interact with the TGF-beta1 signaling pathway and vice versa in vitro and in vivo.
Methods: A luciferase reporter cell line for TGF-beta1 signaling and human microvascular endothelial cells (HMEC) were used for in vitro studies. Cells were incubated with Norrin and/or TGF-beta1 to analyze luciferase activity, cell proliferation, as well as mRNA and protein expression of different mediators of TGF-beta1 or Wnt signaling. In additional experiments, transgenic mice with an ocular overexpression of Norrin (betaB1-Norrin) or TGF-beta1 (betaB1-TGF-beta1) were mated and double transgenic mice were examined by light and electron microscopy, and realtime RT-PCR.
Results: A profound mutual inhibition of Norrin and TGF-beta1 signaling could be observed in different cell culture experiments and in betaB1-TGF-beta1/betaB1-Norrin double transgenic mice. In addition, treatment with Norrin had a distinct influence on the expression of different downstream mediators of the TGF-beta1 signaling pathway. One of the most promising targets of Norrin-inhibition seems to be the TGF-beta inhibitor Smad7.
Conclusion: Norrin and TGF-beta signaling share a mutual inhibition of their pathways which appears to involve, at least partially, Smad7 signaling.
Supported by DFG grant FOR1075

Kategorie: Poster

Poster 48

Rubrik: 4.Zellbiologie
Abstract Nr.:4

Titel: Characterization of putative estrogen receptor binding sites in murine claudin-5 promoter

Autoren: Steinberg K.(1),Foerster C.(1),Burek M.(1),

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Abstract:
Claudin-5 is an integral membrane protein and a critical component of endothelial tight junctions that control paracellular permeability. Claudin-5 is expressed strongly in the vascular endothelium. Recently we have cloned and described a murine claudin-5 promoter. We demonstrated 17beta-estradiol-mediated regulation of claudin-5 in brain and heart microvascular endothelium on promoter, mRNA and protein level. Sequence analysis revealed a putative estrogen response element in the claudin-5 promoter as well as two putative Sp1 transcription factor binding sites. The aim of the present study was to further characterize the estrogen-mediated regulation of claudin-5 promoter. First, we tested whether estrogen receptor subtypes bind in vivo to the claudin-5 promoter region. For this purpose we performed a chromatin immunoprecipitation assays using anti-estrogen receptor antibodies and cellular lysates of endothelial cells. We show that estradiol stimulation resulted in the recruitment of estrogen receptor beta and to a lesser extend recruitment of estrogen receptor alpha. Next, we have introduced point mutations in the putative binding sites in claudin-5 promoter constructs and used them in the luciferase reporter gene assay. We detected an impaired transcriptional activation of the mutated constructs of the claudin-5 promoter after 17beta-estradiol treatment. In summary, this study provides evidence that ER beta is prominently recruited to the claudin-5 promoter and identifies promoter regions necessary for estrogen-mediated regulation of the claudin-5 promoter.

Kategorie: Poster

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Rubrik: 4.Zellbiologie
Abstract Nr.:4

Titel: 17beta-estradiol affects proinflammatory cytokine induced gene transcription of matrix degrading enzymes in meniscal tissue

Autoren: Ewald K.(1),Naujokat H.(1),Schünke M.(1),Kurz B.(1),

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Abstract:
Purpose: Osteoarthritis (OA) affects all articular tissues, including the meniscus, and finally leads to joint failure. The rise in OA prevalence among postmenopausal women suggests a link between OA and the breakdown of estradiol (E2) production. The objective was to evaluate the influence of E2 on the proinflammatory cytokine-dependent transcription of matrix degrading enzymes in meniscal tissues.
Methods: Bovine meniscal tissue was isolated from 2 year-old cows and treated for 24 and 72 hrs with IL-1alpha and E2. Transcription of mRNA for MMP-3, Aggrecan and ADAMTS-4 was analyzed by quantitative RT-PCR. The concentration of glycosaminoglycans (GAG) and NO in the culture supernatant was determined by photometric DMMB and Griess assay, respectively.
Results: E2 (10-11M) decreases significantly the IL-1alpha -dependent transcription of MMP-3 and ADAMTS-4. Aggrecan, which degradation has been associated with the development of OA, increases significantly. The IL-1alpha -dependent GAG release decreases significantly. The E2 receptor antagonist ICI 182,780 inhibits this effect. NO release is also significantly reduced.
Conclusions: E2 seems to play a relevant role in the homeostasis of meniscal tissues under proinflammatory conditions. These effects might be mediated by intracellular estradiol receptors. E2 could be a therapeutic option in joint destructing diseases due to its effects upon different joint structures like the meniscus.

Kategorie: Poster

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Rubrik: 4.Zellbiologie
Abstract Nr.:4

Titel: The effect of different lactate concentrations on the differentiation of c2c12 myoblasts

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Abstract:
In the recent past, lactate (La) has been more and more recognized as a functional molecule with three major tasks: At systemic level, it is a gluconeogenic precursor and an energy substrate. Furthermore it was demonstrated, that on cellular level it functions as a signialing molecule and has therefore been termed 'lactormone'. However, so far to our knowledge no data has been published to its effects on the differentiation of C2C12 myoblasts. As it would be very interesting to investigate how lactate affects the differentiation pattern, undifferentiated C2C12 cells were incubated continuously with normal culture medium (0mM La) as well as with culture medium that contained lactate in two different concentrations (10, 20 mM La) in normal cell culture conditions of 37°C and 5% CO2. In order to apply the lactate signal in context of a more realistic situation, in the next experiment lactate was added to the medium for only 2 h once every 24h (simulating training). The results show that lactate has a delaying effect of the C2C12 myotube formation and an effect of lactate turnover and that these effects are different if lactate is only applied at a certain interval. Further research is necessary to clarify the underlying mechanisms.

Kategorie: Poster

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Rubrik: 4.Zellbiologie
Abstract Nr.:4

Titel: Age dependent effects of atomoxetine: chronic treatment causes differential expression of monoaminergic transporters and glutamatergic receptors

Autoren: Udvardi P.(1),Schaz U.(2),Liebau S.(2),Dreyhaupt J.(3),Fegert J.(1),Böckers T.(2),Ludolph A.(1),

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Abstract:
Objective: Existing in vitro results prove that - besides the well-known inhibition of the norepinephrine transporter (NET) - atomoxetine, a psychotropic substance used in the pharmacotherapy of attention-deficit/hyperactivity disorder (ADHD), acts as an open-channel blocker at the N-methyl-d-aspartate receptor (NMDAR) (Ludolph et al., 2010). Here we present an in vivo study carried out to investigate atomoxetine's effect in rat brain.
Methods: Pregnant Crl:SD(CD) rats were treated with atomoxetine (3 mg/kg, i.p.) and sodium chloride (0,9%, i.p.) respectively. The study was conducted on E12 to E19 thus covering a treatment period equivalent to the human second to third trimenon of pregnancy. After the end of treatment male embryos (E19) and dams were cerebroectomised. Hippocampus, prefrontal cortex, mesencephalon and the striatum were analyzed due to gene-expression and protein-level respectively.
Results: Our in vivo study revealed alterations in gene-expression in the embryonic and adult brain. The monoaminergic and the glutamatergic system were altered after atomoxetine treatment. In the fetal brain altered gene-expression prevailed on the 5-hydroxytryptamine transporter (5-HTT), whereas in the adult brain expression alterations were mainly observed in the NMDAR subunit genes.
Conclusion: The results of this in vivo study indicate that the affinity of atomoxetine to the 5-HTT might be more relevant than discussed before. Gene-expression analysis implies that the glutamatergic system is more affected in adult than in embryonic rodent´s brain. We conclude that besides the inhibitory action at the NET, mechanisms influencing the glutamatergic system might play a major role in the clinical effect of atomoxetine.

Kategorie: Poster

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Rubrik: 4.Zellbiologie
Abstract Nr.:4

Titel: Assessment of satellite cell and myonuclear number in human vastus lateralis muscle in response to different endurance exercise regimens

Autoren: Frese S.(1),Looser P.(1),Konou T.(1),Schiffer T.(2),Tappe K.(1),Bloch W.(1),

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Abstract:
Satellite cells (SC) are mononuclear stem cells of skeletal muscle which play an important role in the muscular adaptive response to exercise. The exact extent of SC involvement in structural und functional remodelling processes in response to endurance exercise induced stimuli has received much less attention.
The aim of this study was to analyze the effect of load-dependent endurance exercise on the number of SC and myonuclei in skeletal muscle of 10 male young cyclists (17.3±0.5yr).
We investigated the training period (TP1, Nov-Feb), the competitive season (CS, Feb-Oct) and the following TP (TP2). After each period muscle biopsies were obtained from the vastus lateralis muscle. SC were labelled with a monoclonal Pax-7 antibody and myonuclear number was analysed from HE-cross-sections (7µm). The muscle fiber diameter was measured. The SC number per muscle fiber at the end of the CS (0.56±0.059) was significantly decreased towards TP1 (0.94±0.269) and TP2 (0.82±0.092; p<0.05). The analysis of myonuclear number illustrated an inverse pattern to the SC number. After CS the number of nuclei/fiber was significantly increased (4.00±0.933) towards TP1 (1.75±0.727) and TP2 (1.75±0.184), while no changes of fiber diameter were observed.
SC content is affected strongly in response to load-dependent endurance exercise. A change of exercise regimen from TP to CS by a reduction of volume and increase of intensity leads to a significant decrease of SC and a massive addition of the myonuclear number. According to the unaltered fiber diameter this indicates a non-hypertrophic hyperplasia.

Kategorie: Poster

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Rubrik: 4.Zellbiologie
Abstract Nr.:4

Titel: Betulinic acid dependent neuronal differentiation of NTera-2/D1 cells correlates to changes in the expression of Retionoic acid receptors-beta and gamma2

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Abstract:
Retinoic acid (RA) dependent neuronal differentiation of the human derived teratocarcinoma cell line NTera2/D1 has been previously shown to be accompanied by changes in expression of RA receptors-beta and -gamma2. We used this cell line here to clarify, whether the experimentally used cytostatic Betulinic acid (BA) besides its known apoptosis inducing effects in neuroectodermally derived tumor cells, is also able to modulate neuronal differentiation, and whether this is accompanied by changes in RA receptors-alpha, -beta and gamma2. As revealed by immunofluorescent staining, BA is indeed able to induce expression of the neuronal differentiation markers Doublecortin and βIII-Tubulin in NTera2/D1 cells, with lower concentrations (10-8 mol/l) being more effective than higher concentrations (10-5 mol/l). In parallel, BA modulates also expression of RA receptors-beta and -gamma2, whereas RA receptor-alpha remains unchanged. Thus, semiquantitative RT-PCR, reveals a significant up regulation of RA receptor-beta by BA, whereas RA receptor-gamma2 is significantly reduced. These results demonstrate for the first time a concentration dependent effect of BA on neuronal differentiation and on expression of RA receptors -beta and gamma2, suggesting the differentiation promoting effects of BA to be at least partially a result of changes in RA receptor expression.

Kategorie: Poster

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Rubrik: 4.Zellbiologie
Abstract Nr.:4

Titel: Detection of the surfactant proteins A, B, C, D in human articular cartilage and chondrocyte cell lines

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Bräuer L.[1], Paulsen F.[3]

Adressen: 1) Institute of Anatomy and Cell Biology, Martin Luther University Halle-Wittenberg 2) Institute of Forensic Medicine, Charite, Berlin 3) Department of Anatomy II, University of Erlangen-Nuremberg

Abstract:
Purpose Aim of the present study was to analyse whether human surfactant proteins (SP-A, B, C and D) are produced by chondrocytes of human articular cartilage as well as two human chondrocyte cell lines (C28/I2 and T/C28a2) and whether this occurs in an age dependent manner.
Methods Articular cartilage was obtained from the tibial plateau of 15 knee-joints of different age (age range: 3 to 90 years). In addition, chondrocytes from the immortalized human chondrocyte cell lines C28/I2 and T/C28a2 were cultured. SP expression was detected and quantified by means of RT-PCR, Western blot analysis, ELISA as well as immunohistochemistry.
Results All four surfactant proteins were detected in all samples of human articular cartilage as well as in the cell lines, both on mRNA and protein levels. ELISA revealed no age dependend expression pattern of SPs and showed the follwing mean values of SP concentration: SP-A = 39.9 ng/mg; SP-B = 169.0 ng/mg; SP-C = 125.0 ng/mg; SP-D = 38,1 ng/mg.
Conclusion All four SPs are produced by articular cartilage chondrocytes. Their production suggest that surfactant proteins might be players of the innate immune system of human articular cartilage and that the small amphiphilic surfactant proteins B and C might have an influence on the rheology of synovial fluid by reducing friction of articular cartilage. However, this must be elucidated in further investigations.

Kategorie: Poster

Poster 55

Rubrik: 4.Zellbiologie
Abstract Nr.:4

Titel:Soluble cea co-stimulates apoptosis in human epithelial and endothelial cells via its interaction with ceacam1

Autoren: Singer B.(1),Scheffrahn I.(1),Muturi H.(1),Ergün S.(1),

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Abstract:
The carcinoembryonic antigen (CEA) is a GPI-anchored glycoprotein found in many types of epithelial cells and the developing fetus. The not membrane anchored version of CEA is associated with malignancies like colon and rectum cancer. The fact that the normal level of soluble CEA in serum is significantly increased in various tumors was already described in 1965. However, until now no functional role was assigned to soluble CEA. Here we show that the interaction of soluble CEA with CEACAM1 in the presence of a non-toxic dose of sodium azide induces apoptosis in epithelial and endothelial cells. Interestingly, only cell-cell-contact inhibited but not proliferating epithelial cells were affected by CEA/sodium azide. Because confluent epithelial cells expressed significantly more CEACAM1 than proliferating cells, the apoptotic effect seemed to be dependent on the abundance of CEACAM1 on the cell surface. The CEA/sodium azide induced signaling led to caspase-3 activation. Taken together, our results demonstrate that the CEACAM1-induced pathway is activated by soluble CEA leading to an increased sensitivity for effect inducing agencies like sodium azide in human epithelial and endothelial cells.

Kategorie: Poster

Poster 56

Rubrik: 4.Zellbiologie
Abstract Nr.:4

Titel:An air-liquid interface (ali) culture of intestinal epithelial cells promotes morphological differentiation

Autoren: Nossol C.(1),Rothkötter H.(1),Diesing A.(1),Post A.(1),Faber-Zuschratter H.(1),Kluess J.(2),Walk N.(1),Kahlert S.(1),

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Abstract:
ALI-cultures are the best in vitro representations of the airway and gastric epithelium. The surface of intestinal epithelium represents a comparable interface. Thus, a novel culture method was developed to study the morphological differentiation of intestinal epithelial cells in a microenvironment closely related to physiological conditions.
In our experiments the effects of different culture conditions on morphological differentiation of intestinal epithelial cells was studied. Intestinal porcine epithelial cells (IPEC-1), derived from ileum and jejunum, were cultured on different substrates. We used Lumox®-dishes, inserts (a membrane based culture system providing an apical and basolateral compartment), and inserts with an ALI-culture. Cultured cells were examined by immunfluorescence and electron microscopy. To compare the intestinal morphology with our cell culture system (in vitro), cryo sections of the gut using the same read-out were studied too.
The ALI-cultures resulted in a significant increase in the cell number, the epithelial layer thickness and in the accumulation of the tight junction protein ZO-1. In contrast, no differences between ALI and gut sections were observed. The cellular volume observed in dish-cultivation of IPEC-1 cells was larger in comparison to cultivation in the ALI-system. Using electron microscopy, flat and long cells were found on dishes, a better differentiation of cells was detected in the ALI-system resulting in a columnar phenotype of epithelial cells.
This first report of an ALI-culture of intestinal epithelial cells suggests that an ALI provides an important factor of the morphological differentiation of intestinal epithelial cells in vitro.

Kategorie: Poster

Poster 57

Rubrik: 4.Zellbiologie
Abstract Nr.:4

Titel:The role of the fractalkine and its receptor cx3cr1 in the interplay between glioma cells and glioma -infiltrating microglia/macrophages (gims)

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Abstract:
Solid tumours have been known for long time to be strongly infiltrated by inflammatory leukocytes, and accumulating evidence has clearly demonstrated a strong correlation between increased numbers of tumour-associated macrophages and growth, malignancy grade, or poor prognosis. Chemokines, and in particular the transmembrane chemokine CX3CL1 (fractalkine, neurotactin) and its receptor CX3CR1 play a pivotal role in the trafficking of immune cells. However, little is known about the expression and function of the CX3CL1 / CX3CR1 axis in human astrocytomas/gliomas, brain tumours that contain 20-30% microglia cells/macrophages. Using immunohistochemistry we found that CX3CL1 is produced in GFAP-expressing glioblastoma regions whereas its receptor CX3CR1 is exclusively expressed on glioma-infiltrating microglia cells/macrophages (GIMs) in situ and in vitro as indicated by co-staining of CX3CR1 and CD11b/CD11c and ionized calcium-binding adapter molecule 1 (Iba1). Additionally, quantitative RT-PCR and Western blot substantiate elevated expression of CX3CR1 in solid glioma samples caused by the invaded Iba-1-positive GIM fraction. In accordance with this, freshly isolated human GIM-enriched fractions separated by CD11b MACS technology displayed high Iba1 and CX3CR1 mRNA expression levels in vitro. Functionally, cultured human GIMs responded to CX3CL1-triggered activation of CX3CR1 with adhesion, migration and enhanced expression of matrix metalloproteinases (MMP) 2, 9, and 14 in vitro. These data indicate that the CX3CL1/CX3CR1 system is responsible for the infiltration of microglial cells/macrophages into glioblastomas and plays a tumour-promoting role.

Kategorie: Poster

Poster 58

Rubrik: 4.Zellbiologie
Abstract Nr.:4

Titel:Neurogenesis in hips cell derived neurons

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Abstract:
Keratinocytes from plucked human hair have already been shown to be a valuable cell source for the reprogramming into hiPS (human induced pluripotent stem) cells. Keratinocytes have a much higher reprogramming efficiency compared to fibroblasts or other cell types. Our group has successfully used this non-invasive method to collect samples from donors of different age. After initial outgrowth the keratinocytes were reprogrammed to hiPS cells via viral infection. Subsequently we differentiated these cells into the neural lineage via the formation of embryoid bodies and neural rosettes. We generated various neuronal subtypes such as glutamatergic, GABAergic or dopaminergic neurons. Additionally we analyzed the progress of neurogenesis of the different hiPS cell derived neuronal subtypes and compared this with already existing models like in vitro cultures of primary rat neurons or neurogenesis of adult neural stem cells. hiPS derived neural cultures might be used as an in vitro model for elucidating the pathogenesis of various neural diseases especially if patient specific cells are used.

Kategorie: Poster

Poster 59

Rubrik: 5.Entwicklungsbiologie
Abstract Nr.:5

Titel: Chondrogenic differentiation of equine adipose tissue-derived stem cells induced by hydrolyzed fish collagen and TGF-beta1

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Abstract:
Adipose tissue-derived stem cells (ADSCs) are multipotent adult stem cells with potential for use in cartilage tissue engineering. Chondrogenic differentiation is associated with cytokines such as transforming growth factor-beta (TGF-beta) and dexamethasone.
The goal of this study was to investigate the effect of hydrolyzed fish collagen in comparison to TGF-beta1 on the chondrogenic differentiation potential of ADSCs.
In vitro chondrogenesis was induced using a three-dimensional pellet culture system. The differentiation medium was either supplemented with TGF-beta1 or hydrolyzed fish collagen for a 3 week period.
After in vitro differentiation, RT-PCR, and histological staining for proteoglycan synthesis and type II collagen were carried out to evaluate the degree of chondrogenic differentiation and of cartilaginous extracellular matrix (ECM) respectively.
ADSCs induced by TGF-beta1 showed a high expression of glycosaminoglycan (GAG). Histological analysis of cultures stimulated by hydrolyzed fish collagen demonstrated an even higher GAG expression than cultures stimulated under standard conditions by TGF-beta1.
The expression of cartilage-specific type II collagen and sox 9 was about the same in both stimulated cultures.
Chondrogenesis of ADSCs was as effectively induced in the presence of hydrolyzed fish collagen as according to the common differentiation protocol with TGF-beta1. These findings demonstrated that hydrolyzed fish collagen alone has the potential to induce chondrogenesis of ADSCs and this is likely to play an important role in articular cartilage repair.

Kategorie: Poster

Poster 60

Rubrik: 5.Entwicklungsbiologie
Abstract Nr.:5

Titel: Interspecies comparison of ATOH8 gene

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Abstract:
ATOH8 is a bHLH domain transcription factor that has been implicated in several developmental and pathological processes, e.g. in the nervous system, kidney, pancreas, retina and muscle. In the present study, we adopted the strategy of comparative genomics to analyze the gene structure and regulatory elements of ATOH8 among different species. We show that ATOH8 is highly diversified, except the conserved bHLH domain. Among 18 species investigated, mammals develop another potential isoform, attested by a human expressed sequence tag (EST). The regulatory elements of ATOH8 convert from TATA-box type into CpG-islands type from the fish, amphibians, birds to mammals. Our gene mapping data show that in the human, ATOH8 is hosted in one chromosome which is a fusion product of two orthologous chromosomes in non-human primates. This unique chromosomal environment of human ATOH8 probably subjects its expression to the regulation at intrachromosomal level. These interspecies diversities might exert delicate regulation on the spatiotemporal expression of ATOH8, thus fine-tuning its function in different tissues, or even different organisms. We have furthermore identified the region of the effective promoter of human ATOH8.

Kategorie: Poster

Poster 61

Rubrik: 5.Entwicklungsbiologie
Abstract Nr.:5

Titel: Exploring the role of bhlh transcription factor atoh8 during embryonic myogenesis and satellite cell differentiation

Autoren: Balakrishnan-Renuka A.(1),Morosan-Puopolo G.(2),Philippi S.(2),Lu Y.(2),Otto A.(3),Yusuf F.(4),Patel K.(3),Brand-Saberi B.(4),

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Abstract:
ATOH8 was severely deregulated in a patient suffering from a congenital myopathy. Our expression analysis shows that in chicken embryos ATOH8 is expressed in the myotome, dorsomedial and ventrolateral lips of the dermomyotome. Its protein is detected in the cytoplasm and nucleus of C2C12 myoblast, rat cardiomyocytes and rat smooth muscle cells. To investigate its role during myogenesis, we have silenced ATOH8 in the somites and analysed its expression in satellite cells and myogenic progenitors in muscle fibres. Furthermore, we looked at the cellular localization of ATOH8 in H2O2 and cardiotoxin induced injury model in C2C12 myoblast and in EDL of mice respectively. Knock-down of cATOH8 in the somites resulted in an effective silencing of ATOH8 and a down-regulated expression of MyoD, Myf5, along with a decrease in MHC expression and an up-regulated expression of Pax3. ATOH8 translocated to the nucleus following H2O2 injury in myoblasts. IHC on muscle fibres revealed the existence of Pax7+/ATOH8-, Pax7+/ATOH8+/- and Pax7+/ATOH8+ subpopulations of satellite cells. Similarly, in the progenitor cells arising from the satellite cells we could also observe three subpopulations expressing Myogenin+/-/ATOH8+, Myogenin+/ATOH8+, Myogenin+/ATOH8- . We conclude that ATOH8 is involved in myogenesis and is expressed during satellite cell differentiation. Cellular injury leads to a nuclear translocation of the protein. Its expression levels increase at the onset of satellite cell differentiation and decreases towards terminal differentiation. This is in line with the decrease of expression observed during maturation of muscles during chicken embryogenesis.

Kategorie: Poster

Poster 62

Rubrik: 5.Entwicklungsbiologie
Abstract Nr.:5

Titel: Effect of varying ca2+-concentrations on the osteogenic differentiation of adult mesenchymal stem cells.

Autoren: Tryankowski E.(1),Reich C.(1),Raabe O.(1),Wenisch S.(1),Kramer M.(2),Arnhold S.(1),

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Abstract:
In human as in veterinary medicine bone implants enhancing and ameliorating osteogenic cell differentiation and graft integration into the bone are equally needed. One of the easily adjustable characteristic of such implant materials is the solubility of Ca2+ ions in Ca2+-enriched biomaterials. In bone micro-compartments high physiologic Ca2+-concentrations ranging from 8 – 40 mM positively influence the migration, proliferation and matrix mineralization of osteoblasts and their progenitor cells in the surrounding area.
The aim of our study was therefore to determine the optimal Ca2+-concentration for the osteogenic differentiation of equine mesenchymal stem cells (MSC). Additionally in canine MSC we compared the Ca2+-responsiveness of stem cells of two different origins, the bone marrow (BM-MSC) and the adipose tissue (AD-MSC).
The outcome of our study shows a dose-depended increase in matrix mineralization of differentiated equine MSCs with an optimal concentration between 5-10 mM Ca2+. Canine BM-MSC reveal an improved mineralization compared to AD-MSC using the same Ca2+-concentration (7,5mM CaCl2).
Our study demonstrates the positive effect of an enhanced Ca2+-concentration on the osteogenic differentiation of MSCs. Following, the pre-differentiation and seeding of equine MSCs on Ca2+-releasing bone graft substitutes could be tested. For canine MSC we have to reassess and refine the possibility to enhance the proven osteogenic differentiation of AD-MSC using further osteogenic factors.

Kategorie: Poster

Poster 63

Rubrik: 5.Entwicklungsbiologie
Abstract Nr.:5

Titel: Expression of cadherins and protocadherins in the developing chicken kidney

Autoren: Lemke C.(1),Lin J.(1),Redies C.(2),

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Abstract:
Cadherins (Cdhs) constitute a family of adhesion receptors, with protocadherins (Pcdhs) being the largest subfamily. We studied the expression of classic cadherins (N-Cdh, R-Cdh, Cdh6-11) and protocadherins (Pcdh1, Pcdh7-10, Pcdh15, Pcdh18-21) during kidney development in chicken from embryonic day (E) 2.5 to mature stages (E20) by in situ hybridization. Of the cadherins studied, N-Cdh, R-Cdh, Cdh6, Cdh11, and Pcdh-1, -8, -9, -10, -18, and -19 were found to be expressed in the chicken kidney. For example, cadherins are expressed by different cell types in the developing glomerulus. Markers for the renal vesicles are R-Cdh and Cdh6, as published previously, mostly for the mouse [Goto, S., E. et al.: J. Am. Soc. Nephrol. 9:1234 (1998), Cho, E.A. et al.: Development 125: 803 (1998)]. At later stages, differentiating podocytes express R-Cdh. In contrast to mouse, we found Cdh6 mRNA signal also in glomerular parietal epithelial cells. Moreover, mesangial cells express N-Cdh. Pcdh1 mRNA signal was observed in glomerular endothelial cells and epithelial cells of the proximal tubule. Pcdh19 and, even more strongly, Pcdh10 are expressed in muture epithelial cells of the distal and collecting tubules; immature tubule segments were also stained for some of these cadherins. Cell type-specific expression patterns were observed also for the other cadherins.
In conclusion, the expression of several (proto-)cadherins is highly regulated in a temporally restricted and cell type-specific manner in the developing and mature kidney of chicken. This result suggests a role for multiple (proto-)cadherins in kidney histogenesis and mature function.

Kategorie: Poster

Poster 64

Rubrik: 5.Entwicklungsbiologie
Abstract Nr.:5

Titel: Localization and expression of vegf and vegf receptor in the rat tooth germ

Autoren: Schwab W.(1),Bramke S.(1),Pehlke K.(1),Kasper M.(1),

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Abstract:
The VEGF VEGF receptor systems a key regulator in angiogenesis. Its biological significance in the dental development is not fully understood. Therefore, the aim of the present study was to monitor the occurence and distribution pattern of VEGF und VEGF receptor in cells of the tooth germs by means of immunocytochemistry.
In slices of newborn rat tooth germs, VEGF-C1 and VEGF receptor 2 (Flk-1) immunoreactivities were detected in cells of the enamel organ and in cells of the dental papilla. In ameloblasts, immunoreactivity for both antigens occurred at the cell membranes, especially at their distal ends and in the contact region to stratum intermedium cells. In addition, differentiating odontoblasts exhibited immunostaining for VEGF and VEGF receptor.
Furthermore, results of double immunofluorescence studies with antibodies against VEGF and podocalyxin, a marker of endothelial cells, showed that blood capillaries come in close proximity to the inner enamel epithelium and to the odontoblast layer, indicating that VEGF in cells of the enamel organ and in odontoblasts regulates the vascularization of the tooth germ. On the other hand the colocalization of both antigens point out that VEGF takes part in the ameloblast and/or odontoblast differentiaton in an autocrine/paracrine manner.

Kategorie: Poster

Poster 65

Rubrik: 5.Entwicklungsbiologie
Abstract Nr.:5

Titel: Insulin induced gastrulation and delayed mesoderm differentiation in rabbit preimplantation blastocysts from diabetic mothers

Autoren: Thieme R.(1),Schindler M.(1),Fischer S.(1),Fischer B.(1),Navarrete Santos A.(1),

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Abstract:
The preimplantation period is a very vulnerable period in embryo development. Metabolic disorders such as diabetes mellitus or obesity negatively influence embryo development. We used an experimentally induced type 1 diabetes model to closer investigate embryo development and gastrulation under diabetic conditions.
Blastocysts grown in diabetic mothers or cultured in vitro without insulin/IGF1 were analysed for mesoderm formation by morphological characterisation of the embryonic disc and gene expression pattern of the mesodermal specific marker Brachyury. Blastocysts from diabetic mothers were developmentally delayed and blastocysts cultured without insulin or IGF1 did not gastrulate, were arrested in early gastrulation (stage 1) or died. The mesoderm inducing molecules Wnt3a and Wnt4 were investigated with decreased Wnt3a levels in blastocysts from diabetic mothers. Wnt4 was not affected. Supplementation of insulin in vitro showed a positive effect on Wnt3a and Wnt4 expression levels. The effect of insulin on Wnt3a was diminished by specific inhibition of MAPK-signalling with PD098059.
We conclude that insulin facilitates Wnt3a, Wnt4 and Brachyury expression. The temporally and stage-dependent induction of Brachyury expression by insulin is mediated via regulation of the Wnts.
Taking together a close relationship exists between insulin/IGF1 and the mesoderm formation network in the rabbit blastocyst. Diabetogenous dysregulations resulting in congenital heart defects, often seen in infants from diabetic mothers, may be explained disclosed by detailed analysis of gastrulating blastocysts.
Supported by DFG NA 418/4-2 and the Wilhelm Roux Programme of the MLU, Faculty of Medicine

Kategorie: Poster

Poster 66

Rubrik: 5.Entwicklungsbiologie
Abstract Nr.:5

Titel: The development of human laryngian muscular tissue in the first life stages – micromorphometric study

Autoren: Epure V.(1),Prundeanu H.(1),Sargan I.(1),Pop E.(1),Bacean A.(1),Folescu R.(1),

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Abstract:
This study evaluates histotological modifications of human's primary phonatory organ – the larynx.
The morphology of the larynx in the first life stages, especially in the stage of fetal development, is less dealt with by the specialty works. Besides specification of the appearance the facts concerning growth rhythm and estimation of cantitative biology are quite sketchy.
This study pursues the morphometric aspects of volume and density of muscular tissue on a number of 41 pieces from human subjects. These pieces were cropped post mortem from fetuses, newborns, children and teenagers, all male subjects, for a better catching of modifications armatured by neurohormonal sexual maturing.
Muscular tissue fragments were fixed in BOUIN liquid or in phormol for histological proccesing.
Tissular micromorphometry like size and volume of muscular fibres, their density on 1 mm2 were accomplished on microscopes wirh eyepieces and object-glasses calibrated with the help of WEIBEL AND MÂRZA – GABOREANU grids.
The statistics accomplished with mathematical skills assisted on the computer, reveal a slow linear progress until puberty, when there appears a qualitative saltation of diametral growth and fibres density.
KEY WORDS: HUMAN LARYNX, MUSCULAR TISSUE, EVOLUTION, MICROMORPHOMETRY

Kategorie: Poster

Poster 67

Rubrik: 6.Reproduktionsbiologie
Abstract Nr.:6

Titel: Hormonally-regulated cftr expression in the murine male reproductive tract

Autoren: Wandernoth P.(1),Mannowetz N.(1),Hornung J.(1),Ruffing U.(1),Raubuch M.(1),Wennemuth G.(1),

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Abstract:
CFTR (cystic fibrosis transmembrane conductance regulator) acts as an HCO3-conducting channel in the reproductive tract. Mutations in the CFTR gene lead to infertility in both sexes. It has been shown that, in females, CFTR RNA expression underlies hormonal regulation. In this work we focused on both expression pattern and protein distribution in prepubertal, pubescent and adult male mice. Additionally, we examined the involvement of CFTR in mature sperm motility.
CFTR RNA expression levels were significantly increased in the reproductive tract of pubescent animals, in comparison to prepubertal and adult mice. However, CFTR protein was localized in epithelial cells of caput, corpus and cauda epididymidis without any significant difference in development stage. A positive reaction was also detected in spermatocytes, spermatids and in the whole sperm tail, isolated from different sections of the epididymis. To assess the importance of CFTR's role in HCO3- entry into sperm with regard to increased motility, we performed single sperm analysis. CFTR inhibitors did not prevent a rise of beat frequency from 3.02 ± 0.05 Hz to 8.18 ± 0.22 Hz during perfusion with buffer containing 15 mM HCO3-. The capacitation-associated protein tyrosine phosphorylation was also not influenced by the presence of CFTR inhibitors.
The results demonstrate that CFTR does not play a major role in HCO3- entry into sperm, early activation of sperm motility or capacitation. The increased expression levels in the male reproductive tract of pubescent mice rather indicate the importance of CFTR in development and maturation of spermatozoa.

Kategorie: Poster

Poster 68

Rubrik: 6.Reproduktionsbiologie
Abstract Nr.:6

Titel: Species specific distribution of pmca4 in the epididymis of bull, rat, marmoset and human

Autoren: Brandenburger T.(1),Post. H.(1),Schwarz A.(1),Aumüller G.(1),Schlatt S.(2),Wilhelm B.(1),

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Abstract:
Ca2+ and Ca2+ -dependent signals are essential for sperm maturation and fertilization. In mouse sperm the plasma membrane Ca2+-ATPase (PMCA) isoform 4 plays a crucial role for male fertility. PMCA4 knockout mice are infertile as their sperm cannot achieve a hyperactivated state of motility. The two major splice variants of PMCA4 are PMCA4a and 4b. PMCA4a shows a much higher basal activity, and its faster activation rate makes it more effective than PMCA4b in returning Ca2+ to resting levels. In bovine epididymal sperm we detected a much higher level of PMCA4a in sperm taken from the cauda compared to those from testis and from caput epididymidis. Spermatozoa acquire their fertilization capacity during their transit through the epididymis. Immunohistochemical staining located the PMCA4 (4b) to the basolateral membrane and the PMCA4a to the apical membrane of the epithelium of the bovine cauda epididymidis, while bovine caput and corpus epididymidis were negative. These findings suggest that PMCA4a is transferred from the epithelial cells onto sperm membranes in cauda epididymidis. Because of the relevance of the epididymis for sperm maturation and the importance of PMCA4 for sperm function, we compared the epididymal PMCA4 protein localization in bull, rat, human and marmoset. Interestingly, a different distribution of PMCA4 was elucidated in the epididymides of the four species, pointing to a species specific involvement of the epididymal parts in sperm maturation.

Kategorie: Poster

Poster 69

Rubrik: 6.Reproduktionsbiologie
Abstract Nr.:6

Titel: Cholinergic receptors in the murine oviductal epithelium

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Abstract:
Acetylcholine (ACh) has been reported in the oviductal epithelium (Steffl et al., 2006), but its function is unknown. Here, we analyzed expression of cholinergic muscarinic (MR) and nicotinic receptors (NR) by RT-PCR in pregnant and cycling mice in whole oviducts and by laser-assisted microdissection in epithelium and smooth muscle, and performed measurements of intracellular calcium concentration in isolated cells of the murine oviduct in response to cholinergic agonists.
In RT-PCR, expression of MR subtypes M1R and M3R was predominant. Their mRNAs were found in the epithelium, but not in the smooth muscle layer, by laser-assisted microdissection. Expression of M1R-M5R receptors was similar in cycling and pregnant animals. NR expression was more variable, most frequently detected were subunits alpha2, 4, 5, 7 and beta4. Expression of the alpha7 subunit was significantly reduced in pregnant animals. In microdissected epithelial samples mRNA coding for subunit alpha7, but not for alpha3 was found. Still, single ciliated epithelial cells are alpha3-subunit positive, judged from DAB-based immunohistochemistry and transmission electronmicroscopy of samples from mice expressing eGFP under the alpha3-subunit promotor. Calcium imaging experiments showed reaction of fractions of ciliated and non-ciliated cells on muscarin, ACh and ATP, but not on nicotine. Reactions to muscarin and ACh could be completely blocked by atropine, but not by mecamylamin. These data demonstrate expression of multiple cholinergic receptors in the oviductal epithelium with MR - presumably M3R and/or M1R – triggering rise in intracellular calcium concentration.

Kategorie: Poster

Poster 70

Rubrik: 6.Reproduktionsbiologie
Abstract Nr.:6

Titel: Testicular phenotype of reelin-deficient reeler mice

Autoren: Morcinek K.(1),Zhou L.(2),Rune G.(2),Haider S.(1),

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Abstract:
Previous studies have shown that Reelin, an extracellular matrix protein provides an inhibitory signal in the migration of gonadotropin–releasing hormone (GnRH) neurons, which results in reduced numbers of hypothalamic GnRH neurons in Reeler mice. To address the question whether reduced fertility of Reeler mice is due to this migratory deficit we studied testicular morphology of adult Reeler mice. Seven homozygous and six heterozygous mice were compared with six wild type B6C3 animals. Using morphometrical and immunohistochemical methods we found no significant differences in diameter of the testes, seminiferous tubules and Leydig cells between the groups. All stages and cell types of spermatogenesis and most importantly, the frequency of stages, indicative of potential arrest in spermatogenesis were similar to the situation in wild type mice. A statistical significant increase of small and large vacuoles within the germinal epithelium, however, was observed in homozygous Reeler mice, compared with heterozygous mutants and the wild type mice. Additionally, the testes of homozygous Reeler mice showed an increased number of androgen receptor positive Sertoli cells. To conclude, in spite of a largely intact process of spermatogenesis in Reeler mice, the vacuoles within the germinal epithelium suggest a loss of cell contacts of Sertoli cells, thus a potential break down of the blood-testis barrier, leading to the dislocation of germ cells towards the lumen of seminiferous tubules. Whether the increase of Sertoli cell number in seminiferous tubules of homozygous Reeler mice represents a compensatory mechanism has to be addressed in further experiments.

Kategorie: Poster

Poster 71

Rubrik: 6.Reproduktionsbiologie
Abstract Nr.:6

Titel: Assembly of avian perivitelline membrane during folliculogenesis – a model system for the study of of the formation of an extracellular matrix

Autoren: Rodler D.(1),Sasanami T.(2),Sinowatz F.(1),

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Abstract:
In birds the perivitelline membrane (PVM), an extracellular matrix covering the growing follicle is regarded to be homologous to the zona pellucida (ZP) in mammals and plays an important role in fertilization. It is placed between granulosa cells and oocyte and consists of a filamentous network of highly conserved structurally related glycoproteins. Whereas three glycoproteins build the zona pellucida of most mammals, five glycoproteins have been identified so far in the perivitelline membrane of chicken and quail (ZP1, ZP2, ZP3, ZP4, ZPD).
In our study, Japanese quail (Coturnix coturnix japonica) was used to get insight into the ZP glycoprotein composition of PVM and its assembly during follicular growth. We analyzed the expression of PVM glycoproteins in the ovary and liver using non-radioactive in situ hybridisation. The accumulation of the PVM glycoproteins on the oocyte surface was studied using anti-quail-ZP-antibodies and transmission electron microscopy. The data obtained clearly shows a characteristic temporal and spatial pattern of quail PVM glycoprotein expression in the zona granulosa, oocyte and liver. The glycoproteins are incorporated into the PVM at different times, suggesting that the early ones (ZP2, ZP3, ZP4) form a kind of prematrix for the late ones (ZP1, ZPD).

Kategorie: Poster

Poster 72

Rubrik: 6.Reproduktionsbiologie
Abstract Nr.:6

Titel: Localization of vegf-receptor 3 in bovine corpus luteum during oestrous cycle, induced luteolysis and pregnancy

Autoren: Kenngott R.(1),Schilffarth S.(2),Schams D.(2),Meyer H.(2),Berisha B.(2),Sinowatz F.(1),

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Abstract:
Physiological lymphangiogenesis in the ovary is still only poorly understood. The aim of this study was the localization of VEGF-R3 in bovine corpora lutea (CL) during different physiological stages. Experiment 1: CL were assigned to following stages: Days 1-2, 3-4, 5-7, 8-12, 13-16, 18 and older (after regression) of oestrous cycle and of pregnancy (months 3-5, 6-7 and older than 8 month). Experiment 2: Induced luteolysis. Cows on days 8-12 were injected with a PGF2alpha; analogue and CL were collected by transvaginal ovariectomy before and 0.5, 2, 4, 12, 24, 48 and 64 h after PGF2alpha injection. Tissue levels of VEGF-R3 mRNA were characterized by qPCR. VEGF-R3 protein measured by ELISA was not detectable in early cyclic CL but increased to higher plateau levels during pregnancy. After induced luteolysis VEGF-R3 protein showed an increase within 2 – 24 h after the injection. VEGF-R3 localization by immunohistochemistry showed immunostaining in the cytoplasm of luteal cells, which was relatively weak in early CL. It increased in late CL, did not change during luteolysis, but showed a further increase during pregnancy, where a positive staining in nucleus and cytoplasm of lymphatic endothelial cells was observed. In conclusion, we assume that local produced lymphangiogenic factors and their receptors may be involved in mechanisms regulating CL function - especially during pregnancy

Kategorie: Poster

Poster 73

Rubrik: 6.Reproduktionsbiologie
Abstract Nr.:6

Titel: Angiogenesis and its role in development of fetal rat Leydig cells

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Abstract:
Two major sources of progenitors of fetal Leydig cells (FLCs) are currently discussed in the literature: mesenchymal fibroblasts migrating from mesonephros, and blood capillaries originating from coelomic epithelium. The aim of the present report is to collect data on the process of angiogenesis and neoangiogenesis in fetal testis, particularly in the peripheral region adjacent to coelomic epithelium. We performed immunohistochemical analyses on tissue sections from rat testis from 14th to 19th fetal day. PAS reaction and immunostaining for Endothelin-1 (ET-1) and Vascular Endothelial Growth Factor Receptor 2, VEGFR 2 (Flk 1) were applied in 5 µm thick serial paraffin sections. In the case of ET-1 the immune reaction in FLCs progenitors within the interstitial was stronger in the later embryonic ages. Prospermatogonia and immature Sertoli cells showed distinct reaction almost equally strong in all ages. The immune reaction in the endothelial cells of small blood capillaries in the testis was also strong. In the case of VEGFR-2 the strongest reaction was found in the cells of interstitial tissue of all embryonic ages. The coelomic epithelium contains moderate immune reaction of ET-1 and VEGFR-2 in the cytoplasm. Also preliminary data on expression pattern of following markers will be shown in the poster: Tie-2, Angiopoietin-1, Angiopoietin-2, VEGF, VEGFR-1, alfa-SMA, TAGLN, NG2, CD31 and CD 73. The observations on the immune reactions of the above markers will help clarify the topical pattern of development of new blood capillaries in the fetal testis, particularly the spiral-like arrangement of the interstitial tissue surrounding the seminiferous cords.

Kategorie: Poster

Poster 74

Rubrik: 6.Reproduktionsbiologie
Abstract Nr.:6

Titel: Impact of DEHP exposure on embryonic cell development and metabolism

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Abstract:
The plasticizer and endocrine disruptor diethylhexylphthalate (DEHP) impairs gonadal development and fertility in the male. Effects on female reproductive health and the developing embryo have not been studied in detail so far. Besides being a risk factor for reproductive health, probably in both sexes, DEHP and its metabolite MEHP, play a role in metabolism, mainly by activation of PPARy, a key mediator of adipogenesis. To investigate the effects of DEHP on early embryonic cells during different developmental stages, we exposed P19 murine ECCs to different concentrations of DEHP (5, 50, 100 µg/ml) during cardiomyogenesis in vitro. The P19 cells were exposed at the undifferentiated stage for four days and subsequently differentiated to beating cardiomyocytes. At different developmental stages the expression of key genes in fatty acid and glucose metabolism (PPARy, FABP4, GLUT4) and developmental and functional markers (alpha-MHC, Cx43) were analyzed by qRT-PCR. Myocardial functionality was investigated using the Multielectrode Array (MEA), measuring the contraction rate of beating embryoid bodies. We found that DEHP interferes with cardiomyocyte differentiation and beating behavior, and disrupts glucose and fatty acid metabolism during cardiomyogenesis. DEHP exposed cardiomyocytes had a 30-50 % higher beating rate compared to vehicle controls. The investigated metabolic markers were significantly altered in later stages of cardiac differentiation. We are currently studying hearts collected from mice exposed to DEHP via dietary and in utero routes. Our experiments will identify stage specific effects of DEHP during ontogenesis, organ differentiation and function, and on key metabolic pathways.

Supported by EU (FP7; REEF #212885)

Kategorie: Poster

Poster 75

Rubrik: 6.Reproduktionsbiologie
Abstract Nr.:6

Titel:Cftr-mediated early activation of sperm by hco3- is regulated hormonally in the murine uterus

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Abstract:
The female genital tract fluid is rich of HCO3- due to the activity of the HCO3--permeant anion channel CFTR. For sperm, HCO3- is an essential factor for both early and late activation. Past work indicates CFTR transcript expression to be regulated hormonally in the female reproductive tract. Investigating pre-pubertal, pubertal and superovulated adult animals, we ask whether CFTR controls the uterine HCO3- content as well as sperm responses to it. CFTR protein and mRNA were absent in uteri of pre-pubertal mice, but clearly detectable in pubertal and adult tissue. These results indicate that CFTR is up-regulated during puberty. The HCO3- content in the uterine fluid of estrus females was elevated 2-, and nearly 4-fold compared with that of diestrus and pre-pubertal animals, correlating with changes in sperm beat frequency. Sperm which were incubated in and recovered from pre-pubertal uteri, showed a flagellar beat frequency that was no different than before incubation. However, sperm recovered from diestrus and estrus uteri, exhibited a beat frequency that was 2- and 4-fold higher. We therefore propose that the uterine HCO3- content has physiological consequences for sperm motility. Although the sperm tail showed CFTR immunoreactivity, two CFTR blockers (GlyH-101 and CFTRinh-172) did neither diminish HCO3- -evoked increases in sperm motility nor capacitation-associated protein tyrosine phosphorylation. We show that in the uterus, both CFTR expression and the supply of HCO3- are upregulated hormonally and suggest that these changes coordinate ovulation and early activation of sperm.

Kategorie: Poster

Poster 76

Rubrik: 7.Immunbiologie
Abstract Nr.:7

Titel: Exercise induced decline in lyve-1 positive lymphatic vessels

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Abstract:
Introduction:The investigation of lymphatic function and biology and its microvascular influence of tissue integrity, failure and tumour metastasis represents a major task of vascular science. To date several investigations investigate exercise induced altererations of lymphatic density in mice or under medical considerations. However, no study investigated exercise induced plasticity of lymphatic vessel density as response to endurance exercise in human skeletal muscle. Purpose: The main purpose was to investigate if alterations of lymphatic density can be observed as a result of extensive and prolonged endurance exercise in human skeletal muscle. Methods: Muscle biopsies were taken from vastus lateralis muscle of male cyclists (n=19) to investigate the distribution of lymphatic capillaries under basal conditions and every 9 days over the time course of a 45 days cycling training intervention (n=12). Lymphatic endothelium was stained by immunohistochemistry using LYVE-1 antibodies. Results: Initially we found an average density of 11,9 ± 4,9 lymphatic vessels per 100 myofibres. The major finding of our study revealed the density of LYVE-1 positive lymphatic capillaries to decrease significantly (p<0,01) over the time course of our exercise intervention. LYVE-1 positive lymphatic vessel density decreased from 11,3 ± 5,4 “Prae Study” to 5,4 ± 3,6 lymphatic vessels per 100 myofibres “Post” study. Conclusion: Our finding of significant decreases of LYVE-1/+ lymphatic vessels in response to exercise gives rise to the assumption, that exercise induced stimuli are able to induce alterations of lymphangiogenetic responses on a structural level.

Kategorie: Poster

Poster 77

Rubrik: 7.Immunbiologie
Abstract Nr.:7

Titel: A new model for intravital imaging of airway inflammation in mice using 2-photon microscopy

Autoren: Pieper M.(1),Kretschmer S.(1),Martensen B.(2),Hüttmann G.(2),König P.(1),

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Abstract:
Following the dynamics of inflammatory cells will increase our understanding of airway inflammation. Although many aspects of cell dynamics can be examined in ex vivo models, certain aspects such as influx of cells can only be examined in living animals indicating a need for an in vivo model.
Mice were anaesthetized and breathed spontaneously or were artificially ventilated via a newly developed nose mask. Body temperature and oxygen saturation were monitored throughout the experiment. The trachea was surgically exposed and imaging was performed using a 2-photon microscope with and without ventilation triggered imaging. Endogenous fluorescence and second harmonic generation signals were used for imaging. Blood flow was detected by FITC-labeled dextrane.
Artificial ventilation via nose mask was essential to maintain sufficient oxygen saturation without damaging the trachea. Synchronizing ventilation and image acquisition greatly reduced motion artifacts. Without exogenous fluorophores immune cells, airway epithelium, vessels, collagen fibers, and elastic fibers between the tracheal rings were visualized giving detailed morphology of the airway. Sufficient tissue perfusion was maintained as judged by visualization of fast moving erythrocytes in blood vessels. Increased numbers of inflammatory cells with the morphology of granulocytes were only detected in the area that was injured by surgery. In the tracheal connective tissue and close to the epithelium, relatively few moving immune cells were observed indicating that no increased inflammation was induced in these areas by the surgical process.
Combining this technique with models of airway disease will increase our understanding of the dynamics of airway inflammation.

Kategorie: Poster

Poster 78

Rubrik: 7.Immunbiologie
Abstract Nr.:7

Titel: A simple method to analyze mrna and lipid content of murine tracheal epithelium

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Abstract:
To better understand immunological signaling in the airway epithelium in situ and in vivo after contact with allergens and bacteria, a simple method to analyze the expression of RNA and the lipid composition of epithelial cells of the mouse trachea was developed. Airway epithelial cells were isolated from the excised mouse trachea by gently rubbing a sterile swab over the epithelial surface of the trachea. After denudation of the epithelium, tracheae were fixed and examined using scanning and transmission electron microscopy. Total RNA was isolated and real time RT-PCR was performed. For determination of the lipid content, lipids were isolated from the swab and analyzed by micro-HPLC ESI FT-ICR mass spectrometry. To assess changes induced by lipopolysaccharide (LPS), the explanted mouse trachea was kept in Hepes-Ringer solution at 37°C and was stimulated with LPS for 2 h.
Electron microscopy revealed that brushing removed epithelial but left the subepithelial tissue unperturbed. About 500 ng of tolal RNA could be extracted from the epithelium of a single trachea. Transcripts for CC10 (expressed in secretory cells), FOXJ1 (expressed in ciliated cells) and CK5 (expressed in basal cells) could readily be detected by RT-PCR. Stimulation with LPS significantly increased the expression of IL6 mRNA in the epithelium. Mass spectrometry allowed to identify and quantify more than 65 different species of lipids including sphingolipids which are important for cell signaling.
This simple technique will allow to examine signal transduction pathways in the airway epithelium after contact with bacteria and allergens ex vivo and in vivo.

Kategorie: Poster

Poster 79

Rubrik: 7.Immunbiologie
Abstract Nr.:7

Titel: Imaging cellular dynamics and tissue morphology in acute allergic airway inflammation by two-photon laser scanning microscopy

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Abstract:
A major characteristic of allergic asthma is the recruitment of granulocytes to the airways. However, their role in airway inflammation is not well understood. To increase our understanding of the dynamics and interactions of granulocytes during airway inflammation, we performed real-time ex vivo imaging of tracheae from mice that were sensitized and challenged to ovalbumin using an adjuvant-free experimental asthma model protocol. Antigen processing cells were labeled by a self-quenched conjugate of ovalbumin added to the bath solution. Imaging was performed using a two-photon laserscanning microscope.
Using autofluorescence alone, we identified large numbers of granulocytes by their lobed non fluorescent nuclei. Granulocytes were often found in cell clusters and were located in the connective tissue and beneath the airway epithelium. Despite close proximity to the epithelial cells no direct interaction or damage of the epithelium was observed. Granulocytes were observed to be stationary and also moving with different velocities. Immunohistochemical staining against Gr-1 and CCR3 after the imaging experiments showed that in addition to eosinophils a substantial number of neutrophils were present. Antigen processing cells with the morphology of dendritic cells were more abundant throughout the tracheal wall of asthmatic mice compared to non-asthmatic animals. Granulocytes and antigen presenting cells were found frequently in close proximity but without indication of prolonged direct contact.
These results indicate that in asthma released granulocyte mediators could act on antigen processing cells and the airway epithelium thereby influencing the course of the subsequent adaptive immune reaction.

Kategorie: Poster

Poster 80

Rubrik: 7.Immunbiologie
Abstract Nr.:7

Titel: Ocular Surfactant Proteins ans their Regulation in dry Eye Disease

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Abstract:
Purpose. Surfactant proteins (SPs) are originally known from lung tissue and have been detected in the meantime in a bulk of different extrapulmonary human tissues. SPs have essential immological and surface-active functions in the lung. SP-A and SP-D play a role in the innate immune system especially against bacteria, virus ans fungiform pathogens and enabling chemotaxis, opsonisation and phagozytosis. In contrast, SP-B and SP-C are able to lower surface tension of interphases and thus support the rheology and disassembly of mucous fluids. Aim of the present study was to investigate the expression pattern of SPs within tear fluid of patients suffering from dry eye disease in comparison to healthy volunteers.
Methods. Tears were collected from altogether 447 persons by Schirmer's strip method. Of these 307 persons suffered from different forms of dry eye disease, whereas 140 volunteers were free of dry eye related symptoms as well as other ophthalmological diseases or traumata. The obtained tear fluid was extracted and quantitatively analyzed for SP-A, -B, -C and -D by means of ELISA.
Results. In cases of dry eye disease the protein concentration of all four surfactant proteins was significantly increased ($p>0,05$) compared to samples from healthy volunteers.
Conclusion. The results suggest possible immunmodulatory effects of SP-A and SP-D at the ocular surface. Furthermore, SP-B and SP-C seem to reduce the surface tension of the tear film. In this context we suppose that surfactant proteins might be considered as players of the innate immune system at the ocular surface and thus could be of special interest for therapeutical approaches for the treatment of dry eye.

Kategorie: Poster

Poster 81

Rubrik: 7.Immunbiologie
Abstract Nr.:7

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

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Abstract:
Antimicrobial peptides are part of the innate immune system in epithelial and non-epithelial surfaces, and may also have important functions in the brain.
However, little is known about the expression of antimicrobial peptides in the CNS and whether glial cells can secrete these peptides. We have performed real-time RT-PCR, ELISA, immunohistochemistry, and Western Blot with samples from cell cultures and an animal model to get more information about the role of antimicrobial peptides in the CNS.
In detail, we have shown the expression of the antimicrobial peptide Psoriasin, which was first identified as an over-expressed peptide in psoriatic skin, in the central nervous system. Additionally, we investigated the occurrence of Psoriasin in an animal model of bacterial meningitis. The next step is to investigate the signalling transduction, which is involved on the expression of Psoriasin in the CNS. We suggest that a G protein-coupled receptor or extracellular-signal-regulated kinases and cytokines mediate the expression of Psoriasin in the CNS. We analyse (i) the role of extracellular-signal-regulated kinases in the expression of Psoriasin and (ii) we investigate the expression of Psoriasin using knock out mice like TNFR1 (tumor necrose factor receptor 1) and IL-6 (interleukin 6) and TNFR1/IL-6. Our results suggest that Psoriasin is an important part of the innate immunity in the brain against bacterial CNS pathogens.
Keywords: antimicrobial peptides, Psoriasin

Kategorie: Poster

Poster 82

Rubrik: 7.Immunbiologie
Abstract Nr.:7

Titel:Hypoxia induces substantial lymphangiogenesis in the mouse lung

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Abstract:
Inflammation can induce lymphangiogenesis. However, the role of accompanying hypoxia in this process it is still unclear. To investigate the effects of hypoxia on lymphangiogenesis in the lung, mice were kept under normoxia (controls) or normobaric hypoxia (10% O2) up to 21 days and changes in lymph vessels and blood vessels were examined using antibodies against CD90 and alpha-smooth muscle actin (α-SMA) on 200 µm thick precision cut lung slices (PCLS). Quantitative analysis of lymph vessel numbers was done on systematically sampled PCLS of the left lung by design-based stereology using an optical fractionator.
After 21 days of hypoxia, the number of lymph vessels in the left lung increased from 6823±698 to 27519±3289 (mean±standard deviation). After 7 days of hypoxia, a strong increase in the number of lymph vessel sprouts primarily around pulmonary veins was detected. After 14 days, branches elongated and further lymph vessels developed. After 21 days of hypoxia, networks of branched lymph vessels around veins and many branched capillaries were found in the alveolar region. As previously described, hypoxia also induced muscularisation of arteries and veins in vessels that were not muscularized in control animals. Furthermore, during hypoxia strong alpha-SMA immunoreactive cells appeared in the wall of alveolar ducts as well as in alveoli after 7 days of hypoxia.
These data indicate that in addition to changes in blood vessels, hypoxia also induces substantial lymphangiogenesis in the lung and leads to changes in the alveolar region. Whether hypoxia directly triggers these changes remains to be determined.

Kategorie: Poster

Poster 83

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

Titel: Spinal cord – motor cortex coculture model: a new technique to study neuronal regeneration in vitro

Autoren: Pohland M.(1),Glumm J.(1),

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Abstract:
After mechanical traumas cortical axons have a substantial potential for axonal growth and regeneration. Today several hippocampal, as well as spinal, in vitro lesion models are used to investigate neuronal differentiation, axonal growth and path finding (Bonnici et al., 2008). Here we describe a new cytoarchitecture-preserving slice coculture technique to analyse neuronal regeneration and axonal outgrowth between the motor cortex and the spinal cord.
Spinal cord (sc) from postnatal (P0-P3) C57Bl/6 mice and motor cortex (mc) dissected from Bl6.GFP pups (P0-P3) expressing green fluorescent protein (GFP) under beta-actin promoter control were chopped either in a sagittal longitudinal plane for the sc or in a coronal plane for the mc. Afterwards the medial cortex zone was orientated to the rostral end of the spinal cord and incubated up to two weeks.
Using nonfluorescent pups as medulla donors and constantly GFP-expressing heterocygote mice as cortex givers, we can easily distinguish ingrowing cortical neurons in non-fluorescent wild type tissue. Our data shows ingrowing fibers and growth cones which are already detectable after 1 day in vitro (DIV). Moreover, the rate of growth was measured using confocal microscopy. In addition, immunhistochemical staining after 1, 3 and 6 DIV suggest a strong neuronal outgrowth and not only a reestablishment of cortical fibers but also their connections by means of microscopical analysis.
Thus, this in vitro method offers possibilities to test axon-regenerative properties of determined compounds or treatments and could provide an important tool to answer a variety of questions in the field of neuronal regeneration.

Kategorie: Poster

Poster 84

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

Titel: Deferoxamine induced neurite outgrowth and synapse formation in postnatal rat dorsal root ganglion (drg) cell cultures

Autoren: Nowicki M.(1),Kosacka J.(1),Spanel-Borowski K.(1),Borlak J.(2),

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Abstract:
Deferoxamine (DFO) was granted orphan drug status for the treatment of traumatic spinal cord injury but its neuroprotective mechanism is not well understood. We therefore investigated DFO's mode of action in serum starved and/or iron stressed cultures of rat dorsal root ganglion cells. We probed for redox signalling by determining hemeoxygenase-1 activity and by measuring expression of intracellular iron-metabolism-related proteins under pro-oxidative conditions. We also employed DNA microarrays to better understand the genomic response of DRG cultures to treatment with DFO thereby enabling hypothesis generation. Essentially, DFO treatment resulted in outgrowth of neurofilament 200–positive neurites and induction of synapse formation as determined by Western immunoblotting, transmission electron microscopy and immunofluorescence confocal microscopy. Furthermore, DFO treatment of DRG cell cultures activated neuroprotective and antioxidative programmes such as matrix metallopeptidase 2 and apolipoprotein D to promote neurite regeneration. Indeed, DFO reduced markedly reactive oxygen species formation, increased the expression of hemeoxygenase-1 and improved iron management through regulation of transferrin-receptor and ferritin. We propose DFO treatment of DRG cell cultures to completely abolish the oxidative effect of ferrous iron (Fe2+). Taken collectively, DFO reduced oxidative stress and induced synthesis of neuroprotective and antioxidative molecules to foster nerve repair and functional recovery. Our findings help to better understand the therapeutic benefit of DFO in the treatment of spinal cord injury.

Kategorie: Poster

Poster 85

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

Titel: A mouse strain with a targeted mutation in pancortin is more susceptible to light-induced photoreceptor damage

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Abstract:
Purpose: To characterize the retinal phenotype of mice with a targeted mutation in Pancortin (strain shows a protection from ischemic neuronal cell death; Cheng et al., JNS 2007), and to design a vector for a conditional knock-out mouse model for Exon 1 of the Pancortin gene.
Methods: The retinal phenotype was analyzed by microscopy, real time RT-PCR and Western blotting. Death of retinal ganglion cells was analyzed after NMDA-mediated excitotoxic damage by counting axons in the optic nerve. In addition, thickness of the outer nuclear layer was analyzed throughout the retina after light-induced photoreceptor damage, an animal model of photoreceptor degeneration. Vector-design for the conditional knock-out was done with conventional cloning.
Results: By immunohistochemistry, Pancortin could be detected in the interphotoreceptor matrix (IPRM) between photoreceptor outer segments. In contrast to data of others (Cheng et al., 2007), we observed that Pancortin mutant mice are not modified by a null mutation, but rather express a mutated form of Pancortin which is secreted. Mutant mice are not protected from NMDA-induced excitotoxic cell death. Still, a significant reduction of neurons in the outer nuclear layer was observed in mutant mice after light-induced damage, when compared to damage of wildtype littermates. To generate mice with a real null mutation in Pancortin a vector that allows a conditional approach was cloned.
Conclusions: Pancortins play an essential role for homeostasis of the IPRM and for survival of photoreceptors after retinal damage. The conditional knock-out system will help us to shed more light into this function.

Supported by DFG Forschergruppe 1075

Kategorie: Poster

Poster 86

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

Titel: Neurodegenerative changes in the retina of the dba2/j mouse

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Abstract:
The DBA2/J mouse (D2J) is commonly used as a model of secondary angle-closure glaucoma accompanied by an age-dependent increase of IOP. Recent studies showed that neurodegenerative processes in those animals individually occur without a stringent correlation to elevated IOP. The aim of our studies is to describe and characterize the neurodegenerative changes and the neuronal function of the DBA2/J retina and to find additional factors which could be involved in the onset or the progression of neurodegenerative changes in the DBA2/J eye. Here we present scotopic flash-ERG and light-adapted flicker-ERG measurements in both strains at different ages. In-situ Hybridization and immunhistochemical staining on sagittal eye sections and/or retina whole-mount preparations in both strains and Aqueous humor (AH) analyses with a Mouse Cytokine Antibody-Array of 10 month old D2J eyes with and without optic nerve neuropathy were done. Our data give evidence that D2J shows lower flicker ERG responses than B6 mice already at younger ages. In both strains the ERG responses decrease as a function of age, but with a stronger decrease in the D2J mice. Immunhistochemical staining showed a partial loss of staining of the outer segments of the cone photoreceptors in the D2J retina at 10 months of age. The Antibody-Array Analysis revealed Osteopontin (OPN) as an age-dependently increased AH factor associated with degenerations of the optic nerve in D2J mice. Therefore, OPN is a promising candidate for future studies to analyze the actual participation of OPN in this model.

Kategorie: Poster

Poster 87

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

Titel: Dynactin-mutations in ALS pathogenesis

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Abstract:
Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease, which affects the upper and lower motor neuron. The pathogenesis of most of the ALS cases remain unknown, but there are a number of proposed mechanisms including oxidative damage, viral mechanisms, excitotoxicity, metabolic defects, mitochondrial dysfunction, protein aggregation as well as genetic factors. Several mutations in different genes such as SOD1 or most recently identified FUS and TDP43 have been accounted for causing or contributing to the development of ALS. Furthermore, a missense mutation (G59S) in the dynactin subunit p150 has been described to cause a slowly progressive adult onset form of lower motor neuron disease.
Based on this finding, a large screening effort of ALS patients and neurological and healthy controls for mutations in the p150 gene was performed and 23 exonic sequence changes leading to an amino acid exchange were detected. The aim of our work was to select two mutations for the generation of transgenic mice models. As a first selection process, we over expressed a recombinant p150 construct in primary motor neurons as well as Cos-7 cells. We then focused on four mutations, all showing a completely different morphological phenotype compared to the wild type p150, which shows a filamentous pattern associated with the microtubule cytoskeleton.
We also investigated varying protein degradation pathways and found out that the dynactin inclusions of the mutated protein were highly ubiquitinated.

Kategorie: Poster

Poster 88

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

Titel:Gabaergic septo-hippocampal neurons and reelin-positive gabaergic hippocampal interneurons are early targets for neurodegeneration in a model of amyloidosis and tauopathy

Autoren: Loreth D.(1),Poirier R.(2),Grueninger F.(2),Bohrmann B.(2),Frotscher M.(1),Metzger F.(2),Kretz O.(1),

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Abstract:
Alzheimer's disease is a neurodegenerative disorder characterized by brain accumulation of amyloid-β peptide, which is believed to initiate a pathologic cascade resulting in progressive impairment of brain plasticity and cognitive functions as well as in neuronal cell death. Here, we analysed a triple-transgenic mouse model of amyloidosis and tauopathy (3tg mice) overexpressing human mutations of amyloid precursor protein, presenilin 2 and tau. We indentified a significant neuronal degeneration affecting GABAergic septo-hippocampal projection neurons. In the axons of these neurons localized in the fimbria fornix, accumulation of hyperphosphorylated tau is detectable at 12 months of age. At 12 months of age, a 30% loss of parvalbumin-positive cells in the medial septum is observed by stereology. At later time points this cell loss is even more pronounced and accompanied by a significant reduction in fimbria-fornix diameter and a disappearance of phospho-tau staining in this region. Moreover, within the hippocampus of 3tg mice Reelin-positive GABAergic interneurons are found to be dramatically reduced in all hippocampal subfields, supported by evidence of reduced NPY, Y1 receptor, and pro-enkephalin RNA expression, whereas Y2R and GABA-A 2 receptors were upregulated. This apparent disequilibrium of inhibitory circuits resulted in strongly enhanced long-term potentiation in the medial-perforant path input to the dentate gyrus at 13 months of age compared with wild-type controls. Our data indicate that inhibitory neurons are early targets of neurodegeneration in a mouse model of amyloidosis and tauopathy and point to a possible role of disinhibition in the pathophysiological cascade of Alzheimer's disease.

Kategorie: Poster

Poster 89

Rubrik: 8
Abstract Nr.:

Titel:Prominin-1 expression highlights distinct cellular populations within the vertebrate retina

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Abstract:
Prominin-1 (CD133) is a pentaspan membrane glycoprotein. Mutations in the human gene have been linked with autosomal progressive retinal degeneration. Although, prominin-1 molecules are encoded by evolutionarily less evolved genomes, there is no detailed information available about their distribution in non-mammalian vertebrate species particularly those endowed with high regenerative abilities. In order to address retinal distribution of prominin-1 in a comparative way, cognates of this molecule were cloned from zebrafish (D.rerio), axolotl (A.mexicanum) and chick (G.gallus). Their expression was investigated in parallel with that of the murine transcript by non-radioactive in situ hybridization. Within the retina of the analyzed species prominin-1 transcripts were confined to two major cellular layers, i.e. outer nuclear layer (ONL), and surprisingly, to the inner nuclear layer (INL). Within the INL prominin-1-positive cells appeared to be aligned either along its vitreal (mouse) or scleral aspect (chick). In mice, nevertheless, INL-associated prominin-1-positive cells represented only a minute population. In zebrafish, where the prominin-1 gene was represented by a pair of co-othologues (prominin-1a and 1b) both distribution patterns were noted. In contrast, axolotl prominin-1 was exclusively confined to the ONL. While expression of prominin-1 in photoreceptors in all vertebrate classes investigated reveals a common phylotypic trait, i.e. an evolutionarily conserved molecular feature, its detected cellular association with non-photoreceptor type retinal cells within the INL appears to be a rather facultative class specific feature. Taken together, this is the first comprehensive analysis on the localization of prominin-1 in the vertebrate retina.

Kategorie: Poster

Poster 90

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

Titel:Neuronal damage by borna disease virus in rats depends on the host genetic background and is prevented by a soluble factor

Autoren: Wu Y.(1),Schwemmle M.(1),Heimrich B.(2),

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Abstract:
While neonatal Borna Disease Virus (BDV)-infected Lewis rats undergo a selective degeneration of dentate granule cells (DGC) and their axons in the hippocampi, BDV-infected newborn Sprague-Dawley (SD) rats have intact structures. In addition, these pathological differences can be also observed ex vivo in hippocampal slice cultures of these rats; however, viral replication efficiency and spread are comparable in these slice cultures. The different phenotypes indicate that BDV-induced neuronal loss is dependent on the host genetic background. To find out whether the hippocampal slices of SD rats can exert a neuroprotective effect on BDV induced degeneration, we performed co-culture experiments, in which hippocampi from different rat strains were either cultured adjacent to or placed 1cm away from each other. Infection of these co-cultures revealed the presence of a soluble protective factor(s) produced by SD hippocampi, which could prevent Lewis slices from BDV-induced degeneration. To further explore the properties of the soluble protective factor, we conducted conditioned medium experiments, in which infected Lewis slices were incubated with nutrient medium derived from SD slices. These results implied that uninfected SD cultures had the ability to prevent neuronal damage in BDV-infected Lewis cultures. A similar protective effect was observed using medium from uninfected Lewis cultures, suggesting that the viral infection may interfere with the production of this protective factor in Lewis rats. In summary, the severity of the virus-induced neuronal damage is dependent on the host genetic background and a soluble cellular factor(s) can prevent the BDV-mediated pathology.

Kategorie: Poster

Poster 91

Rubrik: 9.Peripheres und vegetatives Nervensystem
Abstract Nr.:9

Titel: Rectal prolapse in young male patients is associated with an enteric neuropathy

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Abstract:
Background & Aims:
Obstructed defecation syndrome (ODS) is characterized by chronic constipation with incomplete rectal evacuation primarily due to rectal wall prolapse (RP). RP normally affects elderly women and is generally attributed to a laxity of the rectal support. Here we present a series of young male patients with RP in order to evaluate whether RP in this unusual population is associated with an intestinal innervation disorder.
Material & Methods:
Full-thickness rectal specimens from male patients operated for RP (n=5) and from a male control group (n=15) were processed for immunohistochemistry (HuC/D, pan-neuronal marker). Both the myenteric plexus (MP) and submucosal plexus (SMP) were morphometrically assessed to determine ganglionic density and area, neuronal and glial cell density, neuronal and glial cell numbers per ganglion according to recently published guidelines.
Results:
Patients with RP showed a significantly increased ganglionic area of the MP and SMP and increased neuronal and glial cell numbers per ganglion in the SMP compared to controls. The frequency of submucosal “giant ganglia” (> 7 nerve cells/ganglion) was higher in patients with RP resembling typical histopathological features of intestinal neuronal dysplasia (IND).
Conclusions:
Quantitative analysis of the enteric nervous system reveals an enteric neuropathy in young male patients with RP characterized by enlarged myenteric and submucosal ganglia and elevated neuronal and glial cell content of submucosal ganglia compatible with the diagnosis of IND. The findings suggest that this type of intestinal innervation disorder may contribute to the development of RP in this unusual group of patients.

Kategorie: Poster

Poster 92

Rubrik: 9.Peripheres und vegetatives Nervensystem
Abstract Nr.:9

Titel: Localisation and function of muscarinic receptor m2 in murine airways

Autoren: Jositsch G.(1),Wiegand S.(1),Papadakis T.(1),Hartmann P.(1),Wess J.(2),Krasteva G.(1),Nassenstein C.(1),Kummer W.(1),

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Abstract:
Acetylcholine (ACh) is a major bronchoconstrictor and secretagogue in the airways acting via muscarinic receptors (MR). Highest expression is observed for the M2R subtype. It colocalizes with M3R on the bronchial smooth muscle (1), and is also postulated to be located prejunctionally on the cholinergic nerve terminals, acting as an autoregulatory receptor inhibiting ACh release (2).
Here, we investigated its distribution in murine airways by immunohistochemistry, supported by single-cell-picking and RT-PCR, and analyzed its function in tracheal organ bath experiments utilizing electrical field stimulation (EFS) and pharmacological stimulation, comparing M2R KO mice with corresponding wild-type mice.
Immunohistochemistry with the rat monoclonal M2R antibody 367 localized M2R to airway, but not vascular, smooth muscle cells throughout the airway tree, to cardiomyocytes surrounding intra- and extrapulmonary veins, and to nerve cell bodies of airway ganglia. All labelling was absent in tissue taken from M2R KO mice. RT-PCR revealed M2R-mRNA in picked neuronal cell bodies. In organ bath experiments, neurally (EFS) evoked constriction, standardized to the muscarin response, was reduced rather than enhanced in tracheas of M2R KO mice.
The present data show that, in contrast to a current concept in pathogenesis of asthmatic bronchial hyperreagibility, genetic deficiency of M2R does not result in overshoot of neurally evoked constriction, although neural M2R are clearly demonstrable in cholinergic airway neurons.

(1) Schlenz et al., Am J Physiol Lung Cell Mol Physiol. 2009, 298, 626-636
(2) Fryer and Coulsen, Pharmacol Ther. 2003, 98, 59-69.

Kategorie: Poster

Poster 93

Rubrik: 9.Peripheres und vegetatives Nervensystem
Abstract Nr.:9

Titel: Selective survival of some nitrergic neurons in the myenteric plexus of chagasic megacolon

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

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Abstract:
Chagas disease, triggered by Trypanosoma cruzi, is an infection which may turn acute or chronic. Megacolon is one of the most common manifestations of the chronic phase. Earlier studies demonstrated a marked decrease in neuron number (hypoganglionosis) of the dilated segment. It is not known whether neuron death affects different neuron types proportionally or rather spares specific subpopulations. We compared 4 megacolonic samples derived from Brazilian chagasic with control segments derived from two non-chagasic patient groups: each 6 colonic segments from Brazilian and German patients. The colonic samples from the infected patients were divided into 3 parts, oral to (A), within (B) and anal to the dilated segment (C). Wholemounts of the myenteric plexus were immunohistochemically stained for choline acetyltransferase (ChAT), neuronal nitric oxide synthase (nNOS) and human neuronal protein Hu C/D (HU). We counted the neurons in each 15 ganglia and tested statistically the differences in proportions of nitrergic and cholinergic neurons by chi-square-test. There was no significant difference between the two control groups. Hypoganglionosis in B- and C-segments was evident. Besides this, we found a significant relative increase of nitrergic neurons in all B and C-segments (68% to 86%) as compared to both control groups (54% vs. 57%). The difference of the A-segments vs. controls was not uniform. Since nitrergic neurons act as inhibitory muscle motor neurons, their selective survival could explain the marked dilatation (B-segment) whereas the non-dilated C-segment may already be influenced by excitatory, ascending neurons of more anally located, unaffected regions.

Kategorie: Poster

Poster 94

Rubrik: 9.Peripheres und vegetatives Nervensystem
Abstract Nr.:9

Titel:Gdnf mrna expression is altered in patients with diverticular disease – underlying mechanisms and functional implications

Autoren: Böttner M.(1),Zorenkov D.(2),Bruch H.(3),Roblick U.(3),Egberts J.(4),Schäfer K.(5),Wedel T.(2),

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Abstract:
Background and aims:
Glial cell line-derived neurotrophic factor (GDNF) is essential for developing enteric neurons. Patients with diverticular disease (DD) have been reported to exhibit hypoganglionosis suggesting neurotrophic factor deprivation. Thus we screened mRNA expression of the GDNF system and markers of synaptic plasticity in DD and studied the effects of GDNF on cultured myenteric neurons.
Material and methods:
Samples of tunica muscularis of the sigmoid colon from patients operated for DD (n=16) and controls (n=16) were assessed for mRNA expression levels of the GDNF system and markers of synaptic plasticity. Furthermore, the effects of GDNF treatment on survival and differentiation of cultured myenteric neurons and the mRNA expression of synaptophysin, a marker of synaptic plasticity, were monitored.
Results:
mRNA expression levels of GDNF, GFRalpha2 and synaptophysin were down-regulated in the tunica muscularis of patients with DD compared to controls. GDNF treatment of cultured myenteric neurons increased both the number of neurons and newly formed ganglionic aggregates as well as the growth of interganglionic nerve fibers and mRNA expression of synaptophysin.
Conclusions:
Our results suggest that the GDNF system is compromised in DD. In vitro studies demonstrate that this neurotrophic factor enhances survival, differentiation and synaptic plasticity of enteric neurons. Since patients with DD exhibit hypoganglionosis and decreased expression of markers of synaptic plasticity, we propose that the observed neuronal loss in DD might be due to a lack of neurotrophic support mediated by GDNF.

Kategorie: Poster

Poster 95

Rubrik: 9.Peripheres und vegetatives Nervensystem
Abstract Nr.:9

Titel:The autonomic innervation of the kidney and its role in hypertension

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Abstract:
The renal nerves feature the communication between central nervous system and the kidney. Nerves carrying fibres that run to or from the kidney chiefly stem from the coeliac plexus and its subdivisions, but also from lumbar splanchnic nerves and the intermesenteric plexus. The thoracic sympathetic truncs as well as the vagus nerves send fibres to the coeliac plexus. Afferent fibres are concentrated in the ureter, pelvis, and vascular wall. Afferent renal nerve somata are located in dorsal root ganglia of lower thoracic and lumbar segments. The intrinsic renal nerves enter the hilus in association with the vasculature and end at the juxtaglomerular apparatus. Cortical tubules have contact to terminal axons only in the vicinity of arterioles, and medullary nerve fibres are only found along the vasa recta bundles. Functionally, renal sympathetic nerve activity contributes importantly to homeostatic regulation of renal blood flow, GFR, and epithelial solute and water transport. A major step in translational medicine has recently been achieved by the demonstration of a significant antihypertensive effect of radiofrequency, catheter-based renal denervation in therapy-resistant hypertensive patients (Krum H et al., Lancet 373/1275/2009). Postoperative complications were absent. Norepinephrine spillover and renin activity were reduced to half, and blood pressure was reduced by -27/-17 mm Hg after 12 mo. Differential regrowth of efferent vs. afferent nerve fibres must be discussed. Continued research into therapeutic renal-nerve ablation is of interest and sheds new light on the innervation of distinct structures within the kidney.

Kategorie: Poster

Poster 96

Rubrik: 9.Peripheres und vegetatives Nervensystem
Abstract Nr.:9

Titel:Site-specific gene expression profiles of enteric ganglia by laser microdissection as a new tool for the analysis of enteric neuropathies

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Abstract:
Background:
Enteric neuropathies are associated with a large spectrum of gastrointestinal motility disorders characterized by alterations of enteric ganglia both at morphological and molecular biological level. For the selective gene expression analysis of enteric ganglia we have combined laser microdissection (LMD) with real-time RT-PCR to detect and quantify gene expression profiles relevant for the characterization of enteric neuropathies.
Material & Methods:
Unfixed cryo-sections obtained from human sigmoid colon (n=8) were mounted on membrane-coated slides and ultra-rapidly stained with toluidine blue. mRNA was isolated both from full-thickness sections and myenteric ganglia selectively harvested by LMD. Real-time RT-PCR was performed for neuronal (PGP 9.5), glial (S100ß) and smooth muscle (tropomyosin) cell markers and for selected neurotrophic factors (NGF, NT3) and neurotransmitter receptors (5HT-R3A).
Results:
Collection of 4 mm2 ganglionic tissue by LMD resulted in ct-values allowing a reliable quantitative comparison of gene expression levels. mRNA analysis revealed that NGF, NT3 abd 5HT-R3A were specifically expressed in myenteric ganglia. While full-thickness samples contained high levels of tropomyosin, myenteric ganglia collected by LMD yielded high levels of PGP 9.5 and S100ß expression confirming the selective sampling of ganglionic tissue.
Conclusions:
LMD combined with real-time RT-PCR offers a novel approach to reliably detect and quantify site-specific gene expression profiles related to the human enteric nervous system. This technique expands the spectrum of available tools to further characterize enteric neuropathologies underlying human gastrointestinal motility disorders at the molecular biological level.

Kategorie: Poster

Poster 97

Rubrik: 11.Neuroimmunologie
Abstract Nr.:1

Titel: Correlation of antigen-specific th1/th17 cytokines, serum antibodies and cns histopathology in a murine model for relapsing remitting multiple sclerosis

Autoren: Batoulis H.(1),Lehmann P.(2),Addicks K.(1),Kuerten S.(1),

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Abstract:
During relapsing-remitting experimental autoimmune encephalomyelitis (EAE) mice suffer from transient and finally chronic motor impairment, which resembles the clinical picture of most multiple sclerosis patients. Here we set out to investigate whether there is a correlation between clinical symptoms, the peripheral and central antigen-specific TH1/TH17 response, serum antibodies and CNS histopathology.
SJL mice were immunized with proteolipid protein peptide 139-151 and sacrificed at the preclinical stage, disease onset, remission and relapse. At these different time points the neuroantigen-specific IFN-gamma and IL-17 response was evaluated in the spleen, blood and spinal cord by ELISPOT assays. ELISA was used to detect serum IgG against PLP:139-151. HE-stainings and immunohistochemistry provided information on inflammation, demyelination and axonal damage in the spinal cord of the mice.
Our data demonstrate a correlation between clinical EAE, spinal cord histopathology and the neuroantigen-specific IFN-gamma and IL-17 response in the CNS. On the contrary, the neuroantigen-specific IFN-gamma and IL-17 response in the periphery and the PLP:139-191-specific antibody titers did not correlate with either CNS histopathology or the clinical picture.
We conclude that relapsing-remitting EAE in SJL mice appears to be primarily T cell mediated with IFN-gamma and IL-17 being important effector cytokines. We propose that T cell inflamamtion to the CNS causes downstream demyelination and axonal damage that – taken together – are the structural-morphological correlates for the functional deficits in the mice.

Kategorie: Poster

Poster 98

Rubrik: 11.Neuroimmunologie
Abstract Nr.:1

Titel:The clinical course of experimental autoimmune encephalomyelitis (eae) is reflected by the dynamics of the neuroantigen-specific t cell compartment in the blood

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Abstract:
Immunizing mice with neuroantigen triggers neuroantigen-specific T cells that mediate EAE. Considerable interindividual variability characterizes both T cell reactivity and disease severity. One might hypothesize that the magnitude of the T cell response is the primary variable that defines the disease outcome. However, this hypothesis has not yet been assessed because the limited number of PBMCs that can be obtained from the blood of individual mice has prevented serial testing.
To this end, we developed an ELISPOT-based test system in which 150 microliter of blood was sufficient to measure the frequencies of neuroantigen-specific IFN-gamma and IL-17 secreting cells while continuing to observe the clinical course of EAE.
In PLP:139-151-induced EAE of SJL mice, clinical remission was paralleled by a significant drop in neuroantigen-specific IFN-gamma/IL-17 producing T cells. Likewise, a remarkable re-increase in frequencies indicated the clinical relapse. In MOG:35-55-induced EAE of C57BL/6 mice, the chronic disease was mirrored by a non-fluctuating neuroantigen-specific IFN-gamma/IL-17 response.
We conclude that the limited amount of blood available from individual mice does not prevent systematic studies of the effector cell pool. In two independent models of murine EAE we demonstrate that the dynamics of the neuroantigen-specific blood T cell compartment reflect the clinical course of the disease. As more is learned about the neuroantigens targeted in multiple sclerosis our approach also holds promise to serve as a valuable tool in the quest for more efficient diagnostic and prognostic options in patients where the blood is the primary material available for testing.

Kategorie: Poster