(MP), between the longitudinal and circular muscle, and the submucosal plexus (SP) associated with the mucosal epithelium. Both plexuses are composed of ganglia (contain neurons and glial cells) and interconnecting nerve fibre strands, which consist of the axons of myenteric neurons, the axons of extrinsic neurons that project to the gut wall and glial cells. Over the last decades, several studies dealing with the ENS of different species have revealed that the architecture of the enteric plexuses is more complex in larger animals, including man, than in small animals. The MP forms a continuous network that is continuous around the circumference and extends from the upper oesophagus to the anal sphincter. Its texture and ganglionic density show regional differences in the same individual, and differences between species. The submucous plexus exhibits a limited number of neurons in the oesophagus and gastric compartments, with a more complex intramural structural organization in the ruminant forestomach, and a continuous plexus in the intestine, that is situated on one plane in small animals, and multilayered and functionally distinct in large animals.

GI neurons release a plethora of substances that are chemically different but only partially have been identified functionally.

Combined morphological, electrophysiological, pharmacological, neurochemical and retrograde labelling, has led to identification of GI neurons into different functional classes, i.e., sensory neurons, interneurons, excitatory and inhibitory motor neurons. These neurons are interconnected by chemical synapses into intrinsic neuronal circuits that generate functional reflexes: they are partly independent of the central nervous system (CNS). In the intestine reflex functions arise even if the segment has been isolated from the body.

FLOURESCENT IMMUNOCYTOCHEMISTRY – A METHOD FOR STUDYING GENE EXPRESSION IN A MOUSE BRAIN

Tomaž Büdefeld[1], Neža Grgurevič[1], Stuart A Tobet[2] and Gregor Majdič[1]

[1]Center for Animal Genomics, Veterinary faculty, University of Ljubljana, Gerbičeva 60, 1000 Ljubljana, Slovenia; [2]Colorado State University, College of Veterinary Medicine and Biomedical Sciences, Fort Collins, CO 80523-1680, USA

Fluorescent microscopy techniques are excellent tool for studying cell identity and micro-circuitry in the brain. We are using Steroidogenic factor 1 knockout mice (SF-1 KO) and mice expressing GFP under the influence of SF-1 promotor as models to study neuroendocrine brain development. In the SF-1 KO mice, a very specific disorganization of the ventromedial hypothalamic nucleus (VMH) occurs, with all other parts of the brain being intact.

For studying gene expression in the mouse brain, immunocytochemistry on free-floating sections is used. To obtain brain tissue, mice are perfused with 0.05M PBS and 4% paraformaldehyde. 50?m thick coronal brain sections are cut on an Integraslice vibrotome (Campden instruments) and further processed for immunocytochemistry. For the present study, primary antibodies against calbindin D-28k raised in mouse, estrogen receptor alpha and green fluorescent protein both raised in rabbits, were used. For fluorescent detection of bound antibodies, sections were incubated with secondary antibodies conjugated with Cy2 or Cy3 fluorophores. Bound Cy2 and Cy3 flourophores were visualized under specified excitation wavelengths using confocal microscope. Primary mouse and rabbit antibodies (anti GFP/anti calbindin, anti ERalpha/anti calbindin) were used simultaneously while labelling of GFP/ER alpha coexpressing cells was performed by sequential incubation with each antibodies.

Immunocytochemistry with all three antibodies produced a strong fluorescent signal. Examination of sequential sections revealed that calbindin and ER-alpha are expressed in the same cells both in WT and SF-1 KO mice, even though the location of these cells is altered in SF-1 KO mice, while GFP cells (SF-1 expressing cells) do not co-express either ER-alpha or calbindin.

THE INFLUENCE THROUGH FEEDING ON THE FAT PADS IN THE BOVINE DIGITAL CUSHION

Vera Letter[1], Martin R.L. Scheeder[2], Hans Geyer1, André Chasssot[3], Marta Räber[1]

[1]Institute of Veterinary Anatomy, Vetsuisse-Faculty University of Zürich, Winterthurerstrasse 260, CH-8057 Zürich, Switzerland; [2]Institute of Animal Science, Swiss Federal Institute of Technology Zürich, ETH-Zentrum, CH-8092 Zürich, Switzerland; [3]Agroscope Liebefeld-Posieux, Research Station ALP, rte de la Tioleyre 4, CH-1725 Posieux

The effect of an intensive respectively extensive feed on the fat content and the fatty acid profile of the bovine digital cushion was tested by examining the claws of 32 feedlot animals. In addition, it was examined if, respectively how the fatty acid profiles can affect the claw health. Samples from subcutaneous adipose tissue and the claws of 9 cows served as comparison. Furthermore, the microscopic structure of the fat pads was analyzed and the results were compared with those from previous studies.

The fat pads as well as the subcutaneous adipose tissue showed obvious differences in the fat content and the fatty acid profile between the two different feeding-groups. The fat pads of the intensive fed animals contained a lot less fat and noticeable more omega-6-fatty acids, above all Linoleic and Arachidonic acid. In addition, these animals showed the highest proportion of Eicosapentaenoic acid (EPA) and Docosahexapentaenoic acid (DHA), two omega-3-fatty acids. The extensive fed animals contained more omega-3-fatty acids, mainly α- Linoleic acid. Also the subcutaneous adipose tissue of the intensive fed animals showed a much higher proportion of omega-6-fatty acids, whereas the extensive fed group had a higher proportion of omega-3-fatty acids. The differences in the fatty acid profile are for sure due to the different composition of the feeds.

The claws of the intensive fed animals showed post mortem a significant better claw health than the extensive fed group.

THE SENSA COW, A HAPTIC MODEL FOR RECTAL EXPLORATION TO BRIDGE THE GAP BETWEEN ANATOMY AND CLINICAL WORK: PRESENTATION OF THE WORK OF THE COWBOYS EMMA PROJECT GROUP

Claudia F. Wolschrijn[1], Peter L.A.M.Vos[2], Herman F.Jonkers[2], Onno van Veen[3], Wim Kersten[1]

[1]Department of Pathobiology, division of Anatomy and Physiology, Faculty of Veterinary Medicine, [2]Department of Farm Animal Health, [3]Multimedia department, Faculty of Veterinary Medicine, Utrecht University, The Netherlands

In this presentation the results of the co-operation between the School of Arts and the Faculty of Veterinary Medicine to develop a haptic interface to practice rectal examination will be shown.

The goal of the project was to develop a device that can be used between the classes of our regular course of topographical anatomy and the rectal examination in the clinical phase of the veterinary school teaching program. The major learning goal was to achieve the 3D orientation in the cow. The result is the Sensa cow which has wax elements with sensors. After evaluation it appears that Sensa is very useful in learning the first 3D orientation in the cow.

DIFFERENCES IN SKIN COMPONENTS INSIDE REPTILIAN AND AMPHIBIAN GROUPS

Malan Štrbenc
Institute for Anatomy, Histology and Embryology, Veterinary Faculty, University of Ljubljana, Slovenia
E-mail: malan.strbenc@vf.uni-lj.si

Reptiles and amphibians became increasingly popular pets. In recent years the knowledge on medicine of these animals is improving; however there is a gap between general knowledge on morphology and detailed studies of certain organs on selected species. Comparative study of integument of conventionally kept species, dissected at student practical work was performed. Both reptiles and amphibians demonstrate skin shedding or slough and commonly histology slides show the upper, shedding layer of skin.

While amphibians in terrestrial phase show slightly more prominent keratinisation, the skin is very thin. In dermis there are prominent poison (serous) and mucous glands, and secreting Leydig cells are occasionally encountered in epidermis. While axolotl was not known to posses toxic or irritating skin secretion, we found prominent poison glands. Buffonidae are supposed to have poison glands concentrated on warts. We did find numerous poison glands also on other parts of the body but the size of them increased from abdomen through legs and was greatest at warts on dorsum. In Ranidae the size of poison and mucous glands was approximately the same. In aquatic species Leydig cells were more numerous.

While snakes have similar strength and distribution of scales, in lizards seemingly the skin toughness varies a lot. However, the epidermis on flank skin (excluding ornamental scales) was only twice as thick in green iguana (or tortoise red-eared slider) compared to leopard chamelon, tocke gecko and leopard gecko. The main difference is in dermis. Geckos are colloquially known as scaleless lizards, nevertheless, typical overlaps and hinges were also found.

The black and white subcutaneous glandsî that students found on the neck region in tocke gecko and Rana frog turned out to be at least in part lymphatic tissue.

EPIDERMAL SHEETS - PREPARATION, QUALITY CONTROL, IMUNOHISTOCHEMISTRY AND VISUALISATION BY CONFOCAL MICROSCOPY

Kirsti Witter
Histology and Embryology, Department of Pathobiology, University of Veterinary Medicine Vienna, Veterinärplatz 1, A-1210 Vienna, Austria
E-mail: kirsti.witter@vu-wien.ac.at

Epidermal sheets are often used for studies regarding exclusively the outer skin layer. They can be prepared with inorganic salt solutions or enzymes and used subsequently e.g. for tissue culture and immunohistochemistry. The aim of this study was to test selected methods for preparation of epidermal sheets and to assess conservation of histological structure, stainability including immunohistochemical staining and the possibility of visualisation of staining results by confocal microscopy. Punch necropsy samples (diameter 3 mm) of shaven neck skin of an eight days old piglet euthanised for another study were taken and stored in a moist chamber at 4°C before processing. Epidermal sheet preparation was attempted after incubation with 2 M CaCl2 solution (20 min, 37°C), with 20 mmol/l EDTA-solution (3 hours, 37°C), or with 0.1% trypsin solution (30-120 min, 37°C). Whole mount immunohistochemistry and/or nuclear staining with DAPI was performed without permeabilisation of the sheets, after permeabilisation with chilled acetone, or after permeabilisation with 0.1% Triton X-100. Staining results were visualised using a laser scanning confocal microscope. For quality control, selected samples were embedded in paraffin and epoxy resin for light and electron microscopy, respectively. The easiest and least time consuming method for epidermal sheet preparation was incubation in a CaCl2 solution. The epidermis was firm enough to handle and peeled of the corium without difficulties, including epithelial root sheaths of hairs. Preparation of epidermal sheets with trypsin was unsuccessful, even after prolonged incubation. Only a 0.5†mm margin of the epidermis could be detached from corium, both corium and epidermis were very brittle. CaCl2 as well as EDTA sheets stained well without differences regarding different pretreatment methods. Morphology of epithelium and corium was conserved satisfyingly in all samples. Interestingly, basement membrane material (laminin, PAS-positive material) could be found on both epithelium and corium, indicating a splitting of the membrane itself during preparation. Connective tissue did never remain on epidermal sheets. If the basement membrane was split incompletely during preparation, basal epithelial cells remained on the surface of corium. Confocal microscopy could be used successfully to visualise individual cell layers of the epidermal sheets. However, epithelial root sheaths of hairs caused a wavy appearance of the epidermis and impaired the assessment of e.g. cell numbers and stratification. In conclusion, skin epidermis can be easily detached from corium after incubation in a CaCl2 solution. Whole mount immunohistochemical staining as well as routine histology and staining of sections are possible without disruption of the sheets. For hairy skin removal of root sheaths from the epidermis should be attempted for confocal microscopy.

CONFOCAL MICROSCOPY – A TOOL TO STUDY 7TM RECEPTOR CHIMERAS OF GHRELIN RECEPTOR WITH GABAB RECEPTOR TAIL-SWAP

Valentina Kubale[1,2], Azra Pogačnik1, Birgitte Holst[2], Thue W. Schwartz[2,3], Milka Vrecl[1]
[1]Institute for Anatomy, Histology and Embryology, Veterinary Faculty of University of Ljubljana, Gerbičeva 60, SI-1115 Ljubljana, Slovenia; [2]Laboratory for Molecular Pharmacology, Department of Pharmacology, The Panum Institute, University of Copenhagen, Blegdamsvej 3, DK-2200, Copenhagen, Denmark; [3]7TM Pharma, Fremtidsvej 3, DK-2970, Hørsholm, Denmark

Seven transmembrane receptors (7TM-Rs) also designated as G protein-coupled receptors (GPCRs), were traditionally thought