Institute of Experimental Morphology, Pathology and Anthropology with Museum
Bulgarian Anatomical Society

Acta Morphologica et Anthropologica, 30 (3-4)
Sofia ● 2023

SUPPLEMENT – ABSTRACTS OF THE 26TH NATIONAL CONGRESS OF BULGARIAN ANATOMICAL SOCIETY, SOFIA, 29 SEPT. – 01 OCT. 2023

Morphological Alterations in Tumor and Normal Tissue after Microbeam Radiotherapy

V. Djonov

Institute of Anatomy, University of Bern, Bern, Switzerland

* E-mail: valentin.djonov@unibe.ch

Microbeam-Radiation-Therapy (MRT) is a type of spatially fractionated radiation therapy which modulates the radiation dose on a micrometre scale. This unique dose distribution makes MRT highly efficient even for the treatment of radioresistant tumours. Mice bearing B16-F10 melanoma, human glioblastoma xenografts, developing chick chorioallantois membrane (CAM) were exposed to MRT. Tumor progression and alterations have been documented by Magnetic Resonance Imaging (MRI), micro CT, histological, electron microscopy and molecular-biological analysis at multiple timepoints. In a murine B16-F10 melanoma model, temporally fractionated MRT completely ablated 50% of tumors and prevented organ metastases and local recurrences for 18 months after treatment. In a mouse glioblastoma model, MRT in combination with cisplatin reduced tumor volume 6-fold compared with cisplatin alone and 60-fold compared with untreated mice. The radiation biology underlying the "MRT effect" include novel radiobiological mechanisms: (1) Induction of selective vascular disruption of immature tumor vasculature or transient vascular permeability in a dose-dependent manner (2) Direct cellular damage in the microbeam path that elicits tissue-specific responses. (3) Induction of a unique, tumor-targeted immune response leading to local and systemic anti-tumor immune responses including infiltration of cytotoxic lymphocytes. Spatially fractionated MRT demonstrated one best treatment outcome ever achieved in preclinical models. MRT provides a novel mechanism for drug delivery by increasing vascular transpermeability while preserving vessel integrity and in addition is busting the anti-tumor immune response. These unique features support MRT as a novel therapeutic approach for the treatment of inoperable, radioresistant lesions.

Key words: cancer treatment, spatially fractionated radiotherapy, cell death, micro beams, vascular permeability

Making, defining, and Wiring Cerebellar Inhibitory Interneurons: More Data, More Questions

K. Schilling

Anatomisches Institut – Anatomie & Zellbiologie, Rheinische Friedrich-Wilhelms-Universität Bonn, Germany

* E-mail: karl.schilling@uni-bonn.de

The cerebellum, notably its cortex, is typically described as a highly stereotyped structure and primarily associated with motor control. Yet this view belies the intrinsic complexity of the cerebellum, and it may distract from the fact that our understanding of cerebellar histogenesis, cellular integration and how its circuitry mechanistically implements its diverse functions is still rather fragmentary. Here, I will focus on the characterization and development of cerebellar inhibitory interneurons. Results from different fields and obtained using various technologies over the past two converge to point out that these cells are quite more diverse than traditionally portrayed. As molecular targeting of these cells is still very limited, understanding of their functions or their functional distinction remains challenging. This is particularly true for inhibitory interneurons of the cerebellar nuclei. Indeed, it is not really clear whether interneurons in the classical sense of this expression do exist there, or whether at least some nuclear cells traditionally viewed as interneurons also send collaterals to the cerebellar cortex. I hope that the data summarized, however fragmentary, may stimulate interest and help focus research towards understanding the cerebellum.

Lateralisation, Hubs and Cognition in the Avian Brain

E. Marani

Department of Biomedical Signals & Systems, TechMed Centre, University Twente, Enschede, The Netherlands

* E-mail: enrico.marani@sfr.fr

It is analysed whether similarities in avian and mammalian/human brain development and connectivity result in comparable cognitive capacities as stated e.g. by Güntürkün et al. (2021) “Cognitive functions are similar in birds and mammals”. In the avian brain dominance exists for song and song development: in the left hemisphere in the canary and in the right hemisphere in the Zebra finch. Functional asymmetry of the human brain was clinically found for speech in the left hemisphere and orientation and awareness in the other half. Despite the presence of functional asymmetries in both, differences between mammalian and avian brain concern the cortex structure. The avian cortex contains clusters, while the mammalian cortex consists of laminae. The avian brain halves are restrictedly interrelated by small commissures (anterior and supraoptica), while the mammalian brain has an enormous interhemispheric connection, the corpus callosum. This corpus callosum is absent in the avian brain, meaning that each hemisphere has a separate “mind of its own”. The storage of neurons in the avian forebrain is different, resulting in more neurons/volume. Specific areas of the bird pallium have no connection with the hippocampus, e.g. nidopallium caudolaterale, but contain memory inherently. Hubs contain nodes that are characterized by an overload of connections that can be named according to their module organisation e.g. prefrontal,

premotor or auditory module. The spatial intrahemispherical distribution of the pigeon nodes does not contact the other hemisphere, which is at strong difference with the human brain. The claustrum is regarded as a multi-modal information processing network important in cognition. In humans cognition is codetermined by the callosal bilateral connection between claustrum and contralateral cortical areas and the callosal inter-claustral communication, which is absent in the avian brain. Moreover, the avian claustrum has its own development and topography that is different from mammals and reptiles. Therefore, avian neuroanatomy contradicts the claim of similarity of mammalian and avian cognition.

Güntürkün O, von Eugen K, Packheiser J, Pusch R (2021) Avian pallial circuits and cognition: A comparison to mammals. Curr Opinion Neurobiol 71:29–36

Carotid Bodies and Neuroepithelial Bodies: Polymodal Arterial and Airway Sensors

N. Lazarov

Department of Anatomy and Histology, Medical University of Sofia, Sofia, Bulgaria

* E-mail: nlazarov@medfac.mu-sofia.bg

Oxygen sensing is of paramount importance for the survival, adaptation and maintenance of homeostasis in living organisms. In humans, the carotid body (CB) is the main oxygen sensor in arterial blood while the innervated groups of neuroendocrine cells in the lung called neuroepithelial bodies (NEB) are considered the most important intrapulmonary oxygen sensors. However, recent evidence suggests that both structures are polymodal arterial and airway chemoreceptors, which respond to a variety of environmental stimuli. Indeed, the CB is purposefully situated at the carotid bifurcation and NEBs are typically positioned at branching points along the intrapulmonary pathways. This location is strategic for monitoring intraluminal blood and airway chemicals. They are analogous structures that do not share a common embryonic origin but have a similar structural plan that reflects their unique role as complex sensory receptors. The CB consists of clusters composed of two juxtaposed cell types, neuron-like glomus cells which are considered chemosensory cells and glial-like sustentacular cells which are regarded to be supporting cells and glomus cell precursors. In turn, NEBs are made up of clustered specialized epithelial cells in close association with secretory nonciliated club cells as a potential stem cell source. The receptor cells are dually innervated by both sensory and autonomic nerve fibers. Such an arrangement and innervation are ideally suited for both autocrine and paracrine regulation of cell function. In response to external stimuli, chemoreceptor cells release a broad range of neurotransmitters including peptides and amines which activate chemoafferent nerve endings and send signals to the central nervous system to correct the condition. Moreover, both CBs and NEBs exhibit remarkable structural and neurochemical plasticity, critical for their physiological adaptation to changing environmental and pathological conditions. Knowledge of the morphofunctional characteristics and neurochemical phenotypes of CBs and NEBs is becoming crucial for our better understanding of respiratory homeostasis and cardiovascular responses in health and disease.

Key words: carotid body; neuroepithelial bodies; oxygen sensing; polymodal sensing; structural and neurochemical plasticity

Acknowledgement: This study was funded by the European Union-NextGenerationEU, through the National Recovery and Resilience Plan of the Republic of Bulgaria, project No. BG-RRP-2.004-0004-C01.

Carotid Body Adaptation in a Hypertensive Environment

*D. Atanasova[1,*2], A. Ivanov[1,3], N. Lazarov[1,3]*

[1] Institute of Neurobiology, Bulgarian Academy of Sciences, Sofia, Bulgaria
[2] Department of Anatomy, Faculty of Medicine, Trakia University, Stara Zagora, Bulgaria
[3] Department of Anatomy and Histology, Medical University of Sofia, Sofia, Bulgaria

* E-mail: d.atanasova@inb.bas.bg; dimitrinka.atanasova-dimitrova@trakia-uni.bg

The carotid body (CB), a small structure located at the bifurcation of the common carotid artery, is involved in sensing peripheral chemoreception, which is believed to be a key factor in triggering a number of cardiovascular diseases. There is undisputed evidence in the literature explaining the cause of essential hypertension in a relationship involving both carotid body dysfunction and increased sympathetic innervation. To gain insight into the morphological and neurochemical nature of chemoreceptor glomus cells in hypertensive conditions, we have investigated by stereological methods and immunohistochemical techniques the structural organization, expression and neurochemical profile of certain neuroactive substances in spontaneously hypertensive rats (SHR) compared with age-matched normotensive Wistar rats (NWR). The structural adaptation of the hypertensive CB is associated with an almost two-fold increase in its total volume and the total number of glomus cells as well as in the total length of the capillary network in SHR compared to normotensive animals. In contrast, the globally average cross-sectional area of a capillary in the CB of NWR was 2.8-fold greater than the sectional area in age-matched SHR. Our immunohistochemical experiments demonstrated an increased content of catecholamines, serotonin, neurotrophic factors and their corresponding receptors in the CB of SHR, while the expression of gamma-aminobutyric acid, the density of substance P- and vasoactive intestinal peptide-containing fibers were reduced compared to NWR. It can be inferred that the increased levels of certain neuroactive substances and neurotrophic factors in hypertensive animals modulate chemoreceptor information processing, leading to dysfunction, hyperactivity, and increased blood pressure.

Key words: carotid body, hypertension, plasticity, neurotransmitters, sympathetic innervation

Acknowledgement: This research is financially supported by the European Union-NextGenerationEU, through the National Recovery and Resilience Plan of the Republic of Bulgaria, project No. BG-RRP-2.004-0004-C01 and by the Faculty of Medicine at Trakia University – Stara Zagora (Grant No. 5 /2022).

Neuroepithelial Bodies and Their Role in the Development and Regulation of Pulmonary Hypertension

N. Stamenov[1], D. Atanasova[2, 3], L. Jelev[1], A. Dandov[1], N. Lazarov[1, 2]*

[1] Department of Anatomy and Histology, Medical University of Sofia, Bulgaria
[2]Institute of Neurobiology, Bulgarian Academy of Sciences, Sofia, Bulgaria
[3]Department of Anatomy, Faculty of Medicine, Trakia University, Stara Zagora, Bulgaria

* E-mail: nstamenov@medfac.mu-sofia.bg

Neuroepithelial bodies (NEBs) are a key component of the normal mucosa of intrapulmonary airways. They have a wide array of functions including oxygen sensing, mechanoreception, immunological role and may be involved in the regeneration of normal lung cells. The so-called neuroepithelial body microenvironment (ME) includes their main cells, neuroendocrine cells and also supporting cells, i.e. club or Clara-like cells. The complex innervation of NEBs from both sensory and autonomic nerve fibers makes them even more important for the maintenance of lung homeostasis. For the purpose, NEBs possess a broad range of neurochemicals and they respond to all kind of stimuli including increased blood pressure by changes in this neurochemical profile. Our study focuses on the differences of the neurochemical expression of certain neurochemical substances by NEB in normotensive Wistar rats (NWR) and spontaneously hypertensive rats (SHR). We performed immunohistochemical staining for calcitonin gene-related peptide (CGRP), serotonin (5HT), substance P (SP), gamma-aminobutyric acid (GABA), neuropeptide Y (NPY) and compared their expression levels in both NWR and SHR. We found increased expression of CGRP, SP and GABA in the SHRs compared to the normotensive WR. Those three neurotransmitters may play an important role towards regulation of the blood pressure as they serve as potent vasodilators. Our results suggest that NEBs are very sensitive to changes in the pulmonary blood pressure and are involved in its regulation as well as the systemic blood pressure. The results for the other neuropeptides used in the current experiment were not conclusive and did not show major differences between the two breeds of rats. Further experiments will reveal the detailed mechanisms of their involvement in the maintenance of hypertension.

Key words: neuroepithelial bodies; hypertension; immunohistochemistry; normotensive Wistar rats; spontaneously hypertensive rats

Acknowledgement: This study was funded by the European Union-NextGenerationEU, through the National Recovery and Resilience Plan of the Republic of Bulgaria, project No. BG-RRP-2.004-0004-C01.

Abnormal Hemispheric Lateralization as a Marker for the Neurodevelopmental Origin of Schizophrenia

K. Akabalieva[1], A. Beshkov[2], V. Kotetarov[1], V. Akabaliev[2], S. Sivkov[3,4]*

[1]Department of Psychiatry, Medical University-Sofia, Sofia, Bulgaria
[2]Department of Psychiatry and Medical Psychology, Medical University-Plovdiv, Plovdiv, Bulgaria
[3]Department of Anatomy, Histology, and Embryology, Medical University-Plovdiv, Plovdiv, Bulgaria
[4]Research Institute at Medical University of Plovdiv, Plovdiv, Bulgaria

*E-mail: Stefan.Sivkov@mu-plovdiv.bg

Left-eyedness as a measure of altered hemispheric lateralization and minor physical anomalies appear potential criteria of prenatal neural maldevelopment. To assess the difference in left eye dominance and MPAs between schizophrenic and control subjects and determine the relations between these biological markers of neuronal dysontogenesis in schizophrenia. A case-control study, conducted 2014-2019. Three tests for eye dominance and seven MPAs of the Waldrop scale were used. The study included 180 participants (98 schizophrenic and 82 control) with a mean age of 34.45 years. The refusal rate of potential participants is below 5%, excluding

selectivity bias. Participants were consecutively admitted in-patients, meeting DSM-V criteria for schizophrenia. The control group included volunteers, without previous or present psychiatric disorder. Exclusion criteria for both groups were history of drug or alcohol abuse, neurological disorder, intellectual disability and ocular pathology. The mean left-eyedness is higher in schizophrenic vs. controls: Looking through a hole -.81 vs. .39,p<.001; Looking through a monocle –.78 vs. .39,p<.002; Porta test –.86 vs. .41,p<.002. Importantly, the mean sum of the eye set is more than twice big in schizophrenic vs. control subjects, with marked statistical significance – 2.45 vs. 1.20, p<.000. The Spearman's rank correlation matrix shows that positive correlations are highly predominant between left-eyedness and MPAs. On the continuum of neuroontogenetic disorders any single biological marker may indicate a probable neurodevelopmental disturbance. However, the higher rate of co-occurrence of two biological markers for dysontogenesis, left-eyedness and MPAs, in one subject becomes a stronger reliable index of underlying neurodevelopment disorder.

Keywords: brain, laterality, eyedness, neurodevelopment, schizophrenia

Is the PC12 Cell Line Suitable for Synaptogenesis?

A. Ivanov

Department of Anatomy, Histology, and Embryology, Medical University – Sofia, Sofia, Bulgaria

* E-mail: aivanov@medfac.mu-sofia.bg

In 1975 Tischler and Greene reported the culture of a norepinephrine-producing pheochromocytoma. In the presence of nanogram quantities of NGF, the cells produced processes, grew on collagen, and produced much more dopamine than norepinephrine. This work aimed to determine whether the PC12 cell line may be a suitable cell model for investigating the neuronal accumulation, storage, and release of synaptic vesicles. After treatment with NGF on Day 21 the naïve cells showed characteristics of neuronal differentiation on three levels – light microscopy, immunofluorescence, and electron microscopy levels. The NGF additive was a trigger mechanism for multiple extension growth from virtually every cell in the culture. The cells were visually connected by an extensive network of processes that look like neurites of neurons. The demonstration of pre-and postsynaptic proteins via immunofluorescence circumstantially showed that the PC12 cells synthesize proteins that are associated with synaptic vesicles and functional neuronal junctions. The electron microscopy images show membrane-bound vesicles scattered around the growth cones and varicosities of the cell neurites. The abundance of synaptic vesicles, large dense-core vesicles, and multivesicular bodies indicate that after NGF treatment the PC12 cells show morphological features of differentiated neurons. The PC12 cells turn out to be a potential cell line that under the right conditions may differentiate into cells forming synapses similar to what neurons do **in vivo**. By having neuronal cells, we may investigate morphological changes before the onset of synaptic dysfunction in future models of neurodegenerative diseases.

Key words: PC12, synaptogenesis, synaptic vesicles

Phenotype of Testicular Dysgenesis Syndrome (TDS) – Origin and Cellular Mechanisms

N. Atanassova

Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, Sofia, Bulgaria

* E-mail: ninaatanassova@yahoo.com

Disorders of human male reproductive health manifested at birth (cryptorchidism, hypospadias) or in young adulthood (low sperm count, testicular germ cell cancer) are remarkably common, and they are increasing in incidence. These disorders comprise testicular dysgenesis syndrome (TDS) with a common origin in fetal life related to subtle deficiencies in fetal androgen (testosterone) production. Androgen action during fetal life is important for later correct development of male reproductive organs and their final size. Androgen driven events preferably occurred in a narrow window called masculinization programing window (MPW) during fetal life. For understanding the mechanisms involved in TDS an experimental model for induction of TDS was developed in rat by gestational exposure to anti-androgenic compound dibutyl phthalate (DBP). In fetal testis TDS has a distinct morphology of interstitial cells expressing tubular Sertoli cell markers (AMH, GATA-4). Postnatally, dysgenetic areas are manifested by ectopic expression in seminiferous tubules of 3βHSD, a marker enzyme for steroidogenic interstitial Leydig cell. COUP-TFII (chicken ovalbumin upstream promoter transcription factor-II) was identified as a cellular biomarker for impaired development of fetal Leydig cell (LCs) after in utero exposure to DBP. Abnormal persistence of COUP-TFII in fetal LCs is responsible for their dysfunction (T production) within MPW, being important in determining the risk and severity of down-stream TDS disorders in later life. Molecular mechanism of action of DPB involved downregulation of key genes for steroidogenesis (StAR, Cyp11a, Cytp17a, 3βHSD) in LCs by epigenetic change (increased methylation/H3K27me3), resulting in compensated adult LC failure manifested by low testosterone and elevated luteinizing hormone levels.

Key words: testis, androgens, TDS, Leydig cells, male infertility

Morphometric Analysis of the Myenteric Plexus in the Colorectal Region of Mice-D-Galactose Ageing Model

N. Genov[*1], N. Tomov[2], N. Dimitrov[1], N. Lazarov[3,4], L. Petrov[5], E. Tsvetanova[3], D. Atanasova[1,3]*

[1]*Department of Anatomy, Faculty of Medicine, Trakia University, Stara Zagora, Bulgaria*
[2]*Institute of Anatomy, University of Bern, Bern, Switzerland*
[3]*Institute of Neurobiology, Bulgarian Academy of Sciences, Sofia, Bulgaria*
[4]*Department of Anatomy and Histology, Medical University of Sofia, Sofia, Bulgaria*
[5]*National Sports Academy "Vassil Levski", Sofia, Bulgaria*

* E-mail: nikolay.genov@trakia-uni.bg

Ageing-related diseases are of paramount importance as a socioeconomic factor in the postmodern society. Neuronal degeneration is a known phenomenon in ageing. The D-galactose

model of accelerated ageing is widely used in the field of age-associated neuronal cell death because of the oxidative stress it induces, which correlates to reduced cognitive performance and robustly detectable neuronal shrinkage in the CNS. The aim of this study is to demonstrate the morphological changes of neurons of the myenteric plexus that occur in mice treated with D-galactose. D-galactose was given per os with drinking water, resulting in an average dose of 500 mg/kg daily for six weeks. In the D-galactose accelerated ageing model, a significant size reduction of the neuronal perikarya of the myenteric plexus was observed all along the large intestine. The average soma area at the caecum in the control group was 305 μm^2, which was reduced by approximately 18% in the ageing model. In contrast, at the level of the distal colon in the D-galactose ageing model, the average area of the neuronal soma was 190 μm^2 a reduction of almost 40%. There is a significant reduction of the area of the neuronal bodies in the myenteric plexus among different parts of the rat large intestine in the accelerated ageing model.

Key words: colorectal region, neuronal perikarya, myenteric plexus, D-galactose-induced accelerated ageing model

Acknowledgement: This research is supported by the Bulgarian Ministry of Education and Science under the National Program "Young Scientists and Postdoctoral Students-2" and by the Bulgarian Ministry of Education and Science (Grant D01-17/30.11.2018 and agreements DO1-323/18.12.2019, DO1-358/17.12.2020, and DO1-278/03.12.2021) under the National Research Programme "Innovative Low-Toxic Bioactive Systems for Precision Medicine (BioActiveMed)" approved by DCM # 658/14.09.2018".

When Our Eyes Become Too Dry – Anatomical Insights into the Pathophysiology of Dry Eye Disease

F. Paulsen

Friedrich Alexander University Erlangen-Nürnberg, Institute of Functional and Clinical Anatomy, Erlangen, Germany

* E-mail: friedrich.paulsen@fau.de

Disturbances in the dynamic balance of the lacrimal functional unit (LFU) can lead to hyposecretion (aqueous-deficient dry eye, ADDE) or hyperevaporation (evaporative dry eye, EDE) of the tear film and thus to the development of dry eye disease. Worldwide, the prevalence of this widespread disease is between 5 - 50 % depending on age, gender and ethnic origin. Patients experience a high degree of suffering due to persistent burning and itching of the eyes with a foreign body sensation. If the disease is severe and advanced, more serious complications usually occur, such as visual impairment, inflammation and scarring, and even blindness. The evaporative form, often associated with meibomian gland dysfunction (MGD), is the most common cause of dry eye disease. Here, a deficiency of the outer lipid component of the tear film leads to excessive evaporation of the muco-aqueous component with instability of the tear film hyperosmolarity. Both parameters are considered central pathomechanisms of dry eye disease. The lipids are produced and secreted by the meibomian glands in the eyelids. So far known central causes of meibomian gland dysfunction are a deficiency of androgens (male sex hormones), hyperkeratinisation of the excretory ducts, disturbed signal transduction of a central receptor in lipid metabolism (peroxisome proliferative-activated receptor gamma, PPARγ) and inflammatory reactions. The talk will give an overview of the formation of tears and the tear film as well as the current state of knowledge on the widespread disease dry eye.

Expression of Annulus Fibrosus Collagen Type I and III in Adult Degenerative Scoliosis

B. Landzhov[*1], A. Iliev[1], S. Stanchev[1], N. Stamenov[1], L. Gaydarski[1], GP. Georgiev[2]*

[1] *Department of Anatomy, Histology and Embryology, Medical University of Sofia, Sofia, Bulgaria*
[2] *Department of Orthopaedics and Traumatology, University Hospital 'Queen Giovanna – ISUL', Medical University of Sofia, Sofia, Bulgaria*

* E-mail: blandzhov@medfac.mu-sofia.bg

Adult degenerative scoliosis is a type of adult scoliosis that develops after skeletal maturity in a previously normal spine. Often the degeneration of intervertebral discs (IVD) starts as a result of abnormal mechanical load. Adult degenerative scoliosis (ADS) is the asymmetry of intervertebral discs and facet joints leading to the progressive appearance of low back pain, leg pain, and leg weakness in the advanced stages of the disease. The aim of this study was to evaluate the changes in the expression of annulus fibrosus collagen type I and III in the human IVD of adults with degenerative scoliosis. Our immunohistochemical study was conducted over seven human IVD obtained from cadavers (between 50 and 72 years old) without spine pathology marked as healthy and eight IVD obtained intraoperatively from patients with scoliosis (between 53 and 68 years old). Samples were examined with monoclonal anti-mouse antibodies for collagen type I and collagen type III. The assessment of the content of collagen types I and III was performed using an immunohistochemical study. Both types of collages were observed in the annulus fibrosus of the IVD from the thoracolumbar region. The positive reaction for collagen type I was observed predominantly in samples from IVD without spine pathology. Immunostaining for collagen type I was moderate in disc specimens from patients with ADS. In all IVDs, the expression of collagen type III was moderate and mainly localized in the perilacunar space. In addition, a small number of cells were observed in the examined annulus fibrosus of scoliotic discs. Our study confirmed that the expression of collagen type I in the annulus fibrosus increased in ADS. The immunohistochemical expression of collagen type III is localized in perilacunal space and probably links the isogenous group with the interterritorial matrix. This study sheds more light on the degenerative changes in the IVD and helps to understand the degenerative process in ADS.

Key words: adult degenerative scoliosis, intervertebral discs, collagen type I and type III

Induction of Oxidative Stress in Rat Testicular Cell Populations in Relation to Leydig Cell Function under Condition of Experimental Hyperglycemia

R. Ivanov[*1], E. Pavlova[1], I. Vladov[1], E. Shopova[2], Y. Gluhcheva[1], E. Petrova[1], Y. Tabakov[1], P. Rashev[3], E. Lakova[4], N. Atanassova[1]*

[1] *Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, Sofia, Bulgaria*
[2] *Institute of Plant Physiology and Genetics, Bulgarian Academy of Sciences, Sofia, Bulgaria*

[3] *Institute of Biology and Immunology of Reproduction "Acad. Kiril Bratanov", Bulgarian Academy of Sciences, Sofia, Bulgaria*
[4] *Department of Pathophysiology, Medical University – Pleven, Pleven, Bulgaria*

* E-mail: rosen.ivanov@iempam.bas.bg

Diabetes mellitus (DM) comorbid with oxidative stress and inflammation may affect spermatogenesis and lead to infertility in man. We aimed to investigate the effects of prepubertally induced diabetes on Leydig cell population in tandem with inflammatory and oxidative stress biomarkers in rat testis and blood sera. DM was induced by single i.p. injection of streptozotocin on postnatal day 10 of male Wistar rats. Testicular and blood samples were taken on day 25 (puberty) and day 50 (adulthood). Morphometric, immunohistochemical, immunochemical (ELISA) and biochemical analyses were performed. In adult rats significantly increased glucose levels caused reduction of Leydig cell number corresponding to testosterone insufficiency (40% of control level). Spermatogenesis in adult animals was incomplete, marked by lack of the final stages of germ cell differentiation (elongated spermatids) visualized by specific marker testicular angiotensin converting enzyme. Morphological changes were manifested by reduction in testicular macro-parameters – absolute and relative (gonado-somatic index) testicular weight by 50% and 30% respectively. Experimentally induced diabetes increased protein expression of the pro-inflammatory tumor necrosis factor-α in both ages. Increased protein expression and concentration were also found in oxidative stress markers (3-nitrotyrosine and 4-hydroxy-2-nonenal) in testes and tissue homogenates. Diabetic condition in puberty and in adulthood possibly leads to faster depletion of the antioxidant capacity of cells, evidenced by elevated hydrogen peroxide levels and decreased catalase activity in sera. Prepubertal hyperglycemia induced inflammation and oxidative stress in the testis that are potential risk for reproductive disorders and infertility.

Key words: diabetes mellitus, oxidative stress, Leydig cell, spermatogenesis

Unusual Branching of the External Carotid Artery in a Human Cadaver: A Case Report

K. Stefanov[*1], *M. Dobrev*[1], *I. Bogeva-Tsolova*[1,2], *S. Trifonov*[1]

[1] *Department of Anatomy, Histology, Cytology and Biology, Faculty of Medicine, Medical University – Pleven, Pleven, Bulgaria*
[2] *Department os Surgery, Faculty of Medicine, Medical University – Pleven, Pleven, Bulgaria*

E-mail: stefantrifonov@outlook.com

Comprehensive understanding of the variations in the branching of the external carotid artery (ECA) is essential to minimize vascular complications during cranio-facial and neck surgical procedures. We demonstrate a rare case of unusual branching of ECAs in both carotid triangles and anomalous origin of the left ascending pharyngeal artery (APA). During routine dissection of embalmed female cadaver, the carotid triangles were carefully dissected, the origin of the caudal branches was detected, and the distance between the origin of each artery and the carotid bifurcation (CB) was measured by vernier caliper. The right and left common carotid arteries (CCA) bifurcated at the level of the upper border of the thyroid cartilage, which is the most typical level according to the literature reports. The right superior thyroid artery (STA) originated anterior to the CB, while the left STA originated from the anterior aspect of the left CCA. The right ECA trifurcated into linguofacial trunk (LFT), APA and distal ECA. The

reported incidence of common LFT is around 20%. On the left side, lingual artery and APA arose as a short common linguopharyngeal trunk (LPT). To our knowledge, this is the first report of a common LPT. The left facial and occipital arteries originated anteromedially and posteriorly at the same level. Variations in the branching pattern of ECA are relatively common. Increasing the knowledge of rare variations might help surgeons and interventional radiologists to avoid vascular complications during procedures on the neck and head.

Key words: external carotid artery, ascending pharyngeal artery, linguofacial trunk, superior thyroid artery, linguopharyngeal trunk

Distribution of Variations of Division of the Sciatic Nerve

A. Todorov[*1], M. Dobrev[1], T. Rashev[1], D. Marinova[1], I. Bogeva[1], M. Shoshkova[1], S. Trifonov[1]*

[1] *Department of Anatomy, Cytology, Histology and Biology, Medical University – Pleven, Pleven, Bulgaria*

* E-mail: alex_9020@abv.bg

Sciatic nerve (SN) is the thickest and longest nerve in the body, which leaves the pelvis by passing through the greater sciatic foramen. SN nerve further divides into two terminal branches – tibial (TN) and common peroneal (fibular) nerves (CPN). The point of division of the SN into the TN and the CPN is variable. The common site is at the junction of the middle and lower thirds of the thigh, near the apex of the popliteal fossa. Variations in the anatomical division of the sciatic nerve are not uncommon. During routine dissection course on lower limb we observed 22 extremities of formalin-fixed cadavers. SN usually divides in the upper angle of popliteal fossa to CPN and TN. In the current study, only 16 (73 %) of the SN confirmed to this standard description. High division of SN in the back of thigh was noted in 4 specimen (18 %) and higher division of SN in the pelvis was noted in 2 specimens (9 %). Variations in the anatomical division of the SN have clinical significance, because if this place of division varies from the pelvis to the popliteal fossa this may contribute to piriformis syndrome, sciatica, coccydynia, and muscle atrophy. Knowledge regarding such variations and differences in the course of SN is important in surgery and important for good clinical practice.

Keywords: sciatic nerve, division, variation

Variations of the Branches of Common Hepatic Artery – A Cadaver Case Report

*I. Bogeva-Tsolova[*1,2], K. Stefanov[1], M. Dobrev[1], S. Trifonov[1]*

[1] *Department of Anatomy, Histology, Cytology and Biology, Medical University – Pleven, Pleven, Bulgaria*
[2] *Department of Surgical Diseases, Medical University – Pleven, Pleven, Bulgaria*

* E-mail: irenbogeva@outlook.com

Knowledge of hepatic vascular anatomy is of great importance for the surgeon to perform abdominal intervention. It is known that changes, present at different stages of embryonic development, lead

to large variations in vasculature. The aim of our study is to show variating common hepatic artery in a cadaver study, where multiple variating vessels were found. This is a cadaveric study, performed during the education process at the Department of Anatomy, Histology, Cytology and Biology in Medical University – Pleven. The study we conducted showed unusual variation of the branches of the common hepatic artery. The celiac trunk is presented normally with its three main branches whereas 5 of the branches of the common hepatic artery in this case arise all together from the same segment, resembling a trunk. Observing and describing vascular variations in the body is the key to expend our surgical anatomy knowledge. Such rare branching of the common hepatic artery provides an opportunity to expand the scope of the vascular anatomy of the liver and this impacts the surgical planning and outcome.

Key words: common hepatic artery, celiac trunk, vascular anatomy

Integrating Medical Humanities and History of Medicine: Strategy for Existing Curriculum Implementation

R. Hage, A. Herry

Department of Medical Humanities and History of Medicine, St. George's University, School of Medicine, True Blue, Grenada

* E-mail: RHage@sgu.edu

Integrating Medical Humanities and History of Medicine (MHHM) into medical education enhances communication, empathy, and the physician-patient relationship. Incorporating MHHM as a standalone course in to an existing curriculum may not always be feasible. An alternative approach, presenting intermittent exposure to MHHM content throughout the basic science curriculum with special emphasis in the anatomy and histology courses, was devised to prime students early. The MHHM content was carefully selected to align with the original lecture topics, ensuring a smooth transition. MHHM faculty prepared two PowerPoint slides. Additional information and suggested approaches were provided to the lecturers, granting them flexibility in incorporating the MHHM material. An overview of how St. George's University has incorporated MHHM content in the gross human anatomy and histology course is demonstrated. Attendees learn how to identify lecture content that lends itself to adding MHHM information and to determine the optimal method of introducing two PowerPoint slides into existing lectures. The strategy of incorporating two slides with MHHM content into existing lectures offers a practical solution to integrate MHHM into medical education. What seemed to be a challenging task has shown to be rather simple. Feedback from students and faculty is presented.

Key words: humanities, history, medicine, curriculum,

Otto Lanz; an Uomo Universale and a Humanistic Physician?

R. Hage, S. Gluschitz

Department of Medical Humanities and History of Medicine, St. George's University, School of Medicine, True Blue, Grenada

*E-mail: RHage@sgu.edu

Medical Humanities, an interdisciplinary field encompassing the arts, social sciences, and humanities aims to stimulate qualities of self-efficacy, interpersonal skills, empathy, social and cultural awareness. This abstract explores the life of Otto Lanz (1865-1934), a surgeon, poet, playwright, art connoisseur, and traveler, to investigate whether his diverse interests and pursuits align with the concept of a humanistic physician. An extensive search in archives and libraries in various languages provided a profile of Otto Lanz. Born in Switzerland, Lanz received a culturally rich education before pursuing medical training throughout Europe. He established his medical practice in Bern and later held the Chair of Surgery at the University of Amsterdam. While Lanz's medical contributions, such as the Lanz point and Hautschlitz apparatus, were notable, his interests extended beyond the realm of medicine. Lanz's writings showcased his fluency in multiple languages, incorporating humor, reflections on art, and candid discussions of professional setbacks. Lanz's accomplishments reflect the ideals of a Renaissance man, whether he embodies the principles of a humanistic physician remains subjective. Contemporary descriptions of humanistic physicians emphasize self-reflection, patient connection, teaching, role modeling, and work-life balance. Although these attributes do not directly connect with the Renaissance, Lanz's life exemplifies the concept of a well-rounded individual. However, the extent to which he embodies the principles of humanistic care in his medical practice is subject to interpretation by modern colleagues and medical professionals.

Key words: humanities, history, art, humanistic

Functional and Structural Analyses to Assess Recovery after Facial Nerve Reconstruction Indicate that Injections of Neurotrophic Factors into Paralyzed Facial Muscles Promote Better Quality of Reinnervation and Motor Performance in Rats

S. Rink-Notzon[1], C. Hadjiparaskeva[2], J. Reuscher[2], L. Wollny[2], S. Pavlov[3], L. Sarikcioglu[4], M. Manthou[5], D. Angelov[2]*

[1] *Department of Prosthetic Dentistry, School of Dental and Oral Medicine, University of Cologne, Cologne, Germany*
[2] *Department of Anatomy I, University of Cologne, Cologne, Germany*
[3] *Department of Anatomy and Cell Biology, Medical University of Varna, Varna, Bulgaria*
[4] *Department of Anatomy, Akdeniz University, Antalya, Turkey*
[5] *Department of Histology and Embryology, Aristotle University, Thessaloniki, Greece*

* E-mail: angelov.anatomie@uni-koeln.de

Results from numerous animal experiments indicate that the major problems after peripheral nerve reconstruction are caused by the response of regenerating axons. First, results from pre- and postoperative retrograde neuronal labeling show that regrowing axons are misrouted and fail to rejoin their original nerve fascicles. Second, triple retrograde labeling reveals that each transected axon gives off up to 25 collateral branches within the nerve itself. Such excessive collateral branching leads to re-innervation of several muscle groups, often with antagonizing action, by one single motoneuron which is the reason for abnormally associated movements (synkinesis). Combined histo- and immunocytochemistry studies show a third problem, which

is the intramuscular sprouting of axons. Upon reaching a motor target, axons undergo an intensive intramuscular sprouting. The numerous axonal sprouts reinnervate simultaneously the neuromuscular junctions (NMJ) of many muscle fibers. Intramuscular sprouting has been regarded as an adaptive mechanism to compensate for reduced functional capacity. However, it has also a "maladaptive" side, characterized by the enlargement of the motor units and by the reinnervation of NMJ by more than one axon, a state known as "NMJ-polyinnervation". Since the first two components of misdirected reinnervation, the axonal navigation (pathfinding) and collateral branching at the lesion site can be hardly treated and prevented, we aimed our efforts at the third component, i.e. the polyinnervation of NMJ. Correlating recovery of facial-muscles function in rats (video-based motion analysis of vibrissal whisking) with histological observations, we found, that motor recovery after facial nerve injury is inversely proportional to the percentage of polyinnervated NMJ. Whereas it is established that the cellular correlate of NMJ-polyinnervation is the excessive sprouting of the terminal Schwann cells (TSC) – the molecular mechanisms that govern this excessive sprouting are poorly understood. Nowadays, it is only known that denervated muscles produce a mixture of short-range diffusible sprouting stimuli, some of which have been identified as trophic factors. Based on earlier own data that blind rats from the Sprague Dawley (SD)/RCS strain restore vibrissal whisking after facial nerve injury completely, we recently compared the expression (mRNA and protein) of brain derived neurotrophic factor (BDNF), fibroblast growth factor-2 (FGF2), insulin growth factors 1 and 2 (IGF1, IGF2) and nerve growth factor (NGF) between SD/RCS and SD-rats with normal vision but poor recovery of whisking function after facial nerve transection and suture. We found a complicated time course of expression with (1) a late rise in BDNF protein that followed earlier elevated gene expression, (2) an early increase in FGF2 and IGF2 protein after 2 days with sustained gene expression, (3) reduced IGF1 protein at 28 days coincident with decline of raised mRNA levels to baseline, and (4) reduced NGF protein between 2 and 14 days with maintained gene expression found in blind rats but not the rats with normal vision. In a logical continuation of these experiments, we investigated the effect of injections with different concentrations of trophic factors into the paralysed vibrissal muscles at different postoperative periods after facial (*r. buccalis*) nerve transection and suture (buccal-buccal anastomosis, BBA). We found that regardless of treatment, the range of vibrissal movements (amplitude) remained lower than in intact rats. Nevertheless, blockade of the post-injury-associated collateral axonal branching with BDNF and fostering of elongation in groups with high-dose FGF2 promoted better restoration of motor performance. We conclude that, after peripheral nerve injury and surgical repair, appropriate target reinnervation, and therefore function, can be restored by administering different trophic factors, which need to be applied over a specific time course and at specific concentrations.

Results from Anthropological Analysis of Bone Remains Excavated in 2021 during the Rescue Archaeological Surveys along the Route of „Hemus" Highway, Site 85-2 South, Village of Gradishte, Shumen District, Bulgaria

N. Atanassova

Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences

* E-mail: naditimeva@gmail.com

In 2021, during the rescue archaeological excavations along the route of the „Hemus“ highway in site 85-2 South near the village of Gradishte, Shumen district (Bulgaria), 17 burial graves are discovered, studied and documented. The burial practice is inhumation. With the exception of grave No. 2, which is from the Late Chalcolithic, all other graves are preliminary dated in the 9th-10th c. AD. The present report includes the results of the anthropological analysis of the skeletons from graves No. 1÷17. In the age-sex identification of the individuals, established anthropological methods are applied. The reconstruction of stature and body mass in adults is carried out depending on the condition of the preserved long limb bones. The established pathological changes are identified according to the methods of Aufderheide, Rodriguez-Martin 1998; Ortner, Putschar 1981; Ortner 2003. In the Late Chalcolithic grave, a skeleton of a 6-7 years old child is found. On the skull of the child is established a trabecular form of *Cribra Orbitalia*. Children are found in 56% of the Medieval burials, incl. a fetus at 40 gestational week. In adults predominated males above 50 years. The occipital bones of two children showed *Serpens endocrania symetrica* – vascular impressions found on the endocranial surface. On the skeleton of an young male is observed a cervical rib attachment on the first right rib. In general, the analysis revealed a large number of cranial variations and paleopathological bone changes of the buried individuals.

Key words: late chalcolithic and medieval skeletons; anthropological analysis; paleopathological analysis; cribra orbitalia; serpens endocrania symmetrica

Observing 3D Bioprinted Constructs in Punch Skin Biopsy

I. Bogeva-Tsolova[1,2], M. Dobrev[1], G. Altankov[3]*

[1] Department of Anatomy, Histology, Cytology and Biology, Medical University – Pleven, Pleven, Bulgaria
[2] Department of Surgical Diseases, Medical University – Pleven, Pleven, Bulgaria
[3] Center of Competence Leonardo Da Vinci, Medical University – Pleven, Pleven, Bulgaria

***** E-mail: irenbogeva@outlook.com

In recent years 3-dimensional bioprinting took quite a leap ahead creating tissues, produced in laboratory environment. The problem with bioprinted products occurs when it comes to their translation into the clinical practice and the survival rate once transferred. This is a study which focuses on the results of punch skin biopsy staining methods in order to observe epithelization after translation of 3D bioprinted tissue over to Wistar rats. The study we conducted showed full epithelization of a soft tissue defect, created on the dorsal region of Wistar rats after grafting a construct created from collagen, mesenchymal stem cells and a silicone scaffold, using 3D bioprinter. We used traditional staining techniques – hematoxylin and eosin, Van Gison and fluorescein which allowed to observe all skin layers with focus on the dermis and collagen fibers. The 3D bioprinted patches showed great endurance by the animal organism and no signs of rejection, infection or any other disturbances. In order to find a representative histological proof for the epithelization we found interesting variations in well known staining techniques.

Key words: bioprinting, staining, epithelization, fluorescein

Preoperative Planning for Complex Surgical Treatment of Acetabular Fractures with Patient-Specific 3D Printed Models

P. Valchanov, S. Ivanov

Department of Anatomy and Cell Biology, Medical University of Varna, Varna, Bulgaria

* E-mail: petar.valchanov@mu-varna.bg

Patient-specific 3D printed models in orthopedics and traumatology are a useful instrument in the planning of the surgical operation. They give a better insight regarding the injury patterns, surgical approaches, reduction techniques, and fracture fixation methods. The objective of this study is to evaluate the effectiveness the 3D printed models for optimal preoperative planning of the surgical treatment of complex acetabular fractures. Patients with complex acetabular fractures were assigned to two groups: Conventional group (n = 12) and 3D printed group (n = 10). Both groups included patients with complex acetabular fractures. Datasets from CT scanning were segmented and converted to STL format, with separated bones and fragments for 3D printing. Comparison between the two groups was performed regarding the quality of fracture reduction, functional assessment, operative time, blood loss, and number of intraoperative x-rays. A significant decrease in operative time, blood loss, and number of intraoperative x-rays was observed in the 3D printed group versus the conventional one ($p < 0.01$), with 80% of the patients in the former having good fracture reduction and 20% having fair reduction. In contrast, 50% of the patients in the onventional group had good reduction and 50% had fair reduction. The functional score at 18-month follow-up was better for patients in the 3D printed group.The 3D printing technique can be considered a highly efficient and patient-specific approach for management of complex acetabular fractures, helping to restore patient′s individual anatomy after surgery.

Three-dimensional layered anatomy of the cervical region. Photogrammetry study and practical application of the 3D database

T. Spiriev[1,2], J. Frederick Cornelius[3], A. Mitev [1], V. Stoykov[1], N. Dimitrov[1], V. Nakov[2], I. Maslarski[1]*

[1]Department of Anatomy and Histology, Pathology and Forensic Medicine, University Hospital Lozenetz, Medical Faculty, Sofia University, Sofia, Bulgaria
[2]Department of Neurosurgery, Acibadem City Clinic University Hospital Tokuda, Sofia, Bulgaria
[3] Department of Neurosurgery, University Hospital of Düsseldorf, Heinrich Heine University, Düsseldorf, Germany.

* Email: spiriev@gmail.com

Cervical region is characterized with complex layered anatomical structure comprising vital blood vessels, cranial nerves and organs. Usually, the study of this anatomy requires considering multiple sources to understand this anatomical region– textbooks, videos, dissection practice,

but this process remains difficult. Modern technology in the form of photorealistic three-dimensional (3D) models is another tool to help this process. The neck region was dissected in four fixed cadavers in two anatomical departments. Three of the specimens were color with color injected vessels. Stratigraphical dissections were done on the posterior, antero-lateral and anterior neck region. Every layer of the dissection was photogrammetrically scanned and presented as photorealistic 3D models. In total forty-four 3D models were created. Each model allowed the visualization of the associated anatomy in various visual angles, which might be beneficial for the study process. The models were annotated and uploaded to an online open-access platform allowing visualization in augmented and virtual reality environment. The resulting 3D models we implemented in the medical student's curriculum in the form of VR and AR classes with high positive feedback from the students.The technology of photorealistic 3D scanning allows for the creation of database of anatomical models useful in the teaching process and as a self-study method. Future analyses of the application and of this technology will show its benefits and potential drawbacks.

Key words: Anatomy, cervical region, 3D, virtual reality, augmented reality, scanning

Anxiety-Related Down-Regulation of Oxytocin Receptors in the Bed Nucleus of Stria Terminalis of Spontneously Hypertensive Rats

A. Gradev[1], L. Jelev[1], V. Iliev[1], P. Rashev[2], A. Dandov[1], N. Lazarov[1]*

[1] Department of Anatomy, Histology and Embryology, Medical University of Sofia, Sofia, Bulgaria
[2] Institute of Biology and Immunology of Reproduction "Acad. Kiril Bratanov", Bulgarian Academy of Sciences, Sofia, Bulgaria

* E-mail: a.gradev@medfac.mu-sofia.bg

In the last decades, the neuropeptide oxytocin (OXT) has attracted a great interest in behavioral neuroscience due to its effects on the different aspects of social and emotional behaviors. The bed nucleus of stria terminalis (BNST), a limbic forebrain structure, has been implicated in stress responses such as fear and anxiety but the specific factors that engage it remain elusive. There is recent evidence that the BNST neurons express high levels of oxytocin receptors (OXTR). To determine the anxiogenic effects of OXT in the BNST, we used spontaneously hypertensive rats (SHR), an established animal model of attention-deficit/hyperactivity disorder, also associated with reduced anxiety levels. We first attempted to identify by immunohistochemistry cells expressing OXTR in certain BNST subnuclei of SHR and then compared their expression levels with those in the corresponding BNST nuclei of normotensive Wistar rats (NWR). We found that the OXTR was expressed at lower levels in the oval and the medial-posteromedial nuclei of the BNST of SHR than in neurons of the same subnuclei in Wistar controls. The OXTR expression was reduced about 20% in hypertensive animals compared to controls, and this decrease was statistically significant. Our results suggest that innate lower anxiety levels of SHR are possibly due to the down-regulation of OXTR in BNST neurons, thus confirming the role of BNST oxytocin stimulated neurons in determining anxiety-like behavior. Overally, the SHR strain can be used in behavioral neuroscience as a valuable model of an impaired central oxytocin system and also in anxiety-related research involving BNST.

Key words: anxiety, bed nucleus of stria terminalis, oxytocin receptors, spontaneously hypertensive rats

Acknowledgements: This study was financially supported by the Medical Science Council at the Medical University of Sofia (Grant № Д-171/03.08.2023).

Expression of Neuropeptide-Y in Brattleboro and Wistar Pre- and Postpubertal Rats

A. Dandov[1], A. Gradev[1], P. Rashev[2], N. Lazarov[1,3], L. Jelev[1]*

[1] Department of Anatomy, Histology and Embryology, Medical University of Sofia, Sofia, Bulgaria
[2] Institute of Biology and Immunology of Reproduction "Acad. Kiril Bratanov", Bulgarian Academy of Sciences, Sofia, Bulgaria
[3]Institute of Neurobiology, Bulgarian Academy of Sciences, Sofia, Bulgaria

* E-mail: adandov@medfac.mu-sofia.bg

The bed nucleus of stria terminalis (BNST) is a limbic structure associated with the regulation of stress, hunger, aggression, and drug addiction. Recent studies have shown that neuropeptide-Y (NPY), a neuromodulator in the nervous system, relieves anxiety-like states and regulates hunger and appetite, and its expression is age- and gender-related. It has been revealed that NPY has higher levels in certain limbic structures in Brattleboro rats, a strain lacking vasopressin, and that it plays a certain role in the initiation of puberty. Thus, we set it as a task to follow the expression of NPY in prepubertal (20 days old) and postpubertal (60 days old) Brattleboro rats and compare it with those in Wistar rat controls. For our experiments, we applied immunohistochemistry for NPY using the avidin-biotin method. Our results showed that NPY was expressed in the neuronal bodies of certain areas of BNST in prepubertal Brattleboro rats, and its expression was better seen in females. On the other hand, a very weak expression was observed in prepubertal Wistar rats. In postpubertal Brattleboro rats, however, NPY expression in neuronal bodies of BNST disappeared. It was only detected in nerve fibers adjacent to the perikarya, the former possibly arriving from the hypothalamus. Such immunostaining was also seen in postpubertal Wistar controls. We can infer from our results that NPY does play a significant role during puberty in the Brattleboro strain and it is a matter of future studies to clarify exactly how it modulates anxiety and eating disorders during sexual maturation.

Key words: anxiety, bed nucleus of stria terminalis, Brattleboro rats, neuropeptide-Y, puberty

Acknowledgements: This study was financially supported by the Medical Science Council at the Medical University of Sofia (Grant № Д-171/03.08.2023).

En face Techniques for Observations of the Macrovascular Endothelium

L. Jelev

Department of Anatomy, Histology and Embryology, Medical University of Sofia, Sofia, Bulgaria

* E-mail: ljelev@medfac.mu-sofia.

To study the vascular endothelium from the surface (*en face*), a number of light and electron-microscopic methods have been used and special histological techniques developed for the past nearly 140 years. These methods and techniques allow examination on smaller or larger areas or even the entire endothelium of a vessel and differ in their ability to study particular characteristics of the endothelial cells. For the present work, of particular interest are the *en face* techniques for obtaining permanent preparations for long-lasting light-microscopic observations of the endothelium. Based on the thickness of the preparations produced these techniques are classified into three main groups: 1) techniques for "whole thickness" preparations, 2) techniques for "partial thickness" preparations and 3) techniques for producing endothelial cell monolayers or "Hautchen" preparations. The methodology and potential scientific application of the techniques in each one of the groups is described and they are thoroughly compared.

Key words: vascular endothelium, *en face* techniques, experimental research

Impact of Type 1 Diabetes Mellitus on the Somatotype of Adult Bulgarian Patients

A. Baltadjiev[1], M. Orbetzova[2], S. Sivkov[1], T. Petleshkova[1], Z. Harizanova[1], A. Fusova[1], M. Ilieva-Gerova[2]*

[1]Department of Anatomy, Histology, and Embryology, Faculty of Medicine, Medical University of Plovdiv, Plovdiv, Bulgaria
[2]Department of Endocrinology, Faculty of Medicine, Medical University of Plovdiv, Plovdiv, Bulgaria

* E-mail: atanas.baltadzhiev@mu-plovdiv.bg; dr_atanas@abv.bg

The aim of the study was to determine the somatotype of elderly Bulgarian patients with type 1 Diabetes mellitus. Sixty male and 60 female patients aged 20 to 40 years were involved in the study. A sample of healthy Bulgarian individuals in the same age range was used as a control group. The measurements were done by direct anthropometry. The Heath-Carter anthropometric method was used to rate the somatotype components of each participant. The somatotype of the female patients was mesomorphic endomorph (endo 5.37; meso 4.51; ecto 1.42). The endomorphic component was dominant, followed by the mesomorphic component and the ectomorphic component with the lowest rating. The somatotype of the control females was balanced endomorph (endo 3.74; meso 2.81; ecto 2.98). The endomorphic component was dominant, but the mesomorphic and ectomorphic components were equally presented. The values of endomorphic and mesomorphic components were significantly higher in the

female patients than in the healthy women. Both male patients and controls presented with endomorphic mesomorph somatotype: patients (endo 3.94; meso 4.66; ecto 2.37) and controls (endo 4.34; meso 5.19; ecto 2.22). The mesomorphic component was dominant, followed by the endomorphic component and the ectomorphic component was with the lowest rating. The mesomorphic component was significantly greater in the healthy males than in male patients. The somatotype of the Bulgarian female patients is mesomorphic endomorph, while of the healthy Bulgarian females is balanced endomorph. Both Bulgarian male patients and healthy males have endomorphic mesomorph somatotype.

Key words: anthropometry, body composition, Bulgarians, somatotype, type 1 Diabetes mellitus

Lectin Histochemistry of the Most Superficial Zone of the Normal Articular Cartilage during the Postnatal Reriod.

A. Fedotchenko

Zaporizhzhia State Medical and Pharmaceutical University, Zaporizhzia, Ukraine

* E-mail: afedotchenko@gmail.com

The existence of the most superficial zone (MSZ) of the articular cartilage (AC), is claimed by many authors. Its structure remains controversial. The hip joint of Wistar rats from birth till the 90th day was studied. Histologic sections were stained with hematoxylin and eosin (H&E) as well as lectins conjugated with horseradish peroxidase (HRP): peanut (PNA), soybean (SBA), Helix pomatia (HPA), wheat germ (WGA), Perca fluviatilis (PFA), Sambucus nigra (SNA), Lens culinaris (LCA), and Vicia sativa (VSA), analyzed by light microscopy and a semi-quantitative assessment. The MSZ was clearly distinct from the underlying AC as a layer with intense pink staining. Three sublayers could be seen in it: the outermost (closest to the articular surface) finest sublayer with a strong expression to all studied lectins; the middle (thickest) sublayer, containing cells (with affinity from weak (+) to moderate (++)) and extracellular matrix (expression from minimal (0/+) to weak (+)); the third (the innermost) sublayer as a continuous, relative thick lamina (with a strong expression, from +++ to ++++) that clearly delimited the MSZ from the underlying AC. The lectins affinity of the MSZ did not fluctuate significantly till the 90th day. The constant, strongly expressed double lectin-mediated barrier of the MSZ seems to emphasize its immunobiological protective importance for the AC.

Key words: articular cartilage, hip joint, lectins.

Glucagon- and Insulin Immunopositive Mast Cells in Porcine Gallbladder

I. Stefanov[1,4], S. Stefanov[2], M. Gulubova[3,4]*

[1] Department of Anantomy, Medical Faculty, Trakia University, Stara Zagora, Bulgaria
[2] Sixth year student at Medical Faculty, Trakia University, Stara Zagora, Bulgaria

[3] *Department of General and Clinical Pathology, Medical Faculty, Trakia University, Stara Zagora, Bulgaria*
[4] *Department of Anatomy, Histology and Embryology, Pathology, Medical Faculty, Prof. Dr. Asen Zlatarov University, Burgas, Bulgaria*

* E-mail: ivstefanov@abv.bg

It is well known that mast cells produce and release biologically active substances such as histamine, heparin, proteases, leukotrienes, cytokines, chemokines, and growth factors. However, their capacity to produce insulin and glucagon has not been established yet. We aimed to perform immunohistochemical study to determine the glucagon and insulin positive mast cell existence and distribution in the wall of porcine gallbladder. The collocalization of glucagon and insulin immunopositivity with metachromasy allowed us to detect the presence of glucagon and insulin positive mast cells in the bottom, body and neck of gallbladder in male pigs. In addition, an immunohistochemical detection of tryptase as a better marker for mast cells than toluidine blue dye was used to compare the number of mast cells to glucagon and insulin immunopositive cells. In all layers of gallbladder`s neck, the density of all types of mast cell was highest, followed by the body and bottom of gallbladder. The tryptase immunohistochemistry comparing to toluidine blue staining appeared to be more reliable technic for identifying all glu+ and ins+ mast cells. Glucagon- and insulin expression by mast cell granules allowed us to hypothesize a new unknown by now role of these cells regarding their participation in the regulation of the glucose homeostasis as well as the involvement of mast cells in the pathogenesis of diabetes mellitus. The presence of glucagon and insulin in mast cell granules may be due to the ability of mast cells to accumulate or to synthesize these hormones.

Key words: chromogranin A, gallbladder, glucagon, insulin, mast cells

Association Between Foot Arch Index and Other Morphometric Indices

D. Marinova, M. Angelova, V. Zhekova[1], S. Pavlov*

Department of Anatomy and Cell Biology, Medical University "Prof. Dr. P. Stoyanov"- Varna, Varna, Bulgaria

* E-mail: desislavamarinova81@gmail.com

The foot morphology is crucial for the normal functioning of the locomotor system. Abnormalities and deviations of its shape are associated with various conditions that interfere with and encumber daily life, work and sports activities. Our study is focused on the types of foot among the Bulgarian population. Footprints of randomly selected 150 Bulgarians aged 18 to 60 years were collected. We examined the morphological features of each foot by measuring the arch index, the Clarke angle and the Chippaux-Smirak index. The participants were grouped according to arch index values in three groups: with normal, with high, and with flat arched foot. Our results showed a greater incidence of high arched foot versus low arched foot (29% vs 11%). The study of the arch index once again confirmed the association of the Morton's toe foot with a high medial arch. BMI within the groups with different foot type was compared. In the group with high arched foot, BMI was lower than that in the other groups. However, further investigation of the possible relations between BMI and arch index found no significant correlation between them. We studied

the correlation between the used foot indices and found a particularly well-expressed one between the arch index and Chippaux-Smirak index. Our findings gave us reason to assume that the measurement of Chippaux-Smirak index can successfully replace the measurement of arch index, which would facilitate the assessment of foot type.

Key words: foot, foot indices, arch index

Asymmetry in Body Composition Variables of Youth Athletes

A. Dimitrova

Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, Sofia, Bulgaria

* E-mail: albena_84@abv.bg

Assessing bilateral differences in paired anthropometric features is an important methodological problem in sports anthropology. The present study includes 128 (59 rhythmic gymnasts, 58 tennis players, and 11 swimmers) adolescent female athletes. Body composition components were determined using multi-frequency bioelectrical impedance measurements (analyzer InBody 170). Statistical analysis is made by SPSS 16.00 for Windows. Asymmetry coefficients of muscle and fat mass accumulation in the upper (AA) and lower (AL) limbs were calculated by Nacheva` equation (1986). The percentiles method was applied to distribute the bilaterally studied anthropometric features according to the mean values of the units of asymmetry (UA). Wilkoxon-test is used to assess the statistically significant differences in paired variables ($p \leq 0.05$). Kruskal-Wallis test ($p \leq 0.05$) is applied to determine the differences in UA between three assessed athlete groups, depending on their age. The differences in body composition components between RG, TP, and SW are well expressed in all assessed age groups. The most considerable inter-group differences are observed in terms of the asymmetry coefficient in the lean body mass (LBM) and body fat mass (%, kg) of UL, which have signed the highest values in the tennis players group. Swimmers have significantly the lowest values of UA for all body segments. A close relation is found between asymmetry in body composition variables and the type of sports activity. Tennis was found as a sport with more pronounced inter-limbs asymmetry.

Key words: tennis players, rhythmic gymnasts, asymmetry, body composition, adolescent

Comparison of Inter-Incisive Index Between Bulgarians and Other Balkan Nations

V. Zaykova[*]*, Z. Harizanova, A. Baltadjiev, F. Popova, N. Kadreva, Z. Todorova, M. Kanarev, K. Topalova, S. Sabri*

Department of Anatomy, Histology and Embryology, Faculty of Medicine, Medical University – Plovdiv, Bulgaria

* E-mail: Vyara.Zaykova@mu-plovdiv.bg

In recent years, aesthetic dentistry has become a major focus for the public. Facial attractiveness plays a key role on modern society and the creation of harmonious smile is a purpose for every dentist. The aim of this study was to define certain values of the inter-incisive index in Bulgarians, the sexual dimorphism and bilateral asymmetry and to verify differences of this index between Bulgarians and other Balkan populations. The present study included 121 males and 111 females of Bulgarian origin aged 20-40 years. Mesiodistal dimensions of maxillary central and lateral incisors were measured by Dentistry Sliding Vernier Caliper. We used the technique of direct anthropometry, modified by Prof. Y. Yordanov. We calculated the inter-incisive index as ratio of the mesiodistal dimension of maxillary lateral incisor to the mesiodistal dimension of the maxillary central incisor. The measurements were analyzed with SPSS 23. The level of statistical significance was set at $P< 0.05$. The inter-incisive index showed no statistically significant differences between left and right side of the dental arch in both sexes and between the sexes as well. This justifies the use of average values for the dimensions of left and right maxillary central and lateral incisors. On the other hand, we found statistically significant differences in MD values of incisors between Bulgarians and other Balkan nations. Inter-incisive index shows no sexual dimorphism and bilateral asymmetry in Bulgarians. This can be helpful in aesthetic dentistry, in prosthodontics and in orthodontic treatment planning.

Key words: incisors, proportions, hypodontia, prosthetics, orthodontics

Study on the digital transformation of Histology and Histopathology by Virtual Microscopy (VM) for an innovative medical school curriculum

Z. Harizanova[*]*, F. Popova, S. Novakov, Y. Koeva, N. Penkova, P. Atanasova*

Department of Anatomy, Histology and Embryology, Faculty of Medicine, Medical University – Plovdiv, Plovdiv, Bulgaria

* E-mail: zdravka.harizanova@mu-plovdiv.bg

Medical histology has been a basic science course in the medical school curriculum worldwide. A method such as light microscopy is dependent upon the availability of microscopy lab with rigid opening hours and a teacher. Virtual microscopy (VM) involves digitally photographing tissue sections on microscope slides and provides a physically distant opportunity for histology education. Its software reproduces a high-quality image with meticulous clarity and added features that allow students and teachers to highlight, annotate, and zoom. The aim of this project is the implementation of digital/virtual microscopy (VM)/ whole slide imaging (WSI) in undergraduate and postgraduate students' histology and histopathology education. Experts from Medical Universities of five countries participate in the project – Romania, Poland, Spain, Greece, and Bulgaria. 400 students will participate in a survey giving their opinion on the new method. 200 histology and histopathology slides will be scanned by the Romanian partners performed by Leica Aperio AT2 scanner. The result would be the creation of a large digital slides collection of normal and pathological human tissues and its implementation in a VM library platform in histology and histopathology. The possibility to combine and gather specimens from several universities would be useful both for teaching staff and undergraduate and postgraduate students. Virtual microscopy represents a modern tool, with increased quality

and utility in microscopy education. This project is the first of its kind in Bulgaria and would contribute a lot to the implementation of VM in histology and histopathology education.

Key words: virtual microscopy, light microscopy, histology, medical education

CT and 3D Anatomical Study of the Liver in the Chinchilla (Chinchilla lanigera)

Ö. *Dilek[1], R. Dimitrov[2], K. Stamatova-Yovcheva[2*], M. Ersen[3], D. Yovchev[2], E. Karakurum[1]*

[1]Department of Anatomy, Faculty of Veterinary Medicine, Mehmet Akif Ersoy University, Burdur, Turkey
[2]Department of Veterinary Anatomy, Histology and Embryology, Faculty of Veterinary Medicine, Trakia University, Stara Zagora, Bulgaria
[3]Bucak State Hospital, Department of Radiology, Bucak/Burdur, Turkey

* E-mail: kameliastamatovayovcheva@gmail.com

Single cases of neoplastic processes in the liver of chinchillas have been described. At the same time, the chinchilla is widely used as model for different scientific researches. The aim of the present study was to investigate CT and 3D imaging anatomical features of the chinchilla's liver. Twelve clinically healthy chinchillas (six males and six females) aged eighteen months were used. The animals were positioned in dorsal recumbency. As bone markers were used the vertebrae from Th8 to L2 and the sternum for transverse CT study. The vertebrae, the costal arch, the soft abdominal wall, the diaphragm, the stomach, and the right kidney were used as markers for CT coronal study. 3D reconstructions were made with the aid of a specific imaging program. On transverse and coronal CT images the chinchilla's liver was composed by liver by the left lateral lobe, middle lobe, right lobe and caudate lobe. The middle lobe was separated in left middle lobe and right middle lobe. The right lobe was presented by lateral and medial part. There was anatomical contact between the liver and the fundus and body of the stomach. The caudate process was in close contact with the right kidney. The gall bladder was elongated and ellipsoid. 3D reformat images confirmed the results, obtained by transverse and coronal CT study. The CT density of the liver in HU was 195.6±73.1. The presented CT and 3D reconstructed images are with high resolution. This data could be used as a morphological and imaging base.

Key words: chinchilla, liver, CT, 3D reconstruction

Effect of Aronia Supplementation on Aging Markers in the Testis

E. Daskalova[1], Y. Koeva[1], M. Pencheva[2], M.-F. Kitova[3]*

[1] Department of Anatomy, Histology and Embryology, Medical Faculty, Medical University of Plovdiv, Plovdiv, Bulgaria
[2] Department of Medical Physics and Biophysics, Faculty of Pharmacy, Medical University of Plovdiv, Plovdiv, Bulgaria

[3] *Medical Faculty, Medical University of Plovdiv, Plovdiv, Bulgaria*

E-mail: Elena.Daskalova@mu-plovdiv.bg

Spermatogenesis is a process that continues until the end of an individual's life, although with reduced activity with advancing age. Structural changes in the testicle are manifested by changes in the wall of the seminiferous tubules, a consequence of inflammatory, hormonal or external influences. The aim was to study the effect of Aronia on structural and functional changes in the testis in old rats, by measuring the intensity of expression of Neurotrophic tyrosine kinase receptor 3 (TrkC) and Neurotrophin 3 (NT-3) in Leydig cells and some morphological markers in testis. 18 male Wistar rats were divided into 3 experimental groups: young controls aged 2 months, old controls aged 27 months and 27 months old rats supplemented with chokeberry juice at a dose of 10 ml/kg for 3 months. The animals were bred and delivered from the Vivarium of the MU Plovdiv. The results showed significant differences between young and old animals in expression of the age-related changes that occurred. The morphometrically determined tubules structure data showed no significant differences between the three groups. However, the intensity of the immunoreaction for TrkC and NT-3 was demonstrably higher in the supplemented adult animals compared with the adult controls. These data indicate that supplementation with Aronia juice, thanks to its rich composition of antioxidant ingredients, slows down aging processes in the testis and preserves the functional activity of Leydig cells. Aronia supplementation can be recommended as a natural tool for healthy aging.

Key words: black chokeberry (Aronia melanocarpa), antioxidant activity, functional foods, spermatogenesis, aging

Markers of Oxidative Stress in the Testis are Affected by Aronia Melanocarpa

E. Daskalova[1], Y. Koeva[1], M. Pencheva[2], M.-F. Kitova[3]*

[1] *Department of Anatomy, Histology and Embryology, Medical Faculty, Medical University of Plovdiv, Plovdiv, Bulgaria*
[2] *Department of Medical Physics and Biophysics, Faculty of Pharmacy, Medical University of Plovdiv, Plovdiv, Bulgaria*
[3] *Medical Faculty, Medical University of Plovdiv, Plovdiv, Bulgaria*

***** E-mail: Elena.Daskalova@mu-plovdiv.bg

Inflammation, oxidation, and apoptosis are events considered as predictors of pathogenesis and the development of age-related diseases observed in aged testes. Pro-oxidant and a pro-inflammatory microenvironment are characteristic of aged testes. One of the major reason for diminished semen quality seems to involve the accumulation of reactive oxygen species that accompanies aging. Use of natural compounds with known antioxidant and anti-inflammatory properties have a beneficial effect on the inflammatory and oxidative status of the aged testis. The aim of the present study was to determine the effect of supplementation with antioxidant rich Aronia melanocarpa on markers of oxidative stress in rat testis. 18 male Wistar rats were divided into 3 experimental groups: young controls aged 2 months, old controls aged 27 months and 27 months old rats supplemented with Aronia juice at a dose of 10 ml/kg for 3 months. Suplemented with Aronia group showed increased immunoexpression of NOS1, NOS3 and

MAS1 receptor both in the seminiferous tubules (spermatogenic epithelium, peritibular myofobroblasts) and in the blood vessels wall in comparison to the young and old control groups of rats. Higher expression of NOS1, NOS3, and MAS1 in the testes suggests that animals receiving Aronia have an increased antioxidant capacity. This can provide improved spermatogenic- and steroidogenic functional activity resulting in reduced age dependent testicular disfunction, better spermatogenesis, and preserved androgen production.

Key words: aging testis, oxidative stress, antioxidant activity, black chokeberry (Aronia melanocarpa)

Gastric Mucosal Mast Cell Activity in Children on Longitudinal Enteral Feeding with Percutaneous Endoscopic Gastrostomy, PEG

N. Penkova[1], P. Atanasova[1], I. Yankov[2], V. Yankova[3], P. Hrischev[4]*

[1]. Department of Anatomy, Histology and Embryology, Faculty of Medicine, Medical University of Plovdiv, Plovdiv, Bulgaria
[2]. Department of Pediatrics and Medical Genetics, Faculty of Medicine, Medical University of Plovdiv, Plovdiv, Bulgaria
[3]. Faculty of Medicine, Medical University of Plovdiv, Plovdiv, Bulgaria
[4]. Department of Physiology, Faculty of Medicine, Medical University of Plovdiv, Plovdiv, Bulgaria

* E-mail: nadja_penkova@abv.bg

Longitudinal enteral feeding via percutaneous endoscopic gastrostomy, (PEG) challenges the gastric mucosa. It is carried out in conditions of disturbed physiology - all stages of the digestive process until the food enters the stomach are absent. There is a risk of infection of the gastrointestinal tract (GIT), allergens. The aim of our study is to make a morphological and morphometric analysis of the gastric mucosa of children with longitudinal enteral feeding through PEG with an emphasis on the inflammatory component. The biopsy samples from the stomach of 37 children without GIT disease at age 0-18 years (20 of them are on oral feeding - controls and 17 on enteral feeding duration of 24–48 month through PEG) are examined with light-microscopy and morphometric analysis. We find the scanty amount of loose connective tissue in the lamina propria of children with PEG. The glands are next to each other with slightly hyperemic capillaries between them. The total cell count and the number of parietal cells in the fundic glands of children with PEG is double that the controls. In isolated cases is noticeable an increased number of mast cells some of them degranulate. The predominance of the glandular component over the connective tissue component in the gastric mucosa of children with PEG is probably an adaptation mechanism of the body for maximum absorption of nutrients from the enteral diet. Regarding increased mast cell activity further studies are needed to clarify whether this finding is related to the PEG or to the child‘s underlying disease.

Key words: enteral nutrition, paediatrics, percutaneous gastrostomy, tissue morphometry, mast cell

Morphological Study on the Acute and Subacute Toxic Effects of Tanacetum Parthenium Essential oil After Treatment of Experimental Animals

N. Penkova[1], P. Atanassova[1], L. Peychev[2], P. Hrischev[3]*

[1] Department of Anatomy, Histology and Embryology, Medical Faculty, Medical University of Plovdiv, Plovdiv, Bulgaria
[2] Department of Pharmacology and clinical pharmacology, Medical Faculty, Medical University of Plovdiv, Plovdiv, Bulgaria
[3] Department of Physiology, Medical Faculty, Medical University of Plovdiv, Plovdiv, Bulgaria

* E-mail: nadja_penkova@abv.bg

Tanacetum parthenium (T. Parthenium) is a perennial herbaceous plant of the Asteraceae family reaching. It is native to Europe, specifically the Balkan Peninsula, Asia but cultivation has spread it around the world. T. parthenium had an important role in the traditional medicine. The plant has been used to treat arthritis, asthma, constipation, dermatitis, earache, fever, headache, inflammatory conditions, toothache, etc.. The aim of our study is to determine the toxicity of Tanacetum parthenium essential oil in Wistar rats to detect possible pathological changes in different organs of treated animals. 24 Wister rets were used. 8 treated with Tanacetum parthenium essential oil (EO), 8 – a control group. Using the method proposed by Litchfield and Wilcoxon, the average lethal dose (LD50) of the EO on Wistar rats was determined for two routes of administration: oral and intraperitoneal. The subacute toxicity of the EO was also tested by oral administration of a daily dose of 1.0 g/kg b.w. for 28 days. The control group received saline and olive oil 1ml/kg b.w.. Haematological examination showed no toxicity. Blood and biochemical parameters are within the reference values. Histological examination of the internal organs establishes a normal structure without pathological changes. Histological and haematological studies doesn't show an evidence of toxic effects of Tanacetum parthenium essential oil and it is safe for use in doses below 1 g/kg b.w. for a period not exceeding one month. The wide distribution of this herb in Bulgaria can be used to create a new medicinal products and food supplements.

Key words: Tanacetum parthenium, essential oil, toxicity, biochemistry, histology

Risks in Cyber Environment

J. Aleksovski[1], N. Pirovski[2], I. Georgieva[3]*

[1] Student, Faculty of Medicine, Trakia University, Stara Zagora, Bulgaria
[2] Department of Anatomy, Faculty of Medicine, Trakia University, Stara Zagora, Bulgaria
[3] Philosophy of Science Department, Institute of Philosophy and Sociology, Bulgarian Academy of Sciences, Sofia, Bulgaria

* E-mail: yovanche.aleksovski.18@trakia-uni.bg

Cyber environment is a new type of interaction media. This implies a modification of existing risks and a new type of risks, covered by an instrument for risk evaluation. Existing risk

evaluation instruments, psychology risk questionnaires and cyber risks categories. Instrument for cyber risk evaluation with **a** specific modifications of the existing physical and psychological risks and the new type- cyber risks. Cyber environment is new, but very common and powerful. This creates an opportunity for improved functionality, but also for increased risk. The risk of using a cyber-environment is not limited to the field of information. It is a complex, personal, bio-psycho-social risk with daily and mass exposure and diverse consequences. Its assessment is difficult and currently inadequate in the direction of underestimation.

Key words: risk, cyber, psychology, philosophy of medicine

Acknowledgment: 1. Project of the Medical Faculty of Trakia University №16/2022 Visualization of formal complexes in Wushu through graphic figures of their steps. 2. Contemporary Issues and Discussions in the Philosophy and Sociology of Medicine (2021-2024). Institute of Philosophy and Sociology. Bulgarian Academy of Sciences. Department Philosophy of Science. Project manager: Assoc. Prof. Julia Vasseva-Dikova

Osteogenesis in Rat. Factors Influencing Osteogenesis

N. Kadreva[*], *N. Penkova, P. Atanasova*

Department of Anatomy, Histology and Embryology, Faculty of Medicine, Medical University of Plovdiv, Plovdiv, Bulgaria

* E-mail: n.kadreva09@gmail.com

The process of bone formation is called osteogenesis or ossification. Osteogenesis is a core component of the skeletal system and depends on the well-coordinated and developed proliferation and differentiation of osteogenic cells. Multiple signaling pathways, transcriptional factors, environmental, systemic conditions and many more tightly regulate the process of osteogenesis. Any abnormities in bone formation could cause severe disorders. Bone regeneration, a complex and well-orchestrated physiological process of osteogenesis, remains a medical challenge in the field of orthopedics and maxillofacial surgery as well as tissue engineering approaches promoting regeneration by targeting osteogenesis. The current developments in the research in this field still aim to understand the complexity of the process of osteogenesis and its influencing factors. The current report will examine osteogenesis and development of bone in rats and review some of the factors pointed above that are under current research and show the most common and well described influence on the process and the cells. Over the last 20 years there has been an immense rise in the number of publications on the osteogenic potential of different influencing factors and the review will provide statistical significance a novel classification and latest data on the issue. In terms of influencing factors we point the ones that have inducing or suppressing effect on the process and mainly on the differentiating cells - Mesenchymal stem cells – MSC(s), Osteoblasts, Osteoclasts and Osteocytes. We can classify those factors that commit to differentiation at multiple levels, tuned with the involvement of biochemical pathways, transcriptional and growth factors, signaling molecules, pharmacological substances, hormones and nutrients and newly researched systemic or microenvironmental conditions.

Key words: osteogenesis, influencing factor, bone regeneration

Congenital Neural Tube Defects and the Meaning of their Prophylaxis

P-P. Petrov[1], E. Daskalova[1], T. Kitova[1], M. Zdravcheva[2], S. Kostova[2], S. Todorova [2]*

[1] Department of Anatomy, Histology and Embryology, Faculty of Medicine, Medical University of Plovdiv, Plovdiv, Bulgaria
[2]. Students, Faculty of Medicine, Medical University of Plovdiv, Plovdiv, Bulgaria

* E-mail:dr.petar.preslav@gmail.com

Neural tube defects (NTDs) are the most common birth defects that cause perinatal and infant mortality, lifelong disability, high medical care costs, and psychological trauma for affected families. The global prevalence of DNT is 2 per 1,000 births, with a disproportionately high incidence in developing countries. Risk factors for the occurrence of sporadic DNT include the nutritional status of the mother, pre-pregnancy diabetes, use of antiepileptic drugs and a previous pregnancy affected by DNT. Fetuses result from medically terminated pregnancies, intrauterine deaths, spontaneous abortions, and neonatal deaths after authorization for autopsy and genetic testing. The types of DNT and the embryonic mechanisms for their occurrence, the possibilities for their prenatal diagnosis and their prevention are discussed. The study is based on inter-university research projects: Fetal and placental pathology (project no. HO/DP – 03/15). Fetal morphology, development of the fetal brain, physiology and pathology of the nervous system (project no. P-8514). Exencephaly, anencephaly and cranial meningoencephalocele were examined from cephalic DNTs, and rachischisis and myelomeningocele from spinal ones. The necessary system for research and evaluation of implemented health programs and public health policy necessary to prevent the occurrence of STDs and their adverse outcomes is discussed. Public health policy is needed to implement specialized programs to prevent the occurrence of DNT and involve multidisciplinary teams that provide care to individuals with DNT to enable prevention and improve care and rehabilitation for affected children.

Key words: neural tube, psychological trauma, risk factors

Ostarine (SARM) and Endurance Training Affect Glycogen Content in Rat Liver, Heart and Skeletal Muscle

F. Gerginska[1], S. Delchev[1], V. Vasilev[2], K. Georgieva[2], N. Boyadjiev[2], M. Denev[3], M. Komrakova[4]*

[1] Department of Anatomy, Histology and Embryology, Faculty of Medicine, Medical University of Plovdiv, Plovdiv, Bulgaria;
[2] Department of Physiology, Faculty of Medicine, Medical University of Plovdiv, Plovdiv, Bulgaria;
[3] Student, Faculty of Medicine, Medical University of Plovdiv, Plovdiv, Bulgaria;
[4] Department of Trauma Surgery, Orthopaedics and Plastic Surgery, University Medical Center Goettingen, Goettingen, Germany

E-mail: Fanka.Gerginska@mu-plovdiv.bg

A number of diseases are accompanied by a decrease in muscle mass and physical function. Non-steroidal selective androgen receptor modulators (SARMs) have potent anabolic and androgen-

reducing effects. The information about the effect of SARMs on glucose metabolism and glycogen content in target organs with long-term use and in combination with exercise is insufficient. Male Wistar rats were divided into two groups - endurance trained and non-trained. Half of the trained and non-trained rats received SARM (Ostarine) for 8 weeks. PAS staining for glycogen in liver, heart, soleus and EDL was applied, followed by morphometric and statistical analysis. The trained rats had higher soleus weight and higher glycogen content in liver, heart and skeletal muscles compared to non-trained. SARM administration increased soleus glycogen in trained animals. Rats given Ostarine had significantly lower blood glucose than those treated with vehicle. Non-steroidal SARMs have weak or absent androgenic effects and similar or even better anabolic effects compared to testosterone. The reduction in blood glucose we found confirms the results of other authors. Ostarine increases the glycogen content in the examined muscles in trained rats, which proves the involvement of androgens in glycogen metabolism. These changes are mainly expressed in soleus, who actively participates in endurance training. This fact emphasizes the stimulating effect of SARMs in the adaptation to submaximal training. SARMs enhance the effect of endurance training on glycogen content in type I-predominant muscle fibers, which may improve the physical performance.

Key words: endurance training, glycogen, Ostarine, selective androgen receptor modulators (SARMs), rats

Mast Cells in Rat Pulmonary Pleura

I. Ivanova[1], I. Stefanov[1,2], V. Pilicheva[1]*

[1] Department of Anatomy, Medical Faculty, Trakia University, Stara Zagora, Bulgaria
[2] Department of Anatomy, Histology and Embryology, Pathology, Medical Faculty, Prof. Dr. Asen Zlatarov University, Burgas, Bulgaria

* E-mail: ivelina.ivanova@trakia-uni.bg

Mature and immature Wistar rats have been widely used to study the role of metachromatic and tryptase positive mast cells in pathological conditions of the lung, particularly the lung pleura. However, the knowledge of age-related features in the distribution of different phenotypes of mast cells in the lung pleura of healthy rats is scarce. We aimed to perform immunohistochemical study to determine the metachromatic, tryptase and ghrelin positive mast cells in pulmonary pleura of male rats at different ages. The collocalization of tryptase and ghrelin immunoreactivity with metachromasy allowed us to estimate the number of tryptase, ghrelin positive and metachromatic mast cells in lung pleura of male Wistar rats at different ages. We revealed that the amount of toluidine blue positive mast cells in the lung pleura was significantly less than that of triptase and ghrelin positive mast cells. The number of triptase and ghrelin positive mast cells showed similar values. In the comparative study on serial sections, we observed that some MCTr and GhrC did not show metachromasia. In the subserous layer of the pleura, the percentage of MCTr and GhrC with manifested metachromasia varies in different age groups: 58-59% in 1-year-old, 42% in 3-month-old and 20% in 20-day-old animals. The similar number of tryptase and ghrelin positive mast cells defines mast cells as a main source of ghrelin in pleura. Mast cell tryptase is a known smooth muscle mitogen participating in mast cell-mediated remodeling of the pleural vasculature but mast cell ghrelin may regulate contractility of vascular smooth muscle cells. It was found that immunohistochemical identification of mast cells by demonstration of tryptase in the rat lung is more reliable than toluidine blue staining. Mast cells are the main source of ghrelin in pleura.

Key words: mast cells, ghrelin, tryptase, pleura, rat

Variation of the Temporal Branches of the Facial Nerve Depending on the Branching Pattern

A. Babuci[1], I. Catereniuc[1], Z. Zorina[1], A. Sivreva[2]*

[1] Department of Anatomy and Clinical anatomy, Nicolae Testemitanu State University of Medicine and Pharmacy, Chisinau, Moldova
[2] Department of Medical psychology, social activities and foreign languages, Faculty of Medicine, Trakia University, Stara Zagora, Bulgaria

* E-mail: angela.babuci@usmf.md, t_o_n_y79@abv.bg

The temporal branches of the facial nerve are very susceptible to iatrogenic injures in plastic and reconstructive surgery of the upper third of the facial region. The aim of the study was to determine the distribution of the temporal branches depending on the facial nerve branching pattern. The research was conducted on 75 formalized hemiheads of adult cadavers (59 males and 16 females), at the Department of anatomy and clinical anatomy of „*Nicolae Testemitanu*" University. By ANOVA one-way test were established the intragroup and intergroup variations of the temporal branches depending on the facial nerve branching type. The number of the temporal branches varied from 1 to 4 branches: 1 branch (17.4%), 2 branches (49.3%), 3 branches (20%), 4 branches (13.3%). In males were determined 1-4 temporal branches and in females 1-3 branches. Depending on the branching pattern the mean number of the temporal branches was: Type I – 2.1±1.00 (95% CI 1.5-2.6); Type II – 2.3±0.65 (95% CI 1.9-2.7); Type III – 2.0±0.68 (95% CI 1.6-2.4); Type IV – 2.3±0.90 (95% CI 1.7-2.8); Type V – 2.0±0.82 (95% CI 1.2-2.8); Type VI – 2.8±1.12 (95% CI 2.2-3.4); Type NI (all the bizare branching patterns) – 2.7±1.03 (95% CI 1.8-3.5), p=0.263. The distribution of the temporal branches of the facial nerve was variable depending on the branching pattern. The highest mean value of the temporal branches was characteristic for Type VI and the lowest one for Type II and Type V.

Key words: temporal branches, facial nerve, branching pattern

Variational Morphology of the Pancreas Based on Imaging Study

Z. Zorina[1], I. Catereniuc[1], A. Babuci[1], A. Sivreva[2]*

[1] Department of Anatomy and Clinical anatomy, Nicolae Testemitanu State University of Medicine and Pharmacy, Chisinau, Moldova
[2] Department of Medical Psychology, Social Activities and Foreign Languages, Faculty of Medicine, Trakia University, Stara Zagora, Bulgaria

* E-mail: zinovia.zorina@usmf.md, t_o_n_y79@abv.bg

The pancreas is frequently subjected to surgical interventions and knowledge about its anatomo-topographical peculiarities and variability is of high clinical significance. The purpose of the study was to establish the anatomical variation of the pancreas based on imaging methods. The study was carried out on 35 patients, hospitalized with acute pancreatitis in the Clinical Republican Hospital „Timofei Mosneaga". The computed tomography and cholangiopancreatography images were taken, on the base of which the morphological structure of the pancreas, the

presence and course of the pancreatic ducts, and the type of their fusion with the common bile duct were studied. Using computed tomography, the structural variants of the pancreas were identified in 28.6% of cases: lobulated pancreas – in 11.4%, diffuse pancreatic steatosis – in 8.6%, hypoplasia of the pancreas – in 5.7% and accessory pancreatic lobule, located above neck of the pancreas, with the opening of its duct into the Wirsung duct was revealed in 2.9%. By cholangiopancreatography, variants of the course and fusion of the pancreatic ducts were identified in 14.3% of cases: sigmoid course of the Wirsung duct – in 8.6%, loop-shaped course of the Wirsung duct – in 5.7%, fusion of the Wirsung and Santorini ducts – in 2.9%, and the sigmoid-shaped Santorini duct – in 2.9%. The modern imaging methods are the most informative in identifying the anatomical variants of the pancreas, which detection is important for the efficiency of the therapeutic management of patients with clinical symptoms of acute pancreatitis.

Key words: pancreas, anatomical variants, imaging

The Role of Vascular Endothelial Growth Factor in Myocardial Aging

A. Iliev[1], G. Kotov[2], N. Stamenov[1], B. Landzhov[1], V. Kirkov[3], L. Gaydarski[1], S. Stanchev[1]*

[1] Department of Anatomy, Histology and Embryology, Medical University of Sofia, Sofia, Bulgaria
[2] Clinic of Rheumatology, University Hospital 'St. Ivan Rilski', Department of Rheumatology, Medical University of Sofia, Sofiaq Bulgaria
[3] Department of Health Policy and Management, Faculty of Public Health 'Prof. Dr Tzekomir Vodenicharov', Medical University of Sofia, Sofia, Bulgaria

* E-mail: dralexiliev@abv.bg

Vascular endothelial growth factor (VEGF) is a key signalling protein in angiogenesis. Despite the significant number of studies over the last few decades, its precise role in the complex regulatory mechanisms of cardiac homeostasis is yet to be fully elucidated. Recent advances show that VEGF expression within the heart changes with age which leads to alterations in the microvasculature and associated remodelling processes in the myocardium. The aim of the present research was to study the changes in VEGF expression in myocardial aging. In the present study, we used two age groups of Wistar rats – 6- and 12-month-old; n=6. VEGF expression was visualised through immunohistochemical and immunofluorescent methods and was assessed semi-quantitatively. In 6-month-old, as well as in 12-month-old animals, VEGF expression within the myocardium showed a specific pattern and a difference in the intensity of immunoreactivity was noted. Our semi-quantitative data revealed an increase in VEGF expression in Wistar rats both in the left and in the right ventricle between 6- and 12-month-old animals. Considering the fact that no obvious pathology was seen in the experimental animals, the results in the model studied herein strongly suggest that aging is the major factor causing these changes. The results of the present study point out to the altered expression of VEGF as a possible key mechanism of microvasculature remodelling as myocardial aging advances.

Key words: vascular endothelial growth factor (VEGF), myocardium, aging

Histological Study on Rare Accessory Bone in the Elbow Region

S. Stanchev[1], L. Gaydarski[1], A. Iliev[1], M. Krupev[2], G. Kotov[3], B. Landzhov[1]*

[1] Department of Anatomy, Histology and Embryology, Medical University of Sofia, Sofia, Bulgaria
[2] Clinic of Imaging Diagnostics, University Hospital 'Alexandrovska', Department of Imaging Diagnostics, Medical University of Sofia, Sofia, Bulgaria
[3] Clinic of Rheumatology, University Hospital 'St. Ivan Rilski', Department of Rheumatology, Medical University of Sofia, Sofia, Bulgaria

E-mail: stanchev_1989@abv.bg

Accessory bones in the elbow region are rarely observed. Although they have been previously described, limited data have been reported on their histological characteristics. The aim of the present study was to provide a detailed light microscopic description of an accessory bone in the posterior elbow region, proximal ulna and the surrounding soft tissues. During a routine anatomical dissection of the right elbow joint of a male formol-carbol fixed cadaver an accessory bone was found. The bone and the related neighbouring structures were histologically examined with azocarmine-aniline blue. Our results indicated the accessory bone was connected to the proximal ulna by a well-developed syndesmosis composed of dense regular connective tissue. Moreover, the zones of insertion of the described syndesmosis showed the typical morphological characteristics of enthesis. Several hypotheses have been suggested for the development of accessory bones in the posterior elbow region. The present study supports the congenital origin of the described bone. The current work has an important clinical and scientific significance as it revealed the presence of histologically proven syndesmosis between an accessory bone and the proximal ulna.

Key words: accessory bone, syndesmosis, elbow joint

Association of the Leptin Receptor Q223R (rs1137101) Polymorphism and Obesity in a Cohort of the North Bulgaria Population

T. Rashev[1], V. Nankov[2], D. Marinova[1], M. Dobrev[1], S. Trifonov[1]*

[1] Department of Anatomy, Histology, Cytology and Biology, Faculty of Medicine, Medical University of Pleven, Pleven, Bulgaria
[2] Scientific Research Laboratory, Medical University of Pleven, Pleven, Bulgaria

* E-mail: t_rashev@mail.bg

Q223R polymorphism occurs as a result of a non-conservative A to G substitution at codon 223 resulting in a glutamine to arginine amino acid change. Numerous studies in various populations have replicated the association between Q223R SNPs and obesity with the variant G allele. The aims of the current study it to investigate probable association between the *LEPR* Q223R polymorphism in the leptin receptor gene and obesity in a cohort from Northern Bulgaria. The

study comprised of 157 subjects recruited by random sampling from hospitals in northern Bulgaria. Overweight and obesity were defined as a BMI ≥ 25 kg/m^2 and BMI ≥ 30kg/m^2 respectively, based on criteria of the World Health Organization. Genomic DNA was isolated from peripheral blood leucocytes using column-based DNA extraction (ThermoScientific, USA). Genotyping of LEPR Q223R was carried out using PCR-RFLP assay. The present study included 157 individuals. All participants were divided into two groups: target (obese) and control (non-obese). The target group consisted of 82 subjects (52.6%) and the control group comprised of 74 individuals (44.7%). The frequencies of the A and G alleles were: 0.45 and 0.55 in the obese group and 0.49 and 0.51 in the non-obese group, respectively. No significant differences in the distribution of genotype frequencies were observed between the two groups. In conclusion, this study showed that the *LEPR* Q223R polymorphism in North Bulgarian subjects was not associated with obesity.

Key words: LEPR gene, obesity, body mass index, genotype frequencies

Immunohistochemical Assessment of Vascular Endothelial Growth Factor's Expression in the Myocardium of Spontaneously Hypertensive Rats

L. Gaydarski[1], I. Angushev[1], A. Iliev[1], S. Stanchev[1], G. Kotov[2], N. Stamenov[1], B. Landzhov[1]*

[1] Department of Anatomy, Histology and Embryology, Medical University of Sofia, Bulgaria
[2] Clinic of Rheumatology, University Hospital 'St. Ivan Rilski', Department of Rheumatology, Medical University of Sofia, Bulgaria

* E-mail: lgaidarsky@gmail.com

Vascular endothelial growth factor (VEGF) is an essential for angiogenesis. In humans, five variations of VEGF were reported. High blood pressure is the primary risk factor for cardiovascular disease. However, the etiology and complex pathogenesis of arterial hypertension remain elusive. Existing literature suggests a potential role of VEGF in the compensatory mechanisms of hypertension. Therefore, the present study aims to assess the immunoreactivity of VEGF in the hypertensive myocardium and compare it normotensive controls in order to provide insights into the involvement of VEGF in the pathogenesis of hypertension. We employed 6-month-old spontaneously hypertensive rats as an animal model to simulate arterial hypertension and 6-mont-old normotensive Wister rats as a control. To detect the presence of VEGF, immunohistochemical and immunofluorescent staining were used. Subsequently, a semi-quantitative analysis was conducted to evaluate the expression of VEGF. VEGF immunoreactivity was detected in the predominantly in the cytoplasm of cardiomyocytes in both ventricles. Interestingly, VEGF expression was strongest in the right ventricle of spontaneously hypertensive rats. Additionally, the hypertensive group exhibited significantly higher levels of VEGF expression in both ventricles compared to the controls. The elevated expression of VEGF implies compensatory upregulation of angiogenesis as a desperate attempt of the heart to cope with the increased overload. Our study demonstrates significant elevation of VEGF expression in both ventricles of hypertensive animals, compared to the control group. Thus indicating a potential key role in the pathogenesis of hypertension.

Key words: VEGF; myocardium; hypertension

Some results for anti-GD1a antibodies as a biomarker of neuronal changes in different diseases

V. Kolyovska[1], S. Ivanova[2], D. Drenska[3], D. Maslarov[3,4]*

[1] Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, Sofia, Bulgaria
[2] University Hospital for Neurology and Psychiatry "St. Naum", Sofia, Bulgaria
[3] University First MHAT-Sofia, "St. John Krastitel", Neurology Clinic, Sofia, Bulgaria
[4] Medical University of Sofia, Medical College "Y. Filaretova", Sofia, Bulgaria

* E-mail: verakol@abv.bg

Evidence has been obtained to support the view that gangliosides are involved in the pathogenesis of multiple sclerosis (MS) and its experimental model, chronic relapsing experimental allergic encephalomyelitis (CREAE). GD1a ganglioside is one of the major neuronal gangliosides. It was detected in the spinal cord of Lewis rats with CREAE before the onset of clinical signs and during the first clinical episode. Elevation of serum GD1a gangliosides during the first attacks of the disease has been suggested to be an indicator of neuronal destruction. The aim of the present study is to determine to what extent the value of the anti-GD1a antibody titer can be considered a biomarker for changes in neurons and the need to introduce into clinical practice screening for the levels of GD1a ganglioside in the blood of the healthy population as a marker of neuronal damage in the brain. This will allow early detection of diseases and effective timely treatment. We work with sera from patient with multiple sclerosis, vascular cognitive dementia and elderly people over 90. The blood was taken before any medical intervention. The titer is determined by the enzyme-linked immunosorbent assay (ELISA) technique. A high titer of anti-GD1a antibodies is present only in patients with MS, at the time of an attack. The study was conducted in triplicate. The positive titer of anti-GD1a antibodies can be considered as possible biomarkers only in MS patients in a severe attack. A low titer indicate no neuronal changes.

Key words: anti-GD1a ganglioside antibodies, neurochanges, multiple sclerosis, vascular cognitive dementia, elderly

Assessment of Sex Differences in Size and Shape of Human Mandible Using Geometric Morphometrics

D. Toneva[1], S. Nikolova[1], N. Fileva[2], D. Zlatareva[2]*

[1] Department of Anthropology and Anatomy, Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, Sofia, Bulgaria
[2] Department of Diagnostic Imaging, Faculty of Medicine, Medical University of Sofia, Sofia, Bulgaria

* E-mail: ditoneva@abv.bg

The accuracy and effectiveness of methods for sex estimation depends on the level of sexual dimorphism manifested by human bones. There are various methods for examination of the sex differences in size and shape of the bones. The present study uses a geometric morphometric approach to investigate sexual dimorphism in size and shape of the mandible. Computed

tomography images of 190 adult Bulgarians (98 males and 92 females) were used for generation of surface models of the skulls of individuals. The three-dimensional coordinates of 45 landmarks located on the mandible were acquired. The raw landmark coordinates were superimposed by generalized Procrustes analysis. The statistical significance of sex differences in size (centroid size) and shape (shape variables) of the mandible was evaluated. Multivariate regression, principal component analysis, and discriminant analysis were applied in the study. The accuracy of sex classification was calculated by leave-one-out cross-validation. The size of the mandible showed significant differences between males and females. Significant sex differences were also found in the shape variables including the allometric component. The classification accuracy achieved by the centroid size was 87%. The shape variables provided lower accuracy, which differed considerably depending on the type of shape variables used in the analysis (Procrustes coordinates: 78%; regression residuals: 53%). Male and female mandibles differ significantly in size and shape. Size classifies the mandibles with higher accuracy than shape.

Key words: mandible, sexual dimorphism, geometric morphometrics, computed tomography

Acknowledgement: The study was funded by the Bulgarian National Science Fund, Grant **КП**-06-**Н**51/4–11.11.2021.

Impact of Supernumerary Calvarial Bones on the Neurocranium Morphology: A Comparative Geometric Morphometric Study

S. Nikolova[*]*, D. Toneva*

Department of Anthropology and Anatomy, Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, Sofia, Bulgaria

* E-mail: sil_nikolova@abv.bg

Supernumerary bones in the cranial vault arise during embryogenesis as a result of non-fusion between the normal ossification centers or emerging of additional centers in the cranial sutures and fontanelles. This study aims to assess the impact of metopism and supernumerary bones in the occipital region on the cranial morphology. A series of 245 male crania of adult individuals was investigated. They were divided into four series according to the presence or absence of metopism and supernumerary bones in the occipital region: control (n = 115); metopic crania (n = 32); crania with supernumerary bones in the occipital region (n = 67); and metopic crania with supernumerary bones in the occipital region (n = 31). Polygonal 3D models were generated and 3D coordinates of 35 landmarks of the neurocranium were collected on these models. Size and shape of the assembled landmark configurations were compared using geometric morphometric analyses. Significant intergroup size differences were found in the frontal and occipital bone configurations. All configurations showed significant intergroup shape differences except the occipital one. The supernumerary bones in the occipital region are not associated with significant changes of the cranial morphology, but they are related to an intensification of the typical of metopism modification of the neurocranium shape and size.

Key words: metopism, neurocranium, supernumerary calvarial bones, anatomic variations

Acknowledgement: The study was funded by the Bulgarian National Science Fund, Grant КП-06-Н51/4–11.11.2021.

Zn(II)/Au(I) and Zn(II)/Ag(I) Complexes with Schiff Bases-A New Approach in Treatment of Human Glioblastoma Multiforme Cells

L. Dyakova[1], A. Abudalleh[2], T. Zhivkova[2], G. Marinescu[3], D.-C.-Culita[3], T. Mocanu[3], R. Alexandrova[2]*

[1] *Institute of Neurobiology, Bulgarian Academy of Sciences, Sofia, Bulgaria;*
[2] *Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, Sofia, Bulgaria;*
[3] *Institute of Physical Chemistry "Ilie Murgulescu", Romanian Academy, Bucharest, Romania*

* E-mail: sigma13@abv.bg

The control of glioblastoma multiforme is one of the big challenges in current oncology practice. (Multiple) drug resistance of glioblastoma cells, their escape from the immune response, tumor heterogeneity, limited access of drugs to the tumor due to the blood-brain barrierand high invasiveness of the tumor make treatment of this neoplasia very hard and slow. The aim of our study is to investigate the influence of newly synthesized complexes of Zn (II), Zn (II)/Au(I) and Zn(II)/Ag (I) with Schiff bases (derivatives of 2,6-diformyl cresol (diald) - Aepy, Ampy and Dmen,in 8MGBA human glioblastoma multiforme cells. Cytotoxic/antitumor activity of the compounds was evaluated by: i) short-term (24-72h) experiments –using MTT test, neutral red cytotoxicity assay, crystal violet staining technique, double staining with acridine orange and propidium iodide and ii) long-term experiments (37 days) – by 3Dcolony-forming method. The compounds investigated decrease in time-and concentration-dependant manner viability and proliferation of treated glioblastoma cells. Metal complexes are more effective compared to their ligands alone. They induce cytopathological changes and inhibit the 3D growth of tumor cells in a semi-solid medium. The higher cytotoxic/antitumor activity exhibit ZndmenAu and ZndmenAg. The results obtained show the promising antitumor activity of the compounds investigated. More experiments are needed to clarify their mechanism of action.

Key words: glioblastoma multiforme, cytotoxic/antitumor activity, cell line, metal complexes

Acknowledgements: Grant№ КП-06-М61/3 from 13.12.2022, National Science Fund, Bulgarian Ministry of Education and Science, bilateral project between Bulgarian Academy of Sciences and Romanian Academy.

In vitro and *in vivo* Anticancer Activity of Ethanol Extract of *Epilobium Parviflorum* Schreb on Ehrlich Carcinoma Tumor Model

I. Sulikovska[1], K. Todorova[1], I. Ivanov[2], D. Tasheva[3], I. Iliev[1], A. Vasileva[2], M. Dimitrova[1]*

[1] *Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, Sofia, Bulgaria*
[2] *Medical University of Sofia, Sofia, Bulgaria*

[3] *Sofia University "St. Kl. Ohridski", Sofia, Bulgaria*

* E-mail: inna_sulikovska@ukr.net

Epilobium parviflorum Schreb (EPE) is a flowering plant widely applied in traditional medicine for treatment of different diseases including cancer. The present study aims to assess the antitumor potential of 80% ethanol extract by using both *in vitro* and *in vivo* tumor models. Anticancer activity was investigated on a mouse model of solid and ascites forms of Ehrlich's breast cancer and 5-fluorouracil (5-FU) was used for comparison as a standard chemotherapy medication. The changes in hematological, biochemical biomarkers and histopathological features were analyzed. The *in vitro* activity of EPE was evaluated by the MTT test using Ehrlich's Ascites Carcinoma (EAC) cell lines. The morphological changes induced by the extract in both models were analyzed by light and/or fluorescent microscopy. The results of *in vitro* studies indicated that the EPE induced significant antiproliferative and apoptogenic effects in the EAC cells. *In vivo* studies showed that EPE treated mice had better clinical and histopathological status compared to untreated ones. The plant extract can be used as an auxiliary implement for alleviating the symptoms in breast cancer.

Key words: Ehrlich Carcinoma tumor model, antitumor activity, ethanol extract of *E. parviflorum* Schreb

Acknowledgements: This work is financially supported by the National Science Fund of the Bulgarian Ministry of Education and Science, Grant Nr KP-06-N31/1.

Study of the facial proportions in Young Bulgarians by 3D Laser Scanning

T. Petleshkova[1], S. Sivkov[1], A. Baltadjiev[1], A. Fasova[1], H. Manev[2], R. Raycheva[3], P. Timonov[4]*

[1] *Department of Anatomy, Histology and Embryology, Faculty of Medicine, Medical University of Plovdiv, Plovdiv, Bulgaria*
[2] *Department of Medical Physics and Biophysics, Faculty of Pharmacy, Medical University of Plovdiv, Plovdiv, Bulgaria*
[3] *Department of Social Medicine and Public Health, Faculty of Public Health, Medical University of Plovdiv, Plovdiv, Bulgaria*
[4] *Department of Forensic Medicine and Deontology, Faculty of Medicine, Medical University of Plovdiv, Plovdiv, Bulgaria*

* E-mail: Tsvetanka.Petleshkova@mu-plovdiv.bg

The knowledge of the variations in facial proportions in different populations may find applications in plastic and maxillofacial surgery. The aim of the study was to examine facial proportions in young Bulgarians. The study included clinically healthy individuals of Bulgarian origin (46 men and 49 women), aged between 21 and 30 years. Three-dimensional images were obtained from each subject using a hand-held laser scanner (FastSCAN Cobra, Polhemus Inc., Colchester VT). On each of the obtained images, a set of anthropometric landmarks was placed: nasion (n), stomion (sto), frontotemporale (ft_r, ft_l) and zygion (zy_r, zy_l). From the landmarks, the physiognomic upper facial height (n-sto), bizygomatic breadth (zy_r-zy_l) and smallest forehead breadth (ft_r-ft_l) were measured and averaged for sex. The measurements were used to calculate the Physiognomic upper facial index and Jugofrontal index. The results obtained show that in the

jugofrontal index most of studied men and women fall into the category medium 50% and 51%, respectively. In the category narrow, the percentage of women and men was 32.7% and 28.3%. The highest percentage of men and women in the physiognomic upper facial index fall into the category mesen, 43.5% and 44.9%. In the categories lepten and euryen, the percentage of men was 26.1% and 19.6% and women was 30.6% and 18.4%. For this two indices, no statistically significant gender differences were found in the percentage distribution by categories ($p>0.05$). The highest percentage of studied men and women fall into the categories medium and mesen in the two studied indices.

Key words: 3D laser scanning, facial proportions, Bulgarians

Somatotype of Bulgarian Young Adults

I. Vladimirov[*]*, R. Stoev*

Institute of Experimental Morphology, Patology and Anthropology with Museum of BAS, Sofia, Bulgaria

* E-mail: garet_1991@abv.bg*;* rastesto@abv.bg

An important element of physical development of man is the constitutional type – somatotype. That is why it is object of many studies. Because of complex reasons mass anthropometric studies in Bulgaria are rare in last years and serveys in university students are used to characterize the physical development of young adults. During the period 2016-2023, ananthropometric study was carried out on 190 Bulgarian young adults, students in different universities in Sofia, 75 males and 115 females. The mean age of these students is 21 years for males and 20 years for females. Standard anthropometric measurements and somatotypological characterization after Heath-Carter are applied. The mean height of the investigated students is 177,6 cm in males and 163,1 cm in females. Their weight is 79,2 and 58,5 kg respectively, their Body Mass Index 25,0 and 22,0. Their average somatotype is 3,7: 5,4: 2,1 in males and 4,2: 4,2: 2,5 in females. It is interesting that their somatotype is practically the same as that of the students at the NSA nearly 50 years ago, studied by Maria Toteva. Close are also the somatotypes of students of sports in Warsaw, Turkey and Split, especially in females. Despite the general opinion about the immobilization and obesity of the Bulgarian youth in recent years, we do not find such. The probable reason is the spread of sports activities and more rational nutrition today.

Key words: young adults, somatotype, body height, body mass, body mass index

Exposure to Cobalt Chloride Affects Mouse Testis via Altered Iron Metabolism

E. Pavlova[*]*, Y. Gluhcheva, V. Mihaylova*[2]*, E. Petrova*[1]*, I. Vladov*[1]*, N. Atanassova*

[1] *Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, Sofia, Bulgaria*
[2] *Faculty of Chemistry and Pharmacy, Sofia University "St. Kliment Ohridski", Sofia, Bulgaria*

* E-mail: e_bankova@yahoo.com

Cobalt (Co) exposure is known to interfere with iron (Fe) metabolism. Our aim was to study the effect of chronic cobalt chloride administration on spermatogenesis in tandem with changes in iron homeostasis in adult mice. Pregnant mice were exposed to 75 mg or 125 mg /kg b.w. $CoCl_2 \times 6H_2O$ with drinking water for 3 days before delivery. Treatment continued until postnatal day 90 of the pups. Control animals obtained tap water. Testes of control and Co-treated mice were processed for immunohistochemical and inductively coupled plasma mass spectrometry analyses. Sperm count was performed. Chronic $CoCl_2$ administration resulted in significant Co accumulation and increase of Fe content in the testis that was 2.7-fold higher after treatment with low dose compared to high dose. Significant dose-dependent reduction in testicular index after Co administration was established. Seminiferous tubules diameter was obviously reduced associated with lack of lumen formation. Disorganization of the seminiferous epithelium was accompanied with sloughed off immature germ cells into the lumen and multinuclear germ cells. These findings correspond to significant reduction of sperm count by 2.15-fold for low dose and 3.35-fold for high dose. Stage specific expression of androgen receptor protein in Sertoli cells was not seen after Co exposure. Reduced expression of transferrin receptor-1 protein and increased hepcidin immunoreactivity was also found. Ferroportin expression was not affected by Co administration. Data suggest that cobalt exerts its toxicity on testis possibly through altered iron homeostasis.

Key words: cobalt, testis, iron regulation, androgen receptor

Morphometric Analyses of the Left Atrium

Y. Stoyanov[1], N. Genov[1], N. Dimitrov[1], I. Stefanov[1], D. Atanasova[1,2], I. Ivanova[1], S. Hamza[1], A. Georgieva[1], N. Pirovski[1], V. Pilicheva[1], I. Valkova[1], N. Malcheva[1]*

[1]*Department of Anatomy, Faculty of Medicine, Trakia University, Stara Zagora, Bulgaria*
[2] *Institute of Neurobiology, Bulgarian Academy of Sciences, Sofia, Bulgaria*

* E-mail: yordan.stoyanov@trakia-uni.bg

The left atrium has a thin wall the thickness of which could vary significantly. In normal population four pulmonary veins having four independent ostia drain into that chamber. Anatomical variations and branching patters of the pulmonary veins ostia are not typical but are reported by various studies. The circumferential pulmonary vein line is the circular connection between the ostia at each side and is reported to be thicker on the left side. In addition its anatomical characteristics are vital for pulmonary vein isolation (PVI) during the treatment of atrial fibrillation. The aim of this study is to conduct morphometric analyses on these structures on the formalin-fixed hearts used at our Department of Anatomy. The results of our research showed that 35% of the examined hearts were presenting anatomical variations in the pulmonary venous ostia (PVO). Around 28% showed more than 2 ostia at the right side. On the contrary only 14% of left pulmonary veins showed variation from the anatomical model. The mean value of the thickness of the left atrium was bigger in the hearts without anomaly: 2.67mm, but wasn't showing statically significant difference. In contrast pulmonary vein thickness (PVT) of the ostia (Mean= 1.746 mm) and the circumferential pulmonary vein thickness (Mean=1.736 mm) were showing statically significant thickening in the left side in comparison with the right. PVO variations were presented into one third of the examined hearts. The left pulmonary veins ostia were significantly thicker compared to the right.

Key words: branching pattern, pulmonary veins, left atrium, morphometry

Morphological Changes in Aging Testis in Melatonin Deficiency Conditions

A. Petrova[1], D. Barbutska[1], Y. Koeva[1], K. Georgieva[2], Y. Tchekalarova[3], G. Nanov[4]*

[1] Department of Anatomy, Histology and Embryology, Medical University of Plovdiv, Plovdiv, Bulgaria
[2] Department of Physiology, Medical University of Plovdiv, Plovdiv, Bulgaria
[3] Institute of Neurobiology, Bulgarian Academy of Sciences, Sofia, Bulgaria
[4] Student in Medicine, Faculty of Medicine, Medical University of Plovdiv,Plovdiv, Bulgaria

* E-mail: Anelia.Petrova@mu-plovdiv.bg

In a wide range of research, it has been established that melatonin influences the male hypothalamic-pituitary-gonadal axis, and has a direct effect on the testicular parenchyma through its receptors. Melatonin affacts the testicular Leydig cells (LCs) and functions as a local modulator of steroidogenesis. Moreover, the involvement of melatonin in the processes of maturation and differentiation is discussed in relation to the spermatogenic epithelium. To investigate the potential role of melatonin in terms of testicular function in a melatonin deficiency model of rats of different ages. Male Wistar rats of three age subgroups: 5 months, (sexually mature); 16 months, (adults) and 20 months old (old) were divided into two groups: animals with removed pineal glands, using the Hoffman-Reiter technique (PIN), and a control group (SHAM). Hematoxylin and eosin and Azan (Heidenhaim) staining of paraffin sections of testicular fragments were used to evaluate the morphological changes. By using hematoxylin-eosin staining of testicular fragments of 16- and 20- months old animals with removed pineal glands we found hypertrophy and hyperplasia of LCs in an enlarged testicular interstitium, whereas in the lumen of the seminiferous tubules we established spermatogenic arrest, with almost complete absence of spermatozoa. The Azan staining revealed a folded basal lamina of the seminiferous tubules, with predominant sclerotic changes, especially in the PIN group**.** The results obtained are further evidence of the role of melatonin in the processes of spermatogenesis and steroidogenesis in the male reproductive system.

Key words: Leydig cells, melatonin, steroidogenesis

Fetal MRI and its Meaning for Prenatal Diagnosis of Neural Tube Defects

A. Petrova[1], M. Kanarev[1] M., Shishmanova-Doseva[2], A. Georgiev[3], E. Uchikova[4], K. Kilova[5], A. Tenev[6], T. Kitova[1]*

[1] Department of Anatomy, Histology and Embryolog, Medical university of Plovdiv, Plovdiv, Bulgaria
[2] Department of Pharmacology, Toxicology and Pharmacotherapy, Medical University of Plovdiv, Plovdiv, Bulgaria
[3] Department of Diagnostic Imaging, Medical University of Plovdiv, Plovdiv, Bulgaria
[4] Department of Obstetrics and Gynaecology, Medical University of Plovdiv, Plovdiv, Bulgaria
[5] Department of Medical Informatics, Biostatistics and E-learning, Medical University of Plovdiv, Plovdiv, Bulgaria
[6] Student in Medicine, Faculty of Medicine, Medical University of Plovdiv, Plovdiv, Bulgaria

* E-mail: Anelia.Petrova@mu-plovdiv.bg

The neural tube defects are a heterogeneous group of congenital abnormalities of the central nervous system that occure due to a failed closure of the neural tube. Despite the improvement of the ultrasound methodology, the prenatal examination often provides uncertain findings and the neural tube defects remain a diagnostic challenge. Fetal magnetic resonance is an additional imaging technique that provides clarification of questionable ultrasound data and detection of associated abnormalities not visible by sonographic examination. The aim of the present study was to evaluate the role and significance of fetal magnetic resonance imaging for the prenatal diagnosis of neural tube defects. The magnetic resonance findings of two pregnant women are presented, in which the prenatal ultrasound examination established evidence of malformative syndromes. The study was conducted at the Translational Neuroscience Research Institute at Medical University of Plovdiv, through a translational neuroimaging with a functional 3 Tesla nuclear magnetic resonance. The investigations confirmed the presence of neural tube defects: occipital encephalocele in one, hydrocephalus, Arnold-Chiari type II syndrome and lumbar myelomeningocele in the other. In cases in which the prenatal ultrasound diagnosis suspects an existing central nervous malformation, the magnetic resonance imaging is a recommended method that provides medical professionals and parents with the necessary information to determine the outcome of the pregnancy.

Key words: fetal magnetic resonance imaging, malformative syndromes, neural tube defects

Cavum Vergae and Cavum Septi Pellucidi Cyst–Anatomical Variation, a Case Aeport

M. Kanarev[1], A. Petrova[1], N. Petrova[1], F. Popova[1], Z. Harizanova[1], St. Sivkov[1], T. Lolovski[2]*

[1] Department of Anatomy, Histology, and Embryology, Medical University of Plovdiv, Plovdiv, Bulgaria
[2] Student in Medicine, Faculty of Medicine, Medical University of Plovdiv, Plovdiv, Bulgaria

* E-mail: Marin.Kanarev@mu-plovdiv.bg

Cavum vergae and cavum septi pellucidi are congenital anatomical variations which are common in infancy but usually do not persist until adulthood. If this rarely occurs it is usually asymptomatic. When the condition is pronounced and the variation is of a large size and a diameter of more than a centimeter it can be classified as a cyst. The latter presents as a cystic lesion located in the midline below the body and splenium of corpus callosum above the fornix. If it is large enough in size it can lead to obstructive hydrocephalus. When this occurs, it should be treated radically. The aim of the present case report is to illustrate that anatomical variations are present as asymptomatic cases and can be accidently detected. The accidental magnetic resonance findings of a 29-year-old male subject with an asymptomatic cavum vergae cyst are presented. The study was conducted at the Translational Neuroscience Research Institute at Medical University of Plovdiv, through a translational neuroimaging with a functional 3 Tesla nuclear magnetic resonance. The MRI study was conducted as part of a Doctoral and postdoctoral project No ДПДП-7/2022 at Medical University of Plovdiv. The investigations confirmed the presence of a cavum vergae variation which as long as asymptomatic should not be treated, rather only monitored. MRI is a convenient method for establishing the parameters of CNS variations and malformations.

Key words: cavum vergae, cavum septum pellucidum, cavum vergae cyst, magnetic resonance imaging

Estimation of Humeral and Femoral Length Using Proximal Dimensions in Bulgarian Population

*A. Fasova[*1], P. Timonov[2], A. Baltadjiev[1], T. Petleshkova[1]*

[1] *Department of Anatomy, Histology and Embryology, Medical Universtiy of Plovdiv, Plovdiv, Bulgaria*
[2] *Department of Forensic medicine and Deontology, Medical University of Plovdiv, Plovdiv, Bulgaria*

* E-mail: Antoaneta.Fasova@mu-plovdiv.bg

The role of skeleton is invaluable in determining age, height, sex, race and presence of disease. Reconstruction of length of the long bones is an important part of identification procedure which is related to height estimation. Numerous standards for sex and height determination of bones from different populations have been proposed for forensic and archaeological practice. Due to the presence of racial, population and evolutionary differences, no universal method has yet been created. The current study introduces a method of estimating the length of humerus and femur from proximal dimensions of the bones specific to the Bulgarian population. 139 adult humeri with known age and sex were studied. The proximal anthropometric features include circumference, transverse, and vertical diameters of the head of bones. Simple linear regressions were obtained to define the length. For males, regression formulae, which included head circumference, provided the best fit of the data, resulting in the highest correlation. For females, the vertical diameter of the head showed the best correlation with maximum length. The regression formula suggested that the proximal end is the best estimator of length. The derived formulae are population and sex specific.

Key words: humerus, femur, forensic practice, length estimation

Sectional Area Differences of the Myenteric Ganglia in the Rat Colorectal Region

N. Genov[1], N. Tomov[2], N. Dimitrov[1], N. Lazarov[3,4], D. Atanasova[1,3]*

[1] *Department of Anatomy, Faculty of Medicine, Trakia University, Stara Zagora, Bulgaria*
[2] *Institute of Anatomy, University of Bern, Bern, Switzerland*
[3] *Institute of Neurobiology, Bulgarian Academy of Sciences, Sofia, Bulgaria*
[4] *Department of Anatomy and Histology, Medical University of Sofia, Sofia, Bulgaria*

* E-mail: nikolay.genov@trakia-uni.bg

The myenteric (Auerbach) plexus lying between the longitudinal and circular layers of the muscularis externa in the intestinal tract consists of ganglia interconnected by nerve fibers. The myenteric plexus, which receives innervation from the autonomic nervous system, is primarily responsible for the peristaltic movement of the intestines. This study has been conducted to determine the sectional area differences in the ganglia and their constituent neuronal perikarya

in the myenteric plexus at the levels of the proximal colon, distal colon and rectum in the rat. The results of the morphometric analyzes pointed out a similarity between the sectional area of myenteric ganglia in all three examined regions. More than 30% at the level of the distal colon and more than 20% of the ganglia in the rectum have cross-sectional area ranging from 500 μm^2 to 1500 μm^2. Conversely, at the level of the proximal colon more than 40% of the ganglia had sectional area sizes in the range of 1000 μm^2 to 2000 μm^2. Differences in the size of the neuronal perikarya have been observed as well. Smaller neuronal bodies with a mean value of 50 μm^2 were frequently observed in the proximal colon and the rectum while almost 40% of the neuronal perikarya had an average value of 100 μm^2 at the level of the distal colon. Differences in the sizes of myenteric ganglia and neuronal perikarya are observed in different regions of the large intestine, which determines the presence of regional features and is probably related to certain peculiarities in motility.

Key words: colon, morphometry, myenteric plexus, rectum, rat

Acknowledgement: This research is supported by the Bulgarian Ministry of Education and Science under the National Program "Young Scientists and Postdoctoral Students-2".

Investigations on Cytotoxic and Antiviral Effects of 1,8-Naphthalimide Derivatives

R. Alexandrova[1], H. Hristov[1], A. Abudalleh[1], L. Dyakova[2], D. Staneva[3], M. Georgieva[4], G. Miloshev[4], A. Said[5], I. Grabchev[6]*

[1] *Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, Sofia, Bulgaria*
[2] *Institute of Neurobiology, Bulgarian Academy of Sciences, Sofia, Bulgaria;*
[3] *University of Chemical Technology and Metallurgy, Sofia, Bulgaria*
[4] *Institute of Molecular Biology, Bulgarian academy of Sciences, Sofia, Bulgaria*
[5] *Department of Chemistry, Faculty of Science, Assiut University, Assiut, Egypt*
[6] *Sofia University "St. Kliment Ohridski", Faculty of Medicine, Sofia, Bulgaria*

* E-mail: rialexandrova@hotmail.com

The aim of our study was to evaluate the cytotoxicity, genotoxicity and mitotchondrial toxicity of first generation polypropylene imine dendrimer modified with 1,8-naphthalimide and chitosan modified with 4-amino-1,8-naphthalimide. The following cell lines were used as model systems: MDA-MB-231 (human triple negative breast cancer), MCF-7 (human luminal type A breast cancer), HeLa (human cervical carcinoma) and Lep-3 (non-tumor human embryonic cells). The effect of the compounds on cell viability and proliferation was studied by: i) short-term experiments for 24-72h, performed with monolayer – 2D cell cultures, carried out by MTT test, neutral red uptake assay, crystal violet staining and double staining with acridine orange and propidium iodide); ii) long term experiments – the influence of 1,8-naphthalimide derivatives on viability and 3D growth of cancer cells was assessed during 26-28 days by 3D colony-forming technique. Their genotoxicity was investigated by comet assay. FACS analysis was applied to determine the ability of the compounds to inhibit the mitochondrial membrane potential ($\Delta\Psi m$) in the treated cells. The results obtained revealed that modified chitosan has more pronounced cytotoxic activity than the first generation polypropylene imine dendrimer modified with 1,8-naphthalimide and non-modified chitosan. Non-tumor Lep-3 human cells are less sensitive to the cytotoxic effect of the compounds examined as compared to human breast

cancer and cervical carcinoma cell lines. All examined compounds induce double-strand DNA breaks but only modified chitosan (applied at a concentration of 600 μg/ml for 72h) inhibit the mitochondrial membrane potential in the treated HeLa cells.

Key words: cancer cells, 1,8-naphthalimide derivatives, cytotoxicity, genotoxicity, mitochondrial toxicity

Renal Inflammation Following Chronic Administration of Cobalt Chloride

Y. Tabakov[1], R. Ivanov[1], E. Pavlova[1], E. Petrova[1], A. Tinkov[2,3], O. Ajsuvakova[2,3], A. Skalny[4], Y. Gluhcheva[1]*

[1] Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, Sofia, Bulgaria
[2] Yaroslavl State University, Yaroslavl, Russia
[3] IM Sechenov First Moscow State Medical University, Moscow, Russia
[4] Federal Research Centre of Biological Systems and Agro-technologies of the Russian Academy of Sciences, Orenburg, Russia

* E-mail: yavor.tabakov@iempam.bas.bg

Cobalt (Co) is an essential trace element that can cause severe health effects upon occupational or environmental exposure. Cobalt chloride ($CoCl_2$) has been used in the research of kidney diseases, due to its ability to induce hypoxia. To assess the alterations in mouse kidney following chronic exposure to $CoCl_2$. Pregnant ICR mice were subjected to $CoCl_2x6H_2O$ at a daily dose of 125 mg/kg body weight 2–3 days prior delivery and treatment continued up until day 90 after birth. The compound was administered via drinking tap water. Age-matched mice obtaining regular tap water were used as a control group. Pups were sacrificed, kidneys were excised and processed for histological and immunohistochemical analyses. Myeloperoxidase (MPO) was studied as a marker of inflammation. Cobalt content was measured using inductively coupled plasma mass spectrometry (ICP-MS). Kidney organ weight index (KI) was reduced following chronic $CoCl_2$ administration compared to the controls. Renal Co content was significantly elevated (12.9-fold) compared to the age-matched controls indicating that the kidney is one of the main targets of Co exposure. Pathohistological analysis showed numerous leukocyte infiltrates, altered glomeruli structure and tubular necrosis which may be the cause for the reduced KI. MPO was well expressed in the renal tubules, glomerular membrane and in the vascular endothelial cells of Co-exposed mice suggesting a possible $CoCl_2$-induced oxidative stress as well. The study shows that chronic $CoCl_2$ supplementation induces renal inflammation which may lead to kidney dysfunction and organ damage.

Key words: cobalt chloride, chronic exposure, kidney, inflammation, MPO

Expression of the Transcription Factor ZBTB20 in the Subventricular Zone of Adult Macaque Monkey under Physiological and Ischemic Conditions

L. Veleva [1], D. Stoyanov [1], M. Ivanov [1,2], A. Mladenov [1], S. Pavlov [1], AB Tonchev [1, 2]*

[1] Department of Anatomy and Cell Biology, Faculty of Medicine, Medical University of Varna, Varna, Bulgaria
[2] Research Institute, Medical University of Varna, Varna, Bulgaria

* E-mail: dimo.stoyanov@mu-varna.bg

The subventricular zone (SVZ) is a major neurogenic area in the adult mammalian brain. The mRNA of the transcription factor ZBTB20, important for neurodevelopment, is known to be present in the monkey anterior SVZ (SVZa). This prompted us to study the expression pattern of ZBTB20 in the SVZa and rostral migratory stream (RMS) of non-human primates and the expression changes in a primate model of cerebral ischemia, a strong activator of neuronal progenitors. Adult macaque monkeys underwent either sham surgery or transient global cerebral ischemia. Sections were processed for fluorescent immunohistochemistry. Cell counting was done using ImageJ, statistical analysis was performed in R. ISH images from the database *Monkey Niche* were analyzed using cellDetekt. Here we demonstrate the diverse immunophenotype of ZBTB20+ cells in the adult primate SVZa and RMS, accounting for nearly all DCX+ and GFAP+ cells and the majority of the Ki67+ cells, representing neural stem cells, neuroblasts and transit-amplifying progenitors, respectively. Our data reveal noticeable enhancement of ZBTB20 mRNA expression and higher co-expression of the ZBTB20 protein with DCX and Ki67 following transient global ischemia. Our results suggest a role for ZBTB20 in regulating the activity of neural stem cells and progenitors. This may have implications for stroke treatment and brain tumour biology. The expression of ZBTB20 in all stages of adult neurogenesis suggests that it could regulate the proliferation of neural progenitors in the primate SVZa. Moreover, ZBTB20 may be implicated in the postischemic activation of adult neurogenesis.

Key words: ZBTB20; non-human primate; subventricular zone; neural progenitor

Zbtb20 is Required for Cortical Interneuron Development

D. Stoyanov[1], M. Ivanov[1], S. Pavlov[1], I. Manoylov[3], J. Tchekalarova[3], A. Tchorbanov[3], A. Stoykova[2], A. Tonchev[1]*

[1] Department of Anatomy and Cell Biology, Faculty of Medicine, Medical University of Varna, Varna, Bulgaria
[2] Max Planck Institute for Multidisciplinary Sciences, Göttingen, Germany
[3] Bulgarian Academy of Science, Sofia, Bulgaria

* E-mail: dimo.stoyanov@mu-varna.bg

Zbtb20 is a transcription factor, involved in hippocampal patterning, cortical layering, and olfactory bulb development. Its function in the context of cortical interneuron (CINs) development is unexplored. Brain sections from wild type (WT)/Zbtb20$^{-/-}$ mice were stained

by means of immunofluorescence or in situ hybridisation. BrdU labelling was done to quantify proliferation and birth date. Images were analysed in ImageJ, statistical analysis was done in R. Zbtb20 is expressed in CINs stem cells, found in the VZ of the developing ventral forebrain. Postnatally, Zbtb20$^{-/-}$ mice show sharp reduction of PV^{+} but not SST^{+} CINs, both derived from the MGE. CGE-CINs, expressing Prox1, are also reduced. Ventral forebrain gene patterning is preserved. No premature stem cell differentiation was observed. BrdU labelling revealed lower proliferation in the E12.5 -> E13.5 MGE. BrdU birth dating showed that E12.5 born CINs are fewer in Zbtb20$^{-/-}$ mice. Analysing the upper (UL) and lower layers (LL) separately showed that at E12.5 -> P8 mutants generate fewer LL CINs compared to the WT. Conversely, E16.5 -> P12 mutant animals make more LL CINs than the control, at the expense of UL CINs. Interneurons hold great therapeutic promise. Studying the genetic regulation of their development may allow for more precise stem cell manipulation and their therapeutic application. Zbtb20 is expressed in the germinative zones of CINs. Zbtb20$^{-/-}$ mice show reduction of specific types of CINs. Developing mutant animals have proliferative deficits and delay in the switch from LL to UL CINs production.

Key words: Zbtb20; cortical interneurons; development

Acupuncture Channels and Visible Meridian Phenomena

N. Dimitrov[1], N. Malcheva[1], N. Genov[1], S. Hamza[1], D. Atanasova[1,2]*

[1] Department of Anatomy, Faculty of Medicine, Trakia University, Stara Zagora, Bulgaria
[2] Department of Synaptic Signalling and Communications, Institute of Neurobiology, Bulgarian Academy of Sciences, Sofia, Bulgaria

* E-mail: nikolay.dimitrov@trakia-uni.bg

Acupuncture channels (meridians) are structures on the human body described by traditional Chinese medicine. The meridian phenomena are observed along the course of channels following acupuncture. Their visible manifestations include alterations in the colour of the skin - white lines and red lines, concurring with the channels. The present work aims to demonstrate visible meridian phenomena manifested in human skin and to correlate them to described acupuncture channels. Patients with various diseases on whom acupuncture was applied were observed and photographed. The human skin with visible meridian phenomena manifested was photographed using standard photographic equipment and a mobile camera. We observed that the human skin reacted during acupuncture. In most patients, we observed differently manifested hyperemia around the needles. Hyperemia around the needles usually disappears after 20 minutes, but it lasts longer in some patients. Only a few patients have visible meridian phenomena on the skin observed during acupuncture. Usually, the channels are activated after several times stimulation with acupuncture needles or at the end of the treatment course. White lines remained visible for a shorter period (5 - 15 min) and were observed more often in patients. Red lines have been marked longer (up to one hour after needle removal) in several patients. The observed white and red lines coincided with described acupuncture channels. The observed visible meridian phenomena coincide with described acupuncture channels.

Key words: acupuncture, visible meridian phenomena, acupuncture channels

Acupuncture Needle Tract in Human

N. Dimitrov[1], B. Panova[2], N. Malcheva[1], N. Pirovski[1], I. Ivanova[1], S. Hamza[1], I. Valkova[1], D. Atanasova[1,3]*

[1] Department of Anatomy, Faculty of Medicine, Trakia University, Stara Zagora, Bulgaria
[2] PZU Fizio –Medika, Strumica, Northern Macedonia
[3] Department of Synaptic Signalling and Communications, Institute of Neurobiology, Bulgarian Academy of Sciences, Sofia, Bulgaria

* E-mail: nikolay.dimitrov@trakia-uni.bg

Acupuncture on the human body and tongue is part of Traditional Chinese Medicine. The present study aims to demonstrate the needle tract in the human body and tongue formed during acupuncture. We used the method for needle tract visualization developed by us to demonstrate the needle tract. The moment of intervention to the needles remains "frozen in time" and reveals the tissues in a circumstance, maximally close to the condition during the acupuncture. This method was applied to samples on human cadavers. We could visualize the needle tract in a transverse and longitudinal direction in the human body's tissues and the tongue. The size of the observed needle tract corresponds to the size of used the acupuncture needles. The tissues in the vicinity of the needle tract have been saved, which allows their morphological examination. The needle tract was observed in different tissues like the epidermis, dermis, subcutis and striated muscle tissue. Morphological changes in the cells and tissues are kept near the needle tract. Acupuncture needles during acupuncture form visible needle tracts in the human body and tongue, which can be measured and analyzed.

Key words: needle tract, acupuncture, human, tongue

Assessment of the Basic Body Composition Components in Young Bulgarian Adults in Dependence of Their Nutritional Status Type

Z. Mitova[1], S. Mladenova[2], E. Andreenko[3]*

[1] Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences., Sofia, Bulgaria
[2] Plovdiv University "Paisii Hilendarski"- Branch Smolyan, Department of Natural-Mathematical and Economical Sciences, Smolyan, Bulgaria
[3] Department of Human Anatomy and Physiology, Biological faculty, Plovdiv University "Paisii Hilendarski", Plovdiv, Bulgaria

* E-mail: zorkamitova@gmail.com

This study presents the results of a bioimpedance analysis *(BIA)* on the body composition of representative samples of Bulgarian men and women (students at the University of Sofia, the University of Plovdiv, and its Smolyan Branch). The study is cross-sectional and was conducted in the period 2015-2022. All the subjects examined were measured by height, body weight, waist, and hip circumferences by means of standard anthropometry. In addition, the body mass index *($BMI=weight_{(kg)}/height_{(m)}^2$)* was calculated. Underweight, normal, and overweight (incl. obesity)

were defined according to the cut-off points of BMI by *Cole et al. (2000, 2007)*. The evaluation of body composition was carried out by means of the bioelectrical impedance analyzer of body composition *ABC-01 "Medass"*. Of each person, the individual values of resistance, reactance, impedance, and phase angle at a signal frequency of 50 *kHz* were measured, which are the basis for the evaluation of body composition. It was characterized by the absolute and relative values of its components such as body fat mass, fat-free mass, skeletal-muscle mass, body cell mass, and total body water. Descriptive statistics for calculating mean values and standard deviations of the indicators were used. The data was processed through the software packages *STATISTICA 10.0* and *SPSS 16.0*. Results show that all indices examined have higher values in men with the exception of active resistance, reactance, impedance, and fat mass, which have higher in women. Relationships were established between the type of nutritional status and some of the physiometric indicators.

Key words: bioelectrical impedance analysis, body composition, nutritional status type, young adults men and women, students

Testing of Primers and Optimization of PCR for the Detection of Alternatively Spliced Variants of Mouse ChAT mRNA

D. Marinova, T. Rashev, S. Trifonov*

Department of Anatomy, Histology, Cytology and Biology, Faculty of Medicine, Medical University Pleven, Pleven, Bulgaria

* E-mail: drdesko@gmail.com

Acetylcholine as neurotransmitter plays important functions in the central as well as in the peripheral nervous system. Its biosynthesis is catalyzed by choline acetyltransferase (ChAT). In the mouse, the gene has three 5'-noncoding (R, N and M) and 14 coding exons, from which seven mRNA isoforms (M, N1, N2, R1, R2, R3 and R4) are transcribed. They differ in the 5'-noncoding ends and encode the same protein (common ChAT). Additional isoform that lacks coding exons from 5 to 8 that encodes a smaller protein (peripheral ChAT). Forward and reverse primers targeting M, R3, N1, N2, cChAT and pChAT isoforms were designed. The optimal PCR conditions (primer concentration and annealing temperature) and specificity of the primers were tested in a series of reactions using specially designed templates. The PCR mix (20µl) contained 0,01pmol/ml template, 0.25µM forward and reverse primers, 10µl Luna Universal qPCR Master Mix (BioLabs). The appropriate annealing tº, which yields sufficient specificity for each tested primer pair was as follows: M–65ºC; N1–63ºC; N2–65ºC; R3–60ºC; cChAT–63ºC and pChAT–65ºC. Specially designed gBlock DNA fragments (IDT) or plasmids containing isoform-specific nucleotide sequences were used as templates. The following PCR protocol was applied: 40 cycles at 95°C for 15 sec; 60ºC, 63ºC or 65ºC (primer pair specific) for 10 sec; 60ºC for 30 sec. Primers specific for several ChAT mRNA isoforms were successfully designed and tested. These primers can be used to detect the desired variants in biological samples.

Key words: choline acetyltransferase, primer, polymerase chain reaction, alternatively spliced variants

Immunophenotype of Apelin Receptor Expressing Cells in the Adult Human Subventricular Zone

A. Mladenov[1], M. Ivanov[1,2], L. Veleva[1], D. Stoyanov[1], S. Pavlov[1], A. Tonchev [1,2]*

[1] Department of Anatomy and Cell Biology, Faculty of Medicine, Medical University, Varna, Bulgaria
[2] Research Institute, Medical University, Varna, Bulgaria

* E-mail: andonmladenov5@gmail.com

Adult neurogenesis is the process of generating new functional neurons from pluripotent neuronal stem cells, located in two "canonical" areas: the subventricular zone (SVZ) and the hippocampal subgranular zone. In this ongoing study, we are trying to elucidate the functions of the Apelinergic system, comprising the apelin receptor (APLNR) and its ligands, in the human SVZ, composed of an ependymal layer, a gap zone and an astrocytic ribbon. Fluorescent immunohistochemistry was performed on coronal sections of the SVZ from 3 human brain tissue samples without evidence of neuropathology and neuropsychiatric patient history. Manual cell counting in selected Regions of Interest along the dorso-ventral axis of the SVZ was done using ImageJ. Statistical analysis was completed in R. We found that the APLNR is present in all layers and diverse cell types of the SVZ in the adult human brain, including proliferating cells. While in the gap zone the predominant cell populations, expressing APLNR, are microglia and neuroblasts or immature neurons, in the astrocytic ribbon, the majority of APLNR+ cells represent astrocytes and most probably TAPs. Therefore, our future research will be aimed at phenotyping the SVZ cell populations after ischemic injury and at exploring the effects of the Apelingergic system in neurospheres, organotypic cultures, as well as human and chimpanzee brain organoids. Here we confirm the expression of APLNR in the adult human SVZ and demonstrate the variety of the associated cell types ranging from astrocytes and neural stem cells to neuroblasts.

Key words: adult neurogenesis; subventricular zone; Apelinergic system

eNOS and nNOS Expression in the Wistar and SHR Rat Kidney after Selective Blockade of Endothelin-B Receptors

V. Iliev[1], L. Jelev[1], A. Gradev[1], L. Malinova[1], P. Markova[2], R. Girchev[2]*

[1] Department of Anatomy, Histology and Embryology, Medical University of Sofia, Bulgaria
[2] Department of Physiology and Pathophysiology, Medical University of Sofia, Bulgaria

* E-mail: v.iliev@medfac.mu-sofia.bg

Endothelin-1 (ET-1) is the most potent vasoconstrictor peptide known to date, acting through two type receptors: ETA and ETB. The aim of the current study was to investigate the role of endothelin B receptor subtype in regulation of neuronal (nNOS) and endothelial (eNOS) nitric oxide synthase expression in kidney of normotensive Wistar (W) and spontaneously hypertensive rats (SHR). Experimental animals (12-14 weeks old) were distributed in 4 experimental groups, each consisting of 6 animals: control groups W and SHR and experimental group Wistar and SHR with selective ETB receptors blockade with BQ788 (1mg/kg/min for 1 hour). The expression of nNOS and eNOS in the kidney tissue was determined immunohistochemically on paraffin

embedded tissue slices. In control condition we established higher expression of nNOS in medullar blood vessels in SHR in comparison to normotensive rats. The selective blockade of ETB receptors induced different expression of NOS isoforms in the kidney of normotensive and spontaneously hypertensive rats. In Wistar rats eNOS expressed predominantly in the cortex tubules and blood vessels but in SHR in the medullar blood vessels and tubules and. In addition, in SHR we found enhanced expression of nNOS in the medullar blood vessels after BQ788 application. Some of the effects of ET-1 are associated with the interaction with nitric oxide synthase and production of NO. Our result shown that ET-1, by ETB receptors affect the eNOS and nNOS expression in specific area of kidney in normotensive and spontaneously hypertensive rats.

Key words: Endothelin-B receptor, eNOS, nNOS, kidney, SHR

3D Printing, Histological and Radiological Analysis of Nanosilicate-Polysaccharide Composite Hydrogel as a Tissue-Equivalent Material for Complex Biological Physical Phantom

P. Valchanov, N. Dukov, S. Pavlov, A. Kotny*

Department of Anatomy and Cell Biology, Medical University of Varna, Varna, Bulgaria

* E-mail: petar.valchanov@mu-varna.bg

The nanosilicate-polysaccharide composite hydrogels are a well-studied class of materials in regenerative medicine that combine good 3D printability, staining, and biological properties, which makes them an excellent candidate material for complex bone scaffolds. The aim of this study is to develop a hydrogel, suitable for 3D printing that has biological and radiological properties similar to those of the natural bone, and to develop protocols for their histological and radiological analysis. We synthesized a hydrogel, based on alginate, methylcellulose and laponite and 3D-printed it into a series of complex bioscaffolds. The scaffolds were scanned with CT and CBCT scanners and exported as DICOM datasets. Then they were cut into histological slides and stained with standard histological protocols. From the DICOM datasets the average value of the voxels in HU was calculated and compared with the densities of the natural spongious bone. On the histological sections, we tested different approaches to reduce the background hydrogel staining, which would make the cytological and histological analysis possible. The results confirmed that an alginate/methylcellulose/ laponite based composite hydrogel can be used for 3D printing of complex three-dimensional scaffolds simulating biological and radiological properties of the spongious bone with high fidelity. The development of multipurpose biological physical phantom, mimicking the bone tissue, opens a new avenue for the design of high fidelity dynamic models of the normal and pathological bone.

Purinergic Nerve Structures in the Rat Colorectral Region

T. Kirov[1], D. Atanasova[2, 3], L. Malinova[1], N. Lazarov[1, 2]*

[1] Department of Anatomy and Histology, Medical University of Sofia, Sofia, Bulgaria
[2] Institute of Neurobiology, Bulgarian Academy of Sciences, Sofia, Bulgaria
[3] Department of Anatomy, Faculty of Medicine, Trakia University, Stara Zagora, Bulgaria

* E-mail: tkirov@medfac.mu-sofia.bg

The enteric nervous system with its constituent submucosal and myenteric plexuses has been extensively studied for several decades now. Despite being an essential component of its non-adrenergic, non-cholinergic transmission, purinergic nerve structures remain neglected. Hence, determining the role ATP plays in the regulation of motility and secretion is essential for the proper understanding of enteric neuronal circuitry and various pathophysiological mechanisms. In the current study, the presence, distribution and staining intensity of the ATP-immunoreactive myenteric nerve structures in the colorectal region of the adult rat have been investigated by means of immunohistochemistry at the light microscopical level. Following ATP immunostaining, the myenteric ganglia were easily distinguished, even though no positive neuronal bodies were registered. Both the ganglia and their constituent neurons were markedly outlined by the surrounding immunopositive beaded nerve fiber bundles. ATPergic neuronal structures were readily observed both in the space between the muscle layers and inside them. The immunostained bundles were arranged parallel to the muscle cells. We encountered no statistically significant differences in the staining intensity of the positive nerve fibers. Taken together with previous morphological and pharmacological research the results of this study suggest that the ATP-mediated neurotransmission is of physiological and likely of pathological significance for the colorectal region, which requires further work in future studies.

Key words: ATP, colorectal, rat, myenteric plexus

„Tubarial (salivary) Glands“ – Do They Exist? Preliminary Results

B. Blagova[*]*, L. Malinova*

Department of Anatomy, Histology, and Embryology, Medical University of Sofia, Sofia, Bulgaria

* E-mail: b.blagova@medfac.mu-sofia.bg

In 2020 Valstar et al. reported an accidentally discovered on PSMA PET/CT „new organ at risk“ located above the *torus tubarius*. The authors named it „tubarial (salivary) glands“. The discovery of a „new“ body structure in the 21st century was certainly generating scientific interest and controversy. The proposed study aimed to verify the presence of these structures in humans. Tissue blocks sized ca. 3 *x* 3 *x* 3 cm were obtained from the *torus tubarius* of three cadavers and examined by hematoxylin and eosin (H & E), periodic acid Schiff (PAS), and Alcian blue staining. The dissected area showed submucosal large aggregates of predominantly mucous gland tissue demonstrated by the PAS and Alcian blue staining, a low number of serous acini, myoepithelial cells, and draining ducts. An „organ“ is defined as an anatomical region with a specific shape and structure, consisting of more than one type of tissue that performs specific tasks. It can be argued that the „tubarial glands“ do not have a capsule as opposed to the major salivary glands, however, the sublingual glands also have an unencapsulated part that consists of 8 – 30 minor mixed glands. The discovery of the „tubarial glands“ sparked scientific interest which opened the door to further digging into the thrust area with more evidence from different studies. More research is needed to resolve the controversies about the „tubarial (salivary) glands“ and to formulate a consensus and establish their place in anatomy and clinical practice.

Key words: fourth pair major salivary glands, new organ, tubarial (salivary) glands

A Brief History of the Anatomy Department at the Faculty of Medicine – Stara Zagora

*D. Sivrev**

Department of Anatomy, Faculty of Medicine, Trakia University, Stara Zagora, Bulgaria

* E-mail: dsivrev@abv.bg

On April 8, 1982, the Higher Medical Institute and Department of Anatomy, Histology and Embryology, was opened in the city of Stara Zagora. Its founder and first Head of Department is Prof. Christo Nikolov Chouchkov, MD, PhD, DSc - one of the founders of the University. Prof. Chouchkov was the Head of the Departmen from 1982 to 2000, and the main scientific priority during this period was the study of the structure of the nervous system by tracing, cyto- and histochemical methods, cell cultures and cultivation of epithelium and nerve cells, plastination of organs and anatomical objects for the needs of the education process. In 2000, Prof. Nikolay Lazarov, PhD, DSc was appointed the Head of the Department of Anatomy. He also held the position of Vice-Dean for Research from 2000 to 2003. In May 2007 Assoc. Prof. Irina Stoyanova, MD, PhD temporarily held the position of Head of Department until December. After her departure to Netherlands the temporary Head of the Department is Prof. Maya Gulubova, MD, PhD. After winning a competition in January 2008, Prof. Dimitar Sivrev, MD, PhD is a Head of the Department of Anatomy, a position he held until 2020. Assoc. Prof. Nikolay Dimitrov, MD, PhD since 2020 has been elected Head of the Department of Anatomy at the Medical Faculty of the Trakia University. Assistant-Professors in this period was: Dr Detelin Sokolov, Dr Stefan Stratiev, Dr Hristo Terziev, Dr Marin Marinov, Dr Peter Penkov, Dr Deniza Penkova, Dr Konstantin Trenchev, Dr Pejo Mutavchiev, Dr Roumen Angelov, Dr Jordan Stoyanov, Dr Antoaneta Georgieva, Dr Ivelina Ivanova, Dr Sevinch Hamza, Dr Irena Valkova, Dr Nikolay Genov, Dr Nedelina Malcheva, Dr Vanesa Pilicheva, Dr Ralitsa Plamenova. The scientific work of the Department of Anatomy are presented in more than 200 complete articles in national and foreign periodicals. The lecturers have participated in over 120 scientific symposia, conferences, congresses and other scientific events in Bulgaria and abroad. More than 20 textbooks and manuals and several monographs have been published in the Department of Anatomy.

Key words: department, anatomy, head, professor, history

Endothelin Receptors in Testicular Tissue Homogenate of Normotensive and Spontaneously Hypertensive Rats after Unilateral Testicular Torsion

M. Kozhuharov[1], V. Iliev[2], A. Ivanov[2], A. Gradev[2], Z. Sabit[3], L. Jelev[2], P. Markova[1, 3]*

[1] Department of Anatomy and Physiology, SWU "Neofit Rilski" – Blagoevgrad, Blagoevgrad, Bulgaria
[2] Department of Anatomy, Histology, and Embryology, Medical University of Sofia, Sofia, Bulgaria
[3] Department of Physiology and Pathophysiology, Medical University of Sofia, Sofia, Bulgaria

* E-mail: kozhuharov@swu.bg

A potential role of the endothelium in male idiopathic infertility has been suggested. The endothelium plays an essential role in maintaining the balance between the vasodilator and vasoconstrictor effects of the substances produced in it, which is of fundamental importance for the reproductive function. Endothelin-1, produced by endothelium, is a substance with a powerful vasoconstrictor effect, achieved by activation of 2 type of receptors: ETA and ETB. Research studies have found reduced sexual behavior and impaired spermatogenesis in a rat model of hypertension, as well as abnormal sperm morphology in hypertensive men, indicating a possible link between male infertility and hypertension. The current study investigated the changes of ETA, ETB receptors in testicular tissue of normotensive Wistar rats (WR, n=5) and spontaneously hypertensive rats (SHR. n=5) after unilateral testicular torsion. In testicular tissues homogenate ETA and ETB receptors were quantified by ELISA method (Fine Biotech Co, Ltd). We established decreased the ETB receptor quantity in SHR testicular tissue in comparison to normotensive rats, $p<0.05$. The unilateral torsion increased ET-A and ET-B quantity in both Wistar rats and SHR, $p<0.05$, however affects in different manner endothelin receptor subtype in contralateral testis: in Wistar rats incresed quantity of ET-A whereas in SHR, ETB receptor subtype. The results obtained in the present study indicate the involvement of different mechanisms in the response of the testicular endothelial system to unilateral testicular torsion. In hypertension, ET-B receptor-mediated pathways play a dominant role, while in normotension, mechanisms mediated by the ET-A receptor subclass are important.

Key words: male infertility, spontaneously hypertensive rats, endothelin receptors

Acknowledgement: This work was supported by a scientific project financed by SWU Neofit Rilski, Blagoevgrad, Contract No. RP – B2/23.

Application of Three-Dimensional (3D) Printing in the Preoperative Study and Planning for Neurosurgery

T. Bogdanov[1], D. Ferdinandov[2], T. Hikov[1]*

[1] 3DLab, Department Medical Physics and Biophysics, Medical Faculty, Medical University of Sofia, Sofia, Bulgaria
[2] Neurosurgery clinics, Saint Ivan of Rila University Hospital, Sofia, Bulgaria

* E-mail: tbogdanov@medfac.mu-sofia.bg

The utilization of three-dimensional (3D) printing in neurosurgery preoperative study and planning has revolutionized the field. This abstract explores the multifaceted application of 3D printing technology, enabling the creation of patient-specific anatomical models, surgical guides, and implants. These personalized 3D-printed models enhance surgical precision, reduce operative time, and improve patient outcomes. Additionally, they serve as invaluable tools for education and communication among surgical teams. The integration of 3D printing in neurosurgery underscores its potential in optimizing surgical approaches, minimizing risks, and ultimately advancing the field by providing neurosurgeons with a tangible and accurate representation of each patient's unique anatomy. We employed InVesalius 3D software for medical image segmentation and reconstruction in this study. Patient-specific 3D models were then printed using Fused Deposition Modeling (FDM) printers. These versatile devices ensured the precise replication of anatomical structures from standard medical imaging data. In cases of brachycephaly, the 3D models obtained serve as essential tools for doctors during case study and preoperative planning. These models provide a tangible and detailed visualization of the

patient's cranial deformity, facilitating in-depth analysis, personalized treatment strategies, and improved surgical planning to achieve optimal outcomes in the correction of brachycephalic conditions. In summary, 3D printing, utilizing InVesalius 3D software and FDM printers, offers a revolutionary approach to neurosurgery, with significant potential in brachycephaly cases. These patient-specific models enhance diagnosis, treatment planning, and surgical precision, promising improved outcomes and personalized care in the field of neurosurgery.

Key words: three-dimensional (3D) printing, neurosurgery, InVesalius 3D software