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ABSTRACTS

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SOME PRINCIPLES OF BRAIN EVOLUTION

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The evolutionary changes in all parts of the brain occur in gray and white matter. Many changes in the brain stem have resulted in a diverse set of locomotor patterns. The red nucleus in terrestrial vertebrates was differentiated from the tegmentum of fish and amphibians. The red nucleus structure has changed considerably during vertebrate and mammalian development. In lower vertebrates the larger neurons are the predominate form and during the evolution the number of these neurons diminishes and the number of the small neurons increases constantly. The rubrospinal tract progressively declines from lower mammals to primates. This could be linked to the loss of locomotor activity of the upper extremities.

The differentiation of respective cortical areas is an important process in the evolution of the cerebral cortex. The common sensorimotor cortex in lower mammals is differentiated into sensory and motor areas. Human speech led to the formation of Broca's speech area and Wernicke's area probably half a million years ago. The birth of literacy, about 5000 years ago caused the formation of the Exner's Writing Area, which is still connected to many other areas of the cerebral cortex and other brain structures.

CHRONIC AGOMELATINE TREATMENT SUPPRESSES THE KAINATE-INDUCED GLIAL REACTION DURING EPILEPTOGENESIS IN RATS

N. Lazarov, J. Tchekalarova, D. Atanasova

Medical University-Sofia, Bulgaria

Inflammatory signal molecules are suggested to be involved in the mechanism underlying comorbid depression in epilepsy. The aim of this study was to examine the effect of the novel antidepressant agomelatine (Ago), a potent melatonin MT1 and MT2 receptor agonist and serotonin 5HT2C receptor antagonist, on glial activity during kainate (KA)-induced epileptogenesis.

Brain sections were immunostained with the microglial marker Iba-1 and two astrocyte-specific markers, GFAP and S100 beta, and the glial cell morphology in brain structures vulnerable to KA-induced neurotoxicity was described in the KA-treated groups and their matched controls, respectively.

Both control and Ago-treated groups were characterized with ramified microglial cells with fine processes. After administration of KA, activated Iba-1-immunoreactive microglial cells in the dorsal and ventral hippocampus, dentate gyrus, as well as in the basolateral amygdala (BLA) and piriform cortex were increased in number and displayed enlarged round-shaped bodies and arborization. Chronic Ago treatment during epileptogenesis significantly diminished their number compared to that in the matched vehicle-treated epileptic group, specifically in the CA1a, CA1b, CA1c and CA3c subfields of dorsal hippocampus, BLA and piriform cortex. Conversely, the KA-induced reactive astrogliosis was more restricted and both GFAP- and S100 beta-immunostained cells were observed mainly in the CA1 and CA3c fields of dorsal hippocampus and piriform cortex. The Ago treatment did not significantly reduce the number of immunopositive astrocytes in the CA2, CA3a, CA3b subfields of the dor-

sal and CA1, CA2, CA3 subfields of the ventral hippocampus, the dentate gyrus and the BLA. In addition, only a few immunostained ramified astrocytes were scattered in the ventral hippocampus of the KA-veh group, while the Ago-treated group showed a considerable increase of immunoreactive cells in this area compared to controls.

The present results show that chronic Ago treatment during epileptogenesis prevents the KA-induced microglial activation and suppresses the neuronal cell death in the vulnerable limbic regions. It can provide effective therapy for brain disorders involving neuronal damage, neuroinflammation and oxidative stress such as epilepsy.

KAINATE-INDUCED CHANGES AND THE EFFECT OF LOSARTAN ON ANGIOTENSIN AT1 RECEPTOR EXPRESSION IN LIMBIC STRUCTURES IN A RAT MODEL OF COMORBID HYPERTENSION AND EPILEPSY

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It is known that epilepsy causes elevation of components of the renin-angiotensin system in the hippocampal complex. In this study, we examined the effect of losartan, a selective angiotensin II, type 1 (AT1) receptor antagonist, on the expression of the latter in a model of kainic acid-induced temporal lobe epilepsy and comorbid hypertension in normotensive Wistar rats and naive spontaneously hypertensive rats (SHRs). Immunohistochemistry revealed that in the dorsal hippocampus AT1 receptor protein expression was enhanced in SHRs compared to that in normotensive rats. However, fewer AT1 receptor-immunoreactive cells were seen in the piriform cortex and the basolateral amygdala of SHRs. After kainate-induced status epilepticus, there was an increase in neuronal expression of AT1 receptors in the CA1, CA2, CA3a, and CA3c fields of the hippocampus, and the hilum of the dentate gyrus while the piriform cortex of epileptic SHRs and Wistar rats displayed a decreased AT1 receptor expression. In addition, an elevation in AT1 immunostaining was observed in the basolateral amygdala of epileptic SHRs but not in epileptic Wistar rats. The long-term administration of losartan exerted stronger and structure-dependent suppression of epilepsy-induced up-regulation of AT1 receptor levels in SHRs compared to Wistar rats.

In conclusion, our results confirm that AT1 receptor expression is modified in epilepsy and suggest a role of this receptor subtype in comorbid hypertension and epilepsy.

ASTROGLIA AND GRAFT VASCULARIZATION FOLLOWING INTRACEREBRAL TRANSPLANTATION IN A PARKINSON'S DISEASE MODEL

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The integration of transplanted neural cells with the host brain does not only depend on the newly formed synaptic connections. Vascularization of the graft tissue is crucial for its survival. Despite this, it is known that host glial cells actively interact with the grafted tissue. No extensive study of the glial reaction following intracerebral transplantation and its relationship with angiogenesis has been carried out.

The present study aims to elucidate the role of astroglia in the formation of new vessels following intracerebral transplantation in a rodent model of Parkinson's disease. We investigate the qualitative and quantitative characteristics of GFAP immunoreactivity around grafts and around zones of influence of the transplantation cannula at only two time points – day 7 and day 28 following transplantation.

Our findings demonstrate the formation of new blood vessels in graft cores and penetrating the graft-host interface on day 28 following transplantation. Moreover, astrogliosis surrounding grafts is more pronounced on day 28 following transplantation, as compared to day 7.

These results imply that astrocytes actively participate in the integration of intracerebral grafts. Their presence around newly formed vessels is considered an evidence for the functional maturity of these vessels. The intensive astrogliosis along the graft-host interface can be discussed as a sign of presence of a microcirculatory bed, and therefore for an organotypic integration of the grafts. Clarification of the exact sequence of events in the vascularization process and mechanisms of interaction between astroglia, neurons, and endothelium will shed light on the processes of CNS restoration through transplantation.

SEM REVEALS FILOPODIA-LIKE PROTRUSIONS IN THE EARLY EMBRYONIC DISC OF THE CHICK

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Embryonic development depends on an enormous amount of signaling events between a continuously growing number of cells. According to the classical view, signal transduction is based on either simple diffusion, transcytosis or migrating cells. Very recently the existence of filopodia-like protrusions has been proposed as a novel

mechanism of long-range intercellular signaling during chick somite and limb development. Analogous thinner structures called cytonemes play a role in the development of *Drosophila melanogaster*, as well.

The present study gives new insight into this problem by adding important information using scanning electron microscopy (SEM). Early chick embryos between stages 4 and 5 (representing late gastrulation and early neurulation) were dissected, fixed and critical-point dried and were then fractured in several steps, each step followed by platinum/palladium coating and analysis by SEM. Fracturing was carried out sequentially in the same specimens at different anterior-posterior levels anterior to the primitive node and the ultrastructure of the embryonic disc was analysed with special emphasis on cellular processes. We found cell protrusions of different shape and size in all three layers (mesoderm, epiblast, and hypoblast) of the early embryonic discs. They were most abundant and prominent between the mesodermal cells but also seen in other layers as well as between layers. These protrusions can be roughly classified into cytoplasmic bridges with mid-bodies, cilia-like protrusions, as well as filopodia-like protrusions. As previously shown, cilia of different length are present on the apical surface of epiblast and hypoblast cells. Similarly a plethora of variable protrusions with or without mid-bodies are seen between neighbouring cells within embryonic layers. Unexpectedly, we found that cells of the mesodermal layer form a dense net of filopodia-like contacts with the epithelial cells of the epiblast.

Our data prove that filopodia-like protrusions are present at earlier developmental stages than previously shown. Their abundance indicates a major role in the intercellular communication during early chick embryonic development and may provide a structural basis of cell-to-cell communication during gastrulation and early neurulation in the chick.

OREXIN-A MODULATES NEURONAL NETWORK ACTIVITY IN VITRO

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Orexin A (OXA) is a neuropeptide, isolated from neurons in the hypothalamus, which regulates various brain activities, including wakefulness and higher brain functions like learning and memory. There is a growing interest in OXA's role in neurodegenerative diseases with respect to non-motor symptoms such as sleep, attention and cognitive disorders. Recent studies in Parkinson's and Alzheimer's patients found lower concentrations of OXA in the prefrontal cortex and cerebro-spinal fluid. It is widely assumed that deteriorated cognitive processes are related to impaired network connectivity. However, little is known about the effects of OXA on the network activity and synaptogenesis. Therefore, we investigated the development of activity in dissociated cortical neurons of rat chronically treated with 0.5 µM OXA for three weeks. Network activity was recorded with multielectrode arrays. Additionally, after one, two or three weeks, cultures were stained immunocytochemically for detection of the presynaptic marker synaptophysin. OXA-treated cultures became spontaneously active earlier and the plateau of their activity was higher than in controls. Immunostaining revealed that the synaptic density was much higher in OXA-treated cultures across all age groups. Hence, OXA has a strong stimulating effect on network formation and activity, the latter probably being a consequence of the accelerated synaptogenesis. These results indicate that drugs, based on OXA, are potential candidates for prevention and treatment of disorders associated with neuronal connectivity and activity decline.

AUTOMATED IMAGE PROCESSING OF FLUORESCENT IMAGES: THE END TO MANUAL COUNTING?

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High-throughput experiments are characterized by the generation of large amounts of imaging data in short time which requires fast, automated and reliable methods for processing and analysis. Most of the gold standard methods for quantitative and semi-quantitative analysis of fluorescent images are relying on human decision-making. The existing automated analysis methods require very strict maintenance of constancy in all experimental conditions from animal care and material acquisition to image acquisition. In the high-throughput experiments, however, even the smallest biases introduced by human involvement and the tiniest fluctuations of experimental conditions may be multiplied to unacceptable magnitudes. Thus, any image processing and analysis algorithm, part of such an experiment, must reduce human-produced biases and be robust to variability in experimental conditions. We propose a processing sequence suitable for the analysis of images produced by wide-field epifluorescence RNA-FISH and FIHC experiments which is easily adjustable to the conditions of different experiments. The algorithm involves the following steps: (i) Optimal image acquisition of multichannel z-stack images; (ii) Shading correction of the multidimensional images; (iii) Single manifold extraction of an accurate high-contrast deblurred 2D-projection; (iv) Correction of exposure with emphasis on background normalization in the final image. With this algorithm, we produced images for automated classification of cells expressing different markers. After the initial processing a machine learning algorithm segments the DAPI stained channel and perinuclear regions of interest are produced. Next, the marker channels overlap with the perinuclear RoIs was used to classify each cell into positive or negative for the corresponding marker. The proposed sequence of image processing and analysis steps is performed with an easily procurable open source software (Fiji/ImageJ and Ilastik) which allows full automation thanks to its scripting capabilities.

HOMAGE TO GEORGE E. PALADE: THE HUMAN BODY VIEWED AS A MULTICRINE SYSTEM

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"*Seldom has a field owed so much to a single man*" – in 1971, George Emil Palade wrote these words for Albert Claude, the founder of biological electron microscopy. Now, expressing my profound homage to him, I would like to articulate the same words for George E. Palade, the scientific father of many generations in cell biology research and education. In 1981, I met Palade for first time during the World Physiology Congress held in Budapest, Hungary, and said to him that I know every single word and micrograph published in his articles on pancreatic exocrine secretion. They have driven my research on the secretory pathway in vascular smooth muscle cells (VSMC) studied by electron microscopy (1972-1992). In the present lecture I will focus on two paradigm shifts, namely (i) the transition from light to electron microscopy in studying cell structures and functions made by Albert Claude, George Palade, Christian de Duve and Günter Blobel from the famous Rockefeller group in New York, and (ii) the

transition from a "classical" to secretory phenotype of various cell types, e.g. cardiomyocytes, skeletal muscles and liver, bone, stomach, gut and adipose cells, also VSMC, the latter initiated in the 1970s by Maria Daria Haust in Canada and Russell Ross in USA and by our Laboratory of Electron Microscopy in Varna, later renamed Laboratory of Cell Biology. Altogether, these will be discussed in a sense that the human body may be viewed as a multicrine system.

A CORRELATION BETWEEN POSTNATAL CHANGES IN THE EXPRESSION OF NITRIC OXIDE SYNTHASE (NOS) IN THE RAT MYOCARDIUM AND ITS MORPHOLOGICAL CHARACTERISTICS

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The constitutive production of nitric oxide (NO) by the different isoforms of the enzyme nitric oxide synthase (NOS) is a major factor in the regulation of cardiac function under physiological and pathological conditions alike. As such, it affects various manifestations of the process of myocardial aging in the postnatal period, including hypertrophy and fibrosis of the ventricles and their remodelling, impaired relaxation and decreased cardiac output. The aim of the present study was to establish whether a correlation exists between changes in the expression of neuronal NOS (nNOS) and the morphological characteristics of the myocardium during various periods of postnatal development. We used fifteen male Wistar rats, distributed in five age groups – 2 weeks old, 1 month old, 3 months old, 6 months old, and 12 months old, each group containing 3 animals. We used routine haematoxylin and eosin staining and specific immunohistochemical staining for nNOS. During the early postnatal period (1-3 months), we observed a normal histological appearance of the cardiomyocytes and the interstitial space. As aging progressed (6-12 months), we reported the presence of cardiomyocytic hypertrophy, with narrowing of the interstitial space, focal myocytolysis and pronounced accumulation of collagen fibres. The immunohistochemical staining for nNOS revealed that enzyme activity intensified over the course of postnatal development. It was distributed heterogeneously in the various layers of the myocardium and we noted differences in the enzyme expression between the left and the right ventricle.

In conclusion, the increased expression of nNOS in senescent rats probably represents a protective mechanism against age-related hypertrophy of the cardiomyocytes. Further studies could possibly establish the importance of regional differences in enzyme expression within the myocardium.

A COMPARATIVE QUANTITATIVE ANALYSIS OF POSTNATAL CHANGES IN CARDIOMYOCITES IN NORMOTENSIVE AND SPONTANEOUSLY HYPERTENSIVE RATS

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Differences between the size of cardiomyocytes from normotensive and hypertensive hearts have been studied by a small number of authors and have been limited to measurements of the cellular diameter, length and cross-sectional area. The aim of the present study was to analyse a broader range of morphometric markers in order to assess the size of the cardiac muscle cells and to compare the obtained data between normotensive Wistar rats and spontaneously hypertensive rats. Results were obtained by studying the hearts of 15 male normotensive Wistar rats, distributed in five age groups: 2 weeks old, 1, 3, 6, and 12 months old, each group containing three animals, as well as 6 spontaneously hypertensive rats, distributed in two age groups: 1 month old (young) and 6 months old (adult), each group containing three animals. With the advance in age, the thickness of the free wall of the ventricles and the cross-sectional area of cardiomyocytes from both ventricles increased, as did the cross-sectional area of cardiomyocytic nuclei. Conversely, a decrease in the cardiomyocytic density was noted. These changes were more pronounced in the left ventricle of both normotensive and spontaneously hypertensive rats but occurred at an earlier age and were better expressed in the group of spontaneously hypertensive rats. The morphometric markers analysed in the present study represent a statistically significant assessment, which illustrates the differences between hypertrophy and remodelling of the myocardium induced by advancing age under normotensive conditions and those initiated by arterial hypertension.

ARTERIA CYSTICA ANOMALIES IN LAPAROSCOPIC CHOLECYSTECTOMY

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Aim: **In laparoscopic surgery, knowing the anatomic variations helps being ready for any possibilities. In this study, the positions of the Calot's triangle, and a. cystica and ductus cysticus in relation to each other were evaluated in people from the area who underwent laparoscopic cholecystectomy, and frequency of variation was reviewed.**

Materials and Methods: **In our study, the operation images of 100 patients who underwent laparoscopic cholecystectomy in the General Surgery Clinic at the Medical Faculty of Trakya University were monitored and the anatomy of Calot's triangle was evaluated.**

Results: **In 82% of our cases, the a. cystica was observed as a single branch in the Calot's triangle, whereas two aa. cysticae were clipped in 12% of the cases. In 2 cases, a. hepatica dextra in the Calot's triangle was observed very close to the gallbladder serosa, and there was a very short a. cystica. In one case, a. cystica first passed to the posterior aspect of ductus cysticus and formed a curve at the lower portion of ductus cysticus, then turned to the an-**

terior aspect of the duct, and continued to the gallbladder serosa from the neck part. In 3 cases in this study, a. cystica moved in a caudal direction and was parallel to ductus cysticus, then entered the bladder.

Conclusion: In brief, the anatomy of the Calot's triangle, which acts as a key point in laparoscopic cholecystectomy, varies much. A good understanding of the anomalies of a. cystica in laparoscopic cholecystectomies is important in order to prevent possible complications.

RELEVANCE OF THE TOPOGRAPHIC LOCALIZATION OF THE PERONEAL SUPERFICIAL NERVE BEFORE ANKLE ARTHROSCOPY

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Introduction: The popularity of ankle arthroscopy as a diagnostic and theurapetic method is growing. Numerous pathological conditions serve as indicators for an application of the method. Just a few of those examples are bone and soft-tissue impingement syndromes, osteochondral defects, fractures, osteoarthrosis. Unfortunately, one of the most frequent complications of ankle arthroscopies is the iatrogenic lesion of the peroneal superficial nerve. The reason for this is the close contact between the position of the nerve and the antero-lateral access point for the arthroscopy. The preoperative localization of the nerve reduces greatly the risk of its injury, therefore, reducing the risk of paraesthesia, hypoesthesia, and dissatisfaction for both the patient and the doctor.

Aim: The aim of this paper is to introduce a method for topographic localization of the peroneal superficial nerve.

Materials and Methods: Twenty-two arthroscopies were performed for a period of 6 months (02.2017-08.2017). Preoperative topographic localization of the nerve was applied before all of them.

Results: No clinical signs and symptoms of peroneal superficial nerve lesion were observed in 21 of the cases. Only one case showed postoperative transitory paresthesia for a period of 27 days.

Conclusion: The preoperative topographic localization of the peroneal superficial nerve has a very important part in the prevention of postoperative complications after ankle arthroscopy.

TRANSVERSAL, SAGITTAL AND DORSAL ANATOMICAL MRI STUDY OF THE RABBIT LIVER

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Magnetic resonance imaging (MRI) is an applicable method for the contemporary anatomical visualization of small mammals' abdominal organs. The anatomical object studied is separated into voxel units, depending on the magnetic field. The anatomical objects are obtained on thin scan levels.

We studied the liver of ten sexually mature, clinically healthy New Zealand white rabbits in T2- and T1-weighted sequence.

On a transversal plane, T2-weighted sequence, the anatomical boundary between the left and right liver lobes was not defined. The left medial lobe of the liver was hyperintense compared to the left lateral lobe. The gallbladder demonstrated a hypersignal. Medial to the quadrate lobe was the gallbladder. The cystic duct demonstrated hypersignal to the parenchyma. The right, left medial and left lateral lobes of the liver had dark grey tissue contrast.

On sagittal T2 of the abdominal region at the level of the plane, 10 mm to the left, the left medial lobe of the liver was hypointense. Caudal to the left medial lobe was the lateral one. In the sagittal plane, 20 mm to the left, the medial and left lateral lobes and papillary process were hyposignal anatomical findings. In the sagittal plane, at 10 mm to the right, the right lobe was cranial to the fundus and body of the stomach. The papillary process touched the fundus of the stomach. The caudate process reached the right adipose capsule of the kidney. In the sagittal plane, 20 mm to the right, the left medial and quadrate lobes were caudal to the right lobe. The caudate lobe was dorsal.

In the dorsal plane (T1-weighted sequence), 15 mm ventral to the spine, the right lobe was cranial to the other lobes. Left to the median plane was the left lobe. In the dorsal plane, 30 mm to the spine, the right lobe was covered by the caudate lobe. The left medial lobe covered the left one. In the plane, 45 mm ventral to the spine, the right and left lobes were hyposignal findings in the same anatomical plane.

3D PRINTERS AND MEDICAL EDUCATION

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3D modeling is the process of developing a mathematical representation of the surface of an object in three dimensions with specialized software. The product is called a 3D model. This model can be physically created using 3D printing devices.

In 1981, Hideo Kodama of Nagoya Municipal Industrial Research Institute invented the methods for fabricating three-dimensional plastic models with photo-hardening thermoset polymer. In 1984, Chuck Hull from 3D Systems Corporation produced stereolithography fabrication system, in which layers were added by curing photopolymers with ultraviolet light lasers. The developments in 3D printing gathered attention of engineering fields. The open patents and the use of cheap materials popularized it. It took place in education and production of custom made prosthesis in medicine.

3D bio-printing consists of 3 stages. The first stage is the creation of a 3D model. The second stage is to convert the 3D object data into an STL file format, by approximating the contours of the 3D object with a series of tessellated triangles. Finally, the STL file is sent to a 3D printer for production.

The detailed knowledge of human anatomy is important for medical students and doctors of various departments, especially for those in surgical departments where technical skills are developed based on anatomical knowledge. In various studies, education with 3D products shows positive results. 3D products are relatively cheap, easier to store, scalable, dissectable, ethically unproblematic and able to show rare cases. Education with the use of 3D products is safe and tolerant in regard to mistakes. Also it gives an opportunity for pushing the limits.

COMPARATIVE STUDY ON FINGER-CUN MEASUREMENTS OF THE ACUPUNCTURE POINTS PC6 AND TW5

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Locating acupuncture points reliably and reproducibly is a requisite for the accuracy of scientific research of acupuncture and for assuring the appropriate and effective treatment of patients. Unreliable point location can produce false findings for acupuncture research and clinical practice. The acupuncturists use the one cun measurement as well as various lengths and breadths of the fingers in order to locate acupuncture points.

The finger-cun measurements are;

- **1 cun: the width of the interphalangeal joint of the thumb.**
- **1.5 cun: the width of the index and middle finger, measured at the level of the proximal interphalangeal joint of the index finger.**
- **2 cun: the length of the two distal phalanges of the index finger.**
- **3 cun: the width of all four fingers, measured at the level of the proximal interphalangeal joint of the index finger.**

PC6 and TW5 acupuncture points are 2 cun above the wrist crease anterior and posterior, respectively. There have been several reports on the reliability of traditional acupuncture point location methods.

In this study we have investigated the four different finger-cun measurements to provide accurate estimates for contemporary Turkish adults. The PC6 and TW5 points were accurately detected with an acupuncture detector. One cun, 1.5 cun, 2 cun, and 3 cun were measured with a digital caliper.

The most frequently used finger-cun measurement to find PC6 or TW5 is the 2-cun method. As a result, the 2-finger cun method is more reliable than the 1-cun method to find these two acupuncture points in Turkish adults.

A COMPARISON BETWEEN DIRECTIONAL (FINGER-CUN) AND PROPORTIONAL (PROPORTIONAL BONE CUN) METHODS IN LOCATING ACUPUNCTURE POINTS IN THE FOREARM

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The cun measurement system is an essential component of the traditional point location methods used in acupuncture. This study used the cun system to investigate any variation between the traditional measurements and the sample means for selected finger measurements, and for the forearm length obtained from 110 healthy volunteer subjects randomly selected from students of the Faculty of Medicine and Faculty of Health Sciences, Trakya University, Edirne, Turkey.

This study was conducted in order to determine if finger cun-measurements are consistent with proportional bone methods and to investigate whether both methods can be used to locate acupuncture points in the forearm. The length of the forearm was measured using tape measure. The length of the forearm was compared with 1, 1.5, 2 and 3 finger cuns measured with a digital caliper. Additionally, PC6 and TW5 acupuncture points were found with an acupuncture detector. The distance from the wrist crease to PC6 and TW5 was measured and compared with the measurements obtained from directional and proportional methods.

The results showed that 3 cun measured by the directional method was most significantly different from one cun measured by the proportional method. The directional method is likely less dependable in locating PC6 and TW5 acupuncture points than the proportional method.

THE RELATIONSHIP BETWEEN KIDNEY SIZE AND ANTHROPOMETRY

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The size of the kidney is important for the clinical evaluation of kidney diseases and also helps us to diagnose many diseases related to kidneys. The purpose of this study is to determine the usage of both anthropometric measurement and radiological measurements for prediction of kidney size.

Fifty-four volunteers who were patients at the Urology Clinic of Faculty of Medicine at Trakya University participated in this study. The volunteers did not have any kidney disease. Abdominal ultrasonography was used to measure kidney size. Anterior abdominal wall length, lateral abdominal wall length, and bispinal distance were measured using a caliper.

The average anterior abdominal height, lateral abdominal height, and bispinal distance were 17.59 cm, 17.28 cm, and 27.56 cm respectively. In the same way, the mean values of right kidney length, right kidney transverse diameter, left kidney length, and left kidney transverse diameter were 102.75 mm, 44.72 mm, 99.82 mm, and 46.35 mm, respectively. There was a significant correlation between the left kidney length and the lateral abdominal wall height ($p<0.05$). However, there was no relationship between the right kidney length and any anthropometric measurements. There was a significant correlation between the right kidney transverse diameter and all anthropometric measurements ($p<0.05$), and there was a significant correlation between the left kidney transverse diameter and the anterior abdominal wall height ($p<0.05$).

This study showed that there is a significant relationship between kidney dimensions and some anthropometric measurements. These measurements can be used while examining patients. We suggest expanding this study with more participants to ensure more accurate results.

NEW ANTHROPOMETRIC MEASUREMENTS FOR PREDICTING DIFFICULT INTUBATION: THE GONIOMASTOID DISTANCE AND THE THYROGONIAL DISTANCE

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Aim: **This study aimed to determine the sensitivity and specificity of various anatomic measurements, alone or in combination, in predicting difficult intubation. We hypothesized that goniomastoid distance (GMD), thyrogonial distance (TGD), and tongue movements are sensitive and specific tests for predicting difficult intubation.**

Methods: **We included the data of 170 patients (85 difficult intubations and 85 easy intubations) who underwent an abdominal surgery under general anesthesia. The groups of patients with difficult intubation (n=85) and easy intubation (n=85) were matched by age, BMI, and gender. Intubation difficulty scale (IDS) scores were recorded. Upward and downward maximal protrusions of tongue (UMPT and DMPT, respectively), as the tongue moves, were evaluated in all patients. Anthropometric measurements, including GMD, TGD, and IID were measured.**

Results: **Among the GMD, IID, TGD, interincisal distance, UMPT, DMPT, and their combinations, the GMD had the highest area under the curve (AUC) (AUC=1.0; p<0.001) and predictive values (sensitivity=100%, specificity=100%, PPV=100%, and NPV=100%), with a cut-off value of 53 mm in the classification of difficult intubation. IID achieved the second highest AUC (AUC=0.924; p<0.001) and predictive values (sensitivity=88.24%, specificity=96.47%, PPV=96.1%, and NPV=89.1%). Difficult intubation risk in patients whose UMPT level did not reach the target line was 5.6 times higher, and for those whose DMPT level did not reach the target line, it was 4.9 times higher.**

Conclusion: **GMD is the most powerful test when compared to the other predictive tests. Thus, it may be used in clinical practice for prediction of difficult intubation.**

PREDICTION OF DIFFICULT INTUBATION USING TONGUE MOBILITY

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Aim: **The aim of study is to determine the possible usage of tongue movements out of the mouth in the prediction of difficult intubation.**

Methods: **Two hundred twenty-three patients undergoing abdominal surgery under general anesthesia, between 2014 and 2015, were enrolled in this study. Difficult intubation was evaluated using the intubation difficulty scale (IDS) score. All intubations were performed by the same anesthesiologist to eliminate the differences in the anesthesiologists' experience. IDS scores were evaluated by anesthesiologists who had no idea about the measurements of the tongue movements and performed laryngeal intubation in all patients.**

To define the tongue movements, horizontal lines were used. Horizontal lines were passed through the mid points of the mandible and upper lip height. Patients were asked to protrude the tongue maximally and upward (PTMU), to protrude tongue maximally and downward (PTMD) in anatomically neutral position when sitting down on a chair.

Results: **Mallampati III and IV score in the difficult intubation group was significantly higher than in the groups without difficulty in intubation ($p<0.001$). Similarly, Cormack-Lehane III and IV score in the difficult intubation group was significantly higher than in the groups without difficulty in intubation ($p<0.001$). PTMU and PTMD in the difficult intubation group was significantly higher than in the groups without difficulty in intubation ($p=0.001$ and $p=0.002$, respectively). Patients who could not reach and pass target of PTMD OR=7.3 (95% CI: 2.1 – 25.0) had a higher risk of difficult intubation than the ones who could reach and pass the target ($p=0.001$). Patients who could not reach and pass the target of PTMU OR=4.5 (95% CI: 1.7 – 11.4) had a higher risk of difficult intubation than the ones who could reach and pass the target ($p=0.001$).**

Conclusion: **We investigated the relationship between difficult intubation and tongue movements, which has some advantages like easy application and objectivity.**

SLEEP QUALITY OF THE PATIENTS WITH LUNG CANCER

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Aim: **Similarly to other kinds of cancer, lung cancer has a lot of symptoms that affect the human body in a negative way. One of the worse effects is sleep disturbance. Sleep quality could be changed by many different effects like respiratory and paraneosplastic syndromes.**

In that study, we aimed to study the sleep quality and other factors, which effect the sleep quality of patients with lung cancer, especially in an outpatient setting.

Methods: **Pittsburgh SQI testwas used on 30 lung cancer patients, regardless of their stage.**

Results: **We found that 65.4% of the volunteers included in the study had sleep disturbance. In our opinion, sleep quality is in a close relationship with the stage of cancer and it also depends on other complications like metastasis.**

EXPRESSION OF GHSR1 IN LIVER OF DIETETIC MANIPULATED RATS

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Introduction: **Ghrelin is a gastrointestinal hormone that affects metabolic processes and energy metabolism by a central mechanism and directly via its receptors in peripheral tissues. The involvement of ghrelin in the metabolic syndrome is the subject of a number of studies. One of the frequent disorders of the metabolic syndrome is non-alcoholic hepatic steatosis. The aim of our study is to detect the presence of ghrelin receptor GHSR1 in rat hepatocytes treated with a high-fat-high-carbohydrate diet (HFHCD).**

Materials and Methods: **Female Wistar rats (n=10) were fed HFHCD for 16 weeks. Control rats (n=10) were fed a normal diet for the same period of time. Metabolic control was determined by measuring body weight gain, BMI, adiposity, and plasma biochemical parameters. Immunohistochemical study was performed on the liver of both groups with primary ghrelin receptor antibody GHSR1.**

Results: **The immunohistochemical reaction in the liver of the control group was negative. In the liver of rats treated with HFHCD, we detected expression of GHSR1. The ghrelin receptors were positivized in small groups of hepatocytes located around central veins and periportal spaces.**

Conclusion: **The presence of ghrelin receptors in hepatocytes allows ghrelin to interfere directly in their metabolism and lipogenesis. Expression of the ghrelin receptors in liver can be used as one of the biomarkers for detection of metabolic syndrome and diseases associated therewith.**

ANATOMICAL PREDISPOSITIONS OF MASTICATORY APPARATUS AND UPPER RESPIRATORY TRACT FOR DEVELOPMENT OF OBSTRUCTIVE SLEEP APNEA AND SNORING

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Snoring occurs in the area of the uvula, soft palate, tonsillar pillars and/or pharyngeal walls. While obstructive sleep apnea (OSA) is a secondary collapse of these anatomic levels, but also due to obstruction by the lingual tonsils or epiglottis. The anatomical and structural factors associated with craniofacial bony anatomy predisposing to the OSA are: retrognathia and micrognathia; maxillo-mandibular hypoplasia, large degree of overjet; high-arched hard palate; enlarged "kissing" tonsils - adenotonsillar hypertrophy. These craniofacial skeletal abnormalities can lead to OSA among children and non-obese adults.

The prevalence of sleep disordered breathing (SDB) in the middle-aged general population was reported to be around 9% for women and 27% for men in studies performed in the 1980s and 1990s. Considering the recent improvements in the recording techniques and the various criteria used to define respiratory events, our aim was to re-evaluate the prevalence of SDB using three different apnea-hypopnea definitions. The palatine uvula, usually

referred to as simply the uvula, is a conic projection from the posterior edge of the middle of the soft palate, composed of connective tissue containing a number of racemose glands, and some muscular fibers (musculus uvulae).

Snoring is more closely associated with fat deposition in the pharynx, enlarged tonsils of Waldeyer's ring, or deviated septum problems. The soft palate is distinguished from the hard palate at the front of the mouth in the fact that it does not contain bone. The structural muscles are the five muscles of the soft palate that play important roles in swallowing and breathing. The muscles are: tensor veli palatini, which is involved in swallowing; palatoglossus, involved in swallowing; palatopharyngeus, involved in breathing; levator veli palatini, involved in swallowing; and musculus uvulae, which moves the uvula. These muscles are innervated by the pharyngeal plexus via the vagus nerve, with the exception of the tensor veli palatini.

The tongue is a muscular organ in the mouth of most vertebrates that manipulates food for mastication and is used in the act of swallowing. It plays an important role in the digestive system and is the primary organ of taste in the gustatory system. Adults who habitually breathe through the mouth, attributable to nasal obstruction, are more likely to have sleep disorders and attention-deficit/hyperactivity disorder.

AGE-RELATED CHANGES OF NNOS IMMUNOREACTIVITY IN RENAL CORPUSCLES OF SPONTANEOUSLY HYPERTENSIVE RATS

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The immunohistochemical expression of neuronal nitric oxide synthase (nNOS) in the renal structure is predominantly described in macula densa – a structural component of the juxtaglomerular apparatus, represented by cells of the distal convoluted tubules. Under pathological conditions such as hypertension, the impaired nitric oxide (NO) bioactivity may be an important pathogenetic factor. In addition, the synthesized NO by nNOS is involved in the regulation of the afferent arteriole diameter, tubuloglomerular feedback mechanism, and the renin-angiotensin system. The aim of the present study was to make a detailed description of the nNOS expression in the renal corpuscles under hypertonic conditions in spontaneously hypertensive rats (SHRs). We used nine male SHRs and nine aged-matched male Wistar rats divided into the following groups: 4 months old, 6 months old and 12 months old, each group was represented by three SHRs and three Wistar rats. The immunohistochemical reaction was performed by specific monoclonal antibody for nNOS. In 4-month-old SHRs we observed heterogeneous expression of nNOS in the cells of parietal layer of Bowman's capsule and glomerular capillary tuft (GCT), while the age-matched Wistar group was characterized by weak to missing immunoreactivity. During the period of target organ damage (6- and 12-month-old rats) we found intensified immunoreactivity of nNOS in the cells of the parietal layer of Bowman's capsule and GCT compared to the control groups and noted differences in the distribution of the enzyme in the renal corpuscles of the cortical, midcortical and juxtamedullary nephrons.

In conclusion, there are pronounced age-related differences of nNOS immunoreactivity in renal corpuscles of SHRs and Wistar rats. Most of the studies have discussed the role of macula densa nNOS-derived NO, but the information about the function and significance of this enzyme in the other renal structures is still limited. Future studies may reveal in details the importance of nNOS-derived NO in renal pathology.

CYCLIN D1 AND P53 AND THEIR EXPRESSION IN HUMAN LEYDIG CELLS DURING AGING

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Apoptosis is a complex process, which is adjusted on many levels by a wide range of pro- and anti-apoptotic proteins. It has been established that the long-term damage to the balance between the two groups causes apoptotic changes in cells. Cell apoptosis is also an active process which occurs in the course of aging. The key factors, not only the age-related ones, but also those linked to the overall process of apoptosis affecting the steroidogenic Leydig cells (LCs) and germinative epithelium in the testes are mitochondrial damages caused by free radicals (ROS - Reactive oxygen species). The direct result of the aging of the testis is reduction in the levels of testosterone and the results of this are disruption of the physiological processes and dramatically deteriorated the quality of life. A number of studies reported involvement of the proteins p53 and Cyclin D1 in the regulation of some of the mechanisms of apoptosis. P53 promotes the expression of many apoptotic genes (transcription factor) and has an important role in the apoptosis in normal spermatogenesis and in the response to DNA damage. The role of Cyclin D1 is examined in two directions - cell proliferation and provoking apoptotic changes in major damage to the DNA of the cells. At this stage, the role of p53 and Cyclin D1 in the control of apoptosis in human LCs in the aging testis is not well investigated, which determines the aim of the present study. In this study we used material from human testes from patients between 56, 68 and 80 years of age, embedded in paraffin and prepared for immunohistochemistry. Our results demonstrated an increased intensity of the immunoexpression of p53 and Cyclin D1 in the course of aging. These findings marked the role of these proteins in the regulation of testicular apoptosis.

LIFESTYLE AND ENVIRONMENTAL FACTORS AFFECTING FERTILITY IN MEN

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The studies on the quality indicators of sperm studies have shown deteriorating results in all industrialized countries in the world, prompting scientists to seek the reasons for this. Until now, lifestyle has been an overlooked factor, but nowadays, more and more attention is paid to it as a reason for poor results. It is not possible to prove with uncontested statistical data which of the environmental factors and what part of the daily life of modern man impacts his health and in particular the male reproductive function. But looking at the more recent past and taking into account the changes that have occurred in the last 100 years it is not possible to not take into account the changes in the eating habits, use of hormones, chemicals, antibiotics in the processing of food, lifestyle, and more specifically the predominance of a sedentary life compared to the time spent outdoors performing active physical activity and the stress that we are subjected to daily affect human health and in particular the quality of sperm parameters. The evaluation of the quantitative and qualitative indicators of the ejaculate is conducted using the criteria of the World Health Organization. According to them normozoospermia is defined as sperm with the following characteristics: volume above 2.0 ml; concentration of sperm over 15x106 ml; total sperm number over 40 million; sperm motility more than 40% and sperm with normal morphology over 4%. Unfortunately, more and more young men do not cover these requirements.

MEASUREMENT OF ACE ACTIVITY AND ITS IMPACT OVER MALE STERILITY

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According to recent studies, sterility has been growing progressively, and in about 40% of the cases infertility is caused by changes in spermatogenesis. Sterility does not endanger the physical health but it has an emotional impact on the personality and it influences negatively personal, interpersonal, social and professional attitudes. Of particular importance is the accurately developed spermogram and adequate treatment plan. Regardless of the continuously introduced new methods for diagnosing infertile patients, most of the factors influencing male sterility remain unclear. Between 40 and 50%, and according to some authors, up to 60% of male sterility is diagnosed as idiopathic, i.e. unsolved diagnosis. Increasingly, as a leading cause, the environmental effects of epigenetics are considered, causing an increase in oxidative stress in the sperm membrane, affecting the appearance of a gene without altering gene expression. The testicular form of Angiotensin-converting enzyme (ACE), one of the enzymes for which there are many phenotypic manifestations, has been established but it is difficult to test. The ACE activity has been shown to have a relation to the diagnosis of the causes leading to idiopathic infertility.

CELL CULTURES IN BIOCOMPATIBILITY ASSESSMENT OF NEW MATERIALS FOR BONE IMPLANTS

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The extended life expectancy and the increasing number of overweight people place bone diseases among the most serious health and social problems of our time. To meet this challenge there is a need for advanced new materials for bone implants that maximally resemble the properties and behaviour of natural bones as well as experimental designs to evaluate their biological activity. The aim of our study was to assess the cytocompatibility of different classes of scaffolds for bone implants, including fine calcium phosphate powders, composite materials, cements, and bacterial cellulose-based materials. Murine (bone marrow cells, cell cultures from bone explants, BALB/c 3T3 and L929 fibroblasts) and human (Lep-3 and MRC-5 fibroblasts) cells were used as model systems in our investigations. The effect of the materials on cell viability and proliferation was evaluated in direct (the cells were seeded on the material surface) and/or indirect (the cells were cultured in a medium where the materials were incubated for various periods of time) experiments by the MTT test (the gold standard for cytotoxicity assessment), neutral red uptake cytotoxicity assay, double staining with acridine orange and propidium iodide, SEM, Comet assay, Annexin V (FITC assay, alkaline phosphatase activity assay, alizarin red staining. The results obtained revealed that: the cell cultures used as model systems in our investigations have different advantages and disadvantages and provide complementary information on the biological activity of materials. Some of the examined scaffolds show promising biocompatibility and require additional studies on their osteoinductivity and osteoconductivity.

DEVELOPMENT AND STEROIDOGENIC CAPACITY OF ADULT LEYDIG CELL POPULATION IN EXPERIMENTAL CONDITIONS OF HYPERGLYCEMIA AND OBESITY

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Introduction and Aim: **The metabolic syndrome involves various abnormalities like obesity, insulin resistance/diabetes, hypertension, and hormonal disorders. It is a serious risk factor for male infertility, often associated with compromised hypothalamic-pituitary-gonadal axis. The aim of the present study was to evaluate adult Leydig cell (LC) number and steroidogenesis in tandem with macro biomarkers (body and testis weight, fat accumulation) in experimental conditions of diabetes mellitus (DM) induced on day 1 (neonatally, NDM) or day 10 (prepubertally, PDM); short- and long-term high-fat diet (HFD) with induced obesity in rats since puberty.**

Results: **Significant increase was established in body weight and body fat in short- and long-term HFD. It was associated with a decrease in relative testicular weight in long-term HFD (gonado-somatic index). Epididymal and inguinal adipocyte diameters were increased in the animals from both groups.**

HFD did not affect significantly serum testosterone and LH levels. Nevertheless, expression of LC key steroidogenic factors and intratesticular testosterone levels were significantly lower in long-term HFD rats, associated with a reduction of the LC number.

Hyperglycaemia was confirmed by a significant elevation of blood glucose levels by 15% in adult NDM and by 35% in PDM rats. In NDM body weight and testicular weight were increased but the gonado-somatic index was decreased by 15%. PDM rats had normal body weight but the testicular weight and gonado-somatic index were decreased by 30%. Leydig cell number and testosterone production were in normal range in adult NDM, whereas they were significantly decreased in PDM.

Conclusion: **Our results from the HFD experimental model demonstrated that long-term HFD influenced negatively the number of Leydig cells and testosterone production. Prepubertal DM, but not neonatal DM, suppressed Leydig cell development and steroidogenesis in adulthood. Altogether, our data indicated that diabetes and obesity affected the size of adult Leydig cell population and testicular steroidogenic function and they were associated with reduced macroparameters - body weight, absolute and relative testicular weight.**

APPLICATION AND OPTIMIZATION OF A COMPLEX APPROACH FOR IDENTIFICATION OF MULTI-TARGET ANTICANCER AGENTS

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Drug resistance and toxic side effects are among the main obstacles preventing successful treatment of neoplastic diseases. Development and application of agents that have the capability to interact selectively with two or more macromolecular targets is a promising strategy to overcome these challenges. The identification of compounds with anticancer potential requires a complex approach based on a wide spectrum of model systems and methods with different molecular/cellular targets and mechanisms of action. The optimization and introduction of such an approach in our laboratory was the aim of the study presented.

The cytotoxic activity of various synthetic compounds with different structure and physico-chemical characteristics (cisplatin, disulfiram, Cu(II) complexes with Schiff bases, etc.) was evaluated in short-term (24-72h, with monolayer cultures) and long-term (30-45 days, with 3D cancer cell colonies) experiments by the MTT test, neutral red uptake cytotoxicity assay, crystal violet staining, trypan blue dye exclusion technique, lactate dehydrogenase activity assay, double staining with acridine orange and propidium iodide, Annexin V/FITC assay, Comet assay and colony-forming method. The investigations were performed using permanent cell lines established from chicken (hepatoma), rat (sarcoma) and human (breast cancer, cervical carcinoma, liver cancer, non-small cell lung cancer, glioblastoma multiforme) cancers of different etiology, histological type and expression of hormonal receptors (in the case of breast cancer).

The conditions for carrying out these methods were optimized and their advantages and disadvantages were specified. Information regarding the influence of the compounds investigated on cell survival and proliferation was obtained. Our results indicate that in order to obtain adequate experimental data, the approach described above has to be adapted to each individual "cell line – compound" system.

Acknowledgements: This study was supported by Grant -DCOST 01/16 from 17.08.2017, COST Action CA15135 and a bilateral project between the Bulgarian Academy of Sciences and the Romanian Academy.

METAL COMPLEXES OF URSODEOXYCHOLIC ACID AND ITS METAL COMPLEXES AS POTENTIAL ANTITUMOR AGENTS AGAINST COLON CANCER

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Colorectal cancer (CRC) takes the third place among the most commonly diagnosed cancer in males and the second in females. It is the second leading cause of death among neoplastic diseases worldwide.

The physiological role of bile acids (BAs) is well known. The prevalence and clinical application of ursodeoxycholic acid (UDCA) as well as the data on participation of BAs in the pathogenesis of several liver diseases and gastrointestinal (colon) tumorigenesis provoke interest in the relationship between UDCA and cancer. Experimental evidence (in vitro and animal studies) suggests that ursodeoxycholic acid may have chemopreventive actions in colorectal cancer.

The aim of our study was to evaluate the influence of Cu(II), Zn(II) and Ni(II) complexes of ursodeoxycholic acids on viability and proliferation of cultured human colon cancer cells.

In our investigations, we used the permanent cell line HT29 (human colorectal carcinoma) as a model system. The compounds tested were applied at concentrations of 10-200 μg/mL for 24-96 h (for short-term experiments with monolayer cell cultures) and 30 days (for long-term experiments with 3D cell colonies) and their effect on cell viability and proliferation was evaluated by the MTT test, neutral red uptake cytotoxicity assay, crystal violet staining, trypan blue dye exclusion technique, double staining with acridine orange and propidium iodide and colony-forming method.

Our results showed that the compounds investigated decreased viability and proliferation of the treated cells in a time- and concentration-dependent manner. Metal complexes expressed more pronounced cytotoxic/cytostatic activity compared to the corresponding ligand ursodeoxycholic acid.

The metal complexes examined exhibited promising cytotoxic/antiproliferative properties against HT29 colon cancer cells and deserve further studies to clarify better their anti-tumor potential.

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HISTOLOGICAL AND QUANTITATIVE EVALUATION OF ADULT MOUSE SPERMATOGENESIS AFTER ACUTE TREATMENT WITH SODIUM NITRITE

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Introduction: **Sodium nitrite ($NaNO_2$) is an inorganic salt with various applications. Industrialization and uncontrolled use of nitrate/nitrite salts has increased human exposure to high levels of $NaNO_2$, which can act as a pro-oxidant and pro-carcinogen. The adverse health effects of $NaNO_2$ are typically due to the formation of methemoglobin in the blood and respectively to hypoxia. Most mammals have little tolerance to hypoxia.**

Materials and Methods: **Adult male ICR mice were intraperitoneally injected with a single toxic dose of 120 mg. kg-1 b.w. $NaNO_2$ to evaluate the early effect of acute hemic hypoxia on the testes and sperm count. Treated animals were sacrificed at different time intervals (1h, 5h, 1d, 2d and 5d) following the administration. Testes and epididymides were sampled, weighed and embedded in paraffin using routine histological practice. Spermatozoa were isolated from both vasa deferentia and counted.**

Results: **Apart from the normal morphology of the tubules, histological observations of the testis in all investigated groups demonstrated areas with disorganization of the seminiferous epithelium and collections of undifferentiated germ cells in the luminal region. We observed overgrowth and lumen distension of rete testis, a marker for accumulation of seminal fluid in the testis. Data correspond to increased testicular weight/body weight index. The epididymal index in all groups was reduced bu about 40% compared to controls. Regarding sperm count, we established a reduction in almost all of the groups, statistically significant only 48 h after treatment.**

Conclusion: **The quantitative changes of macro-parameters and sperm count were supported by a histopathological alteration of mice testes even in the early periods after $NaNO_2$-induced hemic hypoxia.**

AMELIORATIVE EFFECT OF SALINOMYCIN ON SOME QUANTITATIVE PARAMETERS OF SPERMATOGENESIS IN CADMIUM-INTOXICATED MICE

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Cadmium (Cd) is a heavy metal of considerable toxicity with destructive impact on most organs. Our previous results and numerous reports demonstrate that Cd induces complete degeneration of the seminiferous tubules with severe depletion of germ cells and spermatogenesis arrest. So far, there are no effective antidotes for treatment of Cd poisoning. In our study, we present the effect of polyether ionophorous antibiotic salinomycin on some quantitative parameters of spermatogenesis in Cd-intoxicated mice.

The experimental animals were divided into four groups as follows: Group 1 consisted of untreated control mice receiving distilled water for 28 days; Group 2 (toxic control group) - received Cd(II) acetate orally at a daily dose of 20 mg/kg b.w. for 2 weeks, followed by 2 weeks of distilled water; Group 3 consisted of mice exposed to Cd(II) ace-

tate for 2 weeks, as described for group 2, and subsequently treated with an average daily dose of 20 mg/kg b.w. of tetraethylammonium salt of salinomycinic acid for 2 weeks; Group 4 consisted of mice who received distilled water for 2 weeks and were subsequently treated with an average daily dose of 20 mg/kg b.w. of tetraethylammonium salt of salinomycinic acid for 2 weeks. All compounds were dissolved and received in the drinking (distilled) water. Animals and testes were weighed to calculate testicular index (testicular weight/body weight ratio). Spermatozoa were isolated from both vasa deferentia and counted.

We found statistically significant increase of the testicular index in Group 4 (salinomycin alone) compared to the Cd-intoxicated group as well as to the control. Sperm count significantly increased (87%) after salinomycin administration to Cd-intoxicated animals. These positive effects correspond to our data for restoration of morphology in other organs of Cd-intoxicated mice and demonstrate the potential application of salinomycin as an antidote for the treatment of Cd-poisoning.

PERIVASCULAR ADIPOSE TISSUE

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Introduction: Obesity is an enormous social problem associated with substantial modulation of adipose tissue structure, involving adipogenesis, angiogenesis, and extracellular matrix remodeling tissue architecture and function. Perivascular adipose tissue (PVAT) is an accumulation of adipose tissue around vasculature, which releases a variety of biologically active molecules, such as adipokines and cytokines, to regulate vascular smooth muscle cells. Undoubtedly, PVAT monitors endothelial function and its role can be protective or detrimental. Cardiovascular disease (CVD) is highly associated with obesity and the metabolic syndrome. Risk factors for CVD are: hypertension, dyslipidaemia, increased visceral adipose tissue mass, diabetes, obesity, etc. It is still unclear how adipose tissue depot contributes to the pathogenesis of CVD and because there is a lack of data regarding the morphogenesis of vascular alterations, we aimed to investigate morphological changes of both blood vessels wall and adipose tissue in cases with CVD and whether peri-coronary epicardial adipose tissue (EAT) is associated with vascular risk factors and coronary atherosclerosis.

Materials and Methods: Material was received from the necropsies of patients who had suffered from a cardiovascular attack. Classical histological technique was applied.

Results: We found alterations in the affected blood vessels; the tunica intima was thickened, which is in fact fibrosis and increased epicardial adipose tissue. The luminal surface of the intima formed a markedly wavy configuration. PVAT was increased in amount and surrounded the blood vessels.

Conclusions: Coronary artery segments involved in cardiovascular disease are characterised by diffuse intimal thickening without lipid or calcium content. The results support the hypothesis that EAT affects coronary atherosclerosis and possibly increases coronary risk.

CYTOTOXIC ACTIVITY OF NEW ZN(II), CO(II) AND NI(II) COMPLEXES WITH KOJIC ACID

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Metals have been used in the treatment of various diseases since ancient times. The discovery of cisplatin in 1960 – one of the most effective and widely used drugs in current clinical oncology, opened a new page in medical chemistry and stimulated scientists to search for new metal compounds with promising antitumor activity and acceptable tolerance.

The aim of our study was to evaluate the influence of Zn(II), Co(II) and Ni(II) complexes with kojic acid on viability and proliferation of human cancer (HeLa uterine cervical carcinoma) and non-cancer (Lep-3 embryonic fibroblasts) cells. The investigations were based on short-term (up to 72 h, with monolayer cultures) and long-term (up to 40 days, with 3D cancer cell colonies) experiments using methods with different cellular/molecular targets and mechanisms of action: MTT test, neutral red uptake cytotoxicity assay, crystal-violet staining, double staining with acridine orange and propidium iodide, Annexin V/FITC assay and colony-forming method. The results obtained revealed that: i) applied at a concentration range of 5 – 200 μg/mL the compounds tested decrease the percent of the viable treated cells (as compared to the control) in a time- and concentration-dependent manner; ii) administered at a concentration of 200 μg/ml the complexes completely inhibit the ability of HeLa uterine cervical carcinoma cells to form 3D cell colonies in a semi-solid medium; iii) cancer HeLa cells have been found to be more sensitive to the cytotoxic effect of the compounds examined as compared to the non-cancer Lep-3 fibroblasts; iv) Co(II) complex with kojic acid shows the highest cytotoxic activity among the compounds investigated and has demonstrated to be more effective than cisplatin.

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NEUROANATOMY OF MUSIC THERAPY AND HEMATOPOIETIC STEM-CELL TRANSPLANTATION

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In Turkey, after a long gap from 1800s to 2000s, music therapy has developed rapidly in recent years. Most of the healthcare providers are suspicious of the effects of music in therapy and try to understand the mechanism of action. Music is now starting to be seen as a therapeutic intervention. In this study, we tried to explain neuroanatomical substrate of music and its possible mechanism of action in patients undergoing hematopoietic stem-cell transplantation (HCT).

Music is a multi-faceted entity that involves complex brain functions such as auditory processing, auditory memory, musical syntax and semantics. Furthermore, music perception may influence the autonomic nervous system

together with the endocrine physiology. Music has important qualities such as tempo, rhythm, melody, harmony and timbre. Melody and harmony are tonal dimensions, whereas meter and rhythm are temporal dimensions, which represent organization of sounds in time. Neuroimaging studies, using PET and fMRI, have shown that superior temporal gyrus is not the only substrate for music. Frontal lobes are also important. For example, violation of tonal context activates Brodmann Area 44 (also known as pars opercularis of the inferior frontal gyrus) together with ventrolateral prefrontal cortex (VLPFC). Perception of consonant versus dissonant musical compositions also engages VLPFC.

In order to stress the power of music therapy in various patient groups, we selected patients undergoing HCT and performed a database search to allocate music therapy studies in this particular patient group. Almost 60,000 HCTs are performed annually worldwide at more than 400 centers. Although, HCT is associated with severe distress and mortality, few studies have investigated the effects of music therapy in these patients. Most of these studies reported that music therapy improved mood disturbances and shortened time for engraftment in these patients. These effects may be attributed to autonomic innervation of bone marrow. Collectively, understanding the neuroanatomy of music may provide new insights and indications for music therapy.

EFFECTS OF KYOTORPHIN ON THE MORPHOLOGICAL CHANGES IN THE SOMATOSENSORY CORTEX AND MENINGEAL BLOOD VESSELS OF ALZHEIMER'S DISEASE MODEL RATS

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Alzheimer's disease (AD) is a neurodegenerative disease associated with a progressive loss of memory, personality changes and deterioration of the intellectual and social functions, which occurs after a prolonged pre-symptomatic phase. Abnormal deposits of beta-amyloid plaques that build up in the spaces between nerve cells and tangles (twisted fibers) that build up inside cells, are prime suspects in damaging and killing nerve cells. Recently, in the cerebrospinal fluid of AD patients, reduced levels of neuropeptide kyotorphin (KTP), accompanied by increase in p-tau, a marker of neurodegeneration, were found.

The goal of the present work was to study the effects of KTP on the morphological changes in the somatosensory cortex and meningeal blood vessels of AD model rats. We used bilateral intracerebroventricular injection of streptozotocin (STZ) in Wistar rats (300-350 g) as a model of sporadic AD. Animals were randomly divided in three groups: a control group (sham-operated and injected with saline), an AD group (injected with STZ) and an AD+KTP group (injected with STZ and treated with KTP). Neuritic plaques in the somatosensory cortex and blood vessels were confirmed using thioflavin S fluorescent staining and visualized by microscopy at 40x magnification. Plaques were quantified using an image analyzer (CUE-2, Olympus America, Center Valley, PA, USA) and recorded as a percentage of the total area. Our results showed that there was a significant increase in the thioflavin-S accumulation in AD and AD+KTP groups compared to controls ($p<0.05$) and a tendency of a decrease in the AD+KTP group compared to the AD group. This data showed slight but significant protective effect of intracerebral KTP against AD-induced morphological abnormalities.

THE CAROTID BODY AND SUPERIOR CAROTID GANGLION IN RATS EXPRESS ANGIOTENSIN AT1 RECEPTORS

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Introduction: **The carotid body (CB) is a small, paired, neural crest-derived neuroendocrine organ that senses the oxygen level in blood and maintains homeostatic regulation in the respiratory and cardiovascular systems. It has a typical dual innervation by both sensory nerve fibers of the primary afferent neurons located in the petrosal ganglion and by the postganglionic sympathetic nerve fibers from the superior cervical ganglion (SCG). Recent evidence suggests that angiotensin II acting via AT1 receptors modulates the activity of chemical substances in a paracrine-autocrine manner.**

Aim and Methods: **The study aims at investigating the immunohistochemical localization of the AT1 receptor in the CB and SCG of intact rats and after treatment with its selective blocker losartan.**

Results: **We found that a population of the CB glomus cells and the vast majority of closely located SCG neurons were richly endowed with AT1 receptors while the nerve fibers supplying the CB did not express the receptor protein. Our data also revealed that the intensity of immunostaining reverberating receptor protein levels was significantly decreased by losartan. In particular, its administration caused a less dense immunostaining of glomus cells in treated rats compared with the control animals treated with a vehicle. Similarly, the intensity of receptor immunostaining in the SCG neurons after losartan treatment was lower than in the ganglion cells of controls.**

Conclusion: **It can be inferred that angiotensin II acting via AT1 receptor activation is involved in CB excitation under normoxic conditions. Understanding the principles by which AT1 receptor activation amplifies CB chemosensory reactivity to oxygen would provide insights in the pathophysiology of certain hypoxemia-related disorders.**

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STRUCTURAL PLASTICITY IN THE ADULT BRAIN DURING VISUAL LEARNING AND MEMORY TASKS

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The human brain connectome is a new and rapidly developing project in neuroscience. The pattern of structural and functional connectivity in the brain is not fixed, but is continuously changing in response to experiences. Exploring these phenomena opens a powerful arsenal of analyses and computational approaches that could provide important new insights into clinical and cognitive neuroscience.

The aim of the present study was to investigate the structural plasticity of the cortical areas of the brain during a memory task performance using functional MRI.

Fifteen right-handed male subjects and fourteen right-handed female subjects were scanned during memory paradigm performance. The scanning of the participants was performed with a 3T MRI system - GE Discovery 750 w with a protocol including structural scan - Sag 3D T1 F-BRAVO FSPGR, slice thickness 1 mm, matrix 256x256, flip angle 10° and standard block design functional scan - 2D EPI, slice thickness 3 mm, matrix 64x64, TR (repetition time) – 3000 msec, TE (echo time) -30, flip angle 90°. Data were analysed using the SPM 12 (Statistical Paramertic Mapping) software running on MATLAB R2015 for Windows. The level of statistical significance was set at $P < 0.05$.

Statistical significance in brain cortical activation was not found between males and females. We found reliable occipital and temporal signal response across the block design contrasts with statistically significant differences within the groups in both genders. The results highlighted several detailed differences between males and females and potential future directions in brain activation studies.

NITRIC OXIDE-CONTAINING STRUCTURES IN THE CAROTID BODY AND SUPERIOR CERVICAL GANGLION IN RATS

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Introduction: The carotid body (CB) is a major peripheral chemoreceptor organ that registers the arterial blood levels of pO_2, pCO_2, and pH, and responds to their changes by regulating respiration. It is dually innervated by sensory fibers from petrosal ganglion (PG) neurons, and autonomic fibers from postganglionic sympathetic neurons of the superior cervical ganglion (SCG) and parasympathetic vasomotor fibers of intrinsic ganglion cells in the CB.

Aim and Methods: The presence and distribution of nitrergic structures in the adult rat CB and SCG have been examined by means of NADPH-d histochemistry, and NOS immunohistochemistry with antibodies against its constitutive isoforms.

Results: Initially, we identified the sites of origin of afferent and efferent innervation of the CB by retrograde tracing with Fast Blue, and then by combining NADPH-d staining and immunohistochemical staining for NOS we demonstrated at the light microscopic level the presence of nitrergic structures in the rat CB and SCG. Our results revealed that the majority of large- and medium-sized PG and SCG cells were NADPH-d positive and displayed a strong NOS immunostaining. We also found that the glomus cells were surrounded in a basket-like manner by NADPH-d reactive as well as by nNOS- and eNOS-immunoreactive varicose nerve fibers of PG and SCG origin. A relatively small number (4.9%) of nitrergic SCG neurons projected to both the glomus cells and blood vessels in the rat CB.

Conclusion: The present findings provide histochemical and immunohistochemical evidence that the rat glomus cells receive dense sensory and autonomic nitrergic innervation while CB blood vessels are exclusively supplied by sympathetic and parasympathetic postganglionic NOS-containing fibers. It is likely that NO is involved in CB inhibition by way of its direct action on chemosensory glomus cells through nNOS and indirectly on its vasculature via both nNOS and eNOS.

INFLUENCE OF THERMAL STRESS ON THE EXPRESSION OF CB1 CANNABINOID RECEPTORS IN THE RAT MESENCEPHALIC TRIGEMINAL NUCLEUS

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The mesencephalic trigeminal nucleus (Me5) is a unique brain structure that plays a crucial role in orofacial proprioception, and receives synaptic input from various brainstem regions regulating feeding behavior. It is composed mainly of large pseudounipolar neurons, which are glutamatergic, and a subset of smaller multipolar cells of GABAergic nature. Evidence shows that cannabinoid (CB) receptors and their endogenous ligands are widely distributed in brainstem areas that are involved in pain sensation and feeding by influencing the glutamate and GABA neurotransmitter systems. This study aims at investigating the presence of a CB1 receptor, the major cannabinoid receptor subtype in the brain, in Me5 neurons after acute exposure to thermal stress, and pretreatment with its selective agonists and antagonists. We found that the administration of the endogenous CB1 agonist anandamide evokes an intense expression of CB1 receptors in the mesencephalic trigeminal neurons in rats exposed to short-term (up to 1 hour) thermal stress at 32-36°C, while injecting the synthetic CB1 antagonist AM251 decreases the levels of protein expression of this receptor in a hot-plate test. Our results provide immunohistochemical evidence that mild thermal stress triggers endocannabinoid release in a subset of Me5 neurons which utilize glutamate and GABA for synaptic transmission but lack CB1 receptor expression under normal conditions. These findings suggest a possible role for the Me5 endocannabinoid system in providing additional control to the complex neural network which modulates feeding in response to pain-triggering stimuli.

NERVE STRUCTURES IN ZUSANLI (ST36) ACUPOINT IN RATS

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Acupuncture points (also called acupoints) are locations on the body, which are the focus of treatment with acupuncture. Acupuncture point (acupoint) "Zusanli" (ST36) is one of most important and most widely used acupoints in humans and animals. Nerve structures of the peripheral nervous system are presumably responsible for some acupuncture effects. The aim of the present study is to examine the nerve structures in tissues in ST36 acupoint. The experiments were carried out on adult male Wistar rats. Samples from ST36 acupoint with size 5x5x5 mm were obtained and sectioned in 5 µm or 30 µm sections, alternatively. Classical histological techniques of staining were used: H&E, Bodian, Bielschowsky-Gross, Mallory, van Gieson, and Heidenhein's Azan; immunohistochemistry for S-100 protein was also used. Free nerve endings, muscle spindles and nerve fibers were observed in tissues in ST36 acupoint. Free nerve endings were seen in the epidermis. In the dermis small nerve fibers and free nerve endings in a palisade manner around hair follicles and sebaceous glands were visualized. The observed nerve fibers in the dermis are in the proximity of small blood vessels. Moreover, smaller nerve fibers were seen in the subcutaneous tissue. Muscle spindles, small nerve fibers and large nerve fibers were also observed deeper, in the muscular layer. The larger nerve fibers in the deep muscular layer were observed in vicinity of large blood ves-

sel. The muscle spindles were observed mostly in the proximity of blood vessels and small nerve fibers. The nerve structures (free nerve endings, muscle spindles and nerve fibers) in the tissues of ST36 acupoint in rats were with normal appearance. These nerve structures possibly play a role in the effect of acupuncture via needle-nerve interaction.

ZBTB20 MAY REGULATE THE POSTNATAL MATURATION OF PARVALBUMIN INTERNEURONS

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The onset of expression of the transcription factor (TF) Nkx2.1 at the ninth embryonic day signals the start of subdivision of the mouse forebrain into its two progenitor zones: the pallium and the subpallium. The pallium being the origin of the cortical excitatory neurons and the subpallium, comprising four progenitor domains: tree ganglionic eminences (GE) known as Lateral, Medial and Caudal GE, respectively (LGE, MGE, CGE) and a small region named the preoptic area, giving rise to almost all cortical and striatal interneurons (IN). MGE, gives rise to 60% of all cortical IN, namely parvalbumin (PV) and somatostatin positive INs, and thus was the first zone we investigated. Of all subpallial progenitor zones the LGE does not contribute to neocortical neurogenesis, but generates neurons only to the olfactory bulb and striatum. Zbtb20 is a zinc finger BTB domain-containing TF related to normal development of the mammalian forebrain with mutant mice having severe defects in the formation of the hippocampus and the normal formation of the neocortical layers due to problems during the neurogenesis of the pallial glutamatergic neurons. The strong expression of this TF in the GE poses the question of its role in cortical IN development. In order to address this problem we employed a loss-of-function strategy by means of *Zbtb20*$^{lacZ/lacZ}$ knock-out mouse line. We have already demonstrated almost complete absence of PV INs in the neocortex at postnatal day (P)12. We then wanted to explore the PV levels at later stages and were able to do so only at stage P15 due to early postnatal mortality of the homozygous mutants. We then found a more substantial number of PV+ INs compared to P12, but severe reduction was still present. We analyzed the levels of PV+ INs in the WT and heterozygous *Zbtb20*$^{lacZ/+}$ mice at P28 and found a substantial reduction on the heterozygous mutants. The data so far hints both at the possibility that the phenotype seen for PV+ INs can be in part due to delayed postnatal maturation and in part to a dose-dependent effect of Zbtb20 on PV+ IN generation.

NEUROANATOMY OF ROMANTIC LOVE AND NERVE GROWTH FACTOR AS A LOVE PAINKILLER

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Romantic love, a universal, cross-cultural phenomenon, has been a focus of attention and theme of research for neuroscientists. Romantic love involves a collection of activities associated with the acquisition and retention of emotions. It is also characterized by distinct physiological hormonal changes and psychological behaviours. Technologic advancements in neuroimaging have led to the identification of specific brain areas related to the

genesis of love. Recent studies showed that the activated areas during love include cortex, medial insula, anterior cingulate, hippocampus, and nucleus accumbens. These regions are also parts of the brain reward system, which makes love a reward experience. Some other regions are deactivated during love and these include amygdala, frontal cortex, prefrontal cortex, and middle temporal cortex. Deactivation of the amygdala may be related to reduced fear response and inhibition of xenophobia, whereas deactivation of the frontal cortex may reduce negative emotions and judgement. Interestingly, these deactivations are lateralized to the right side. In terms of neuroanatomy, there are some differences between maternal love and romantic love. The hypothalamus - linked to sexual arousal - is active in romantic love as compared to maternal love. Another neural substrate is the left dorsal anterior cingulate cortex, which seems active during watching photographs of a romantic partner. Apart from neuroimaging studies, investigating hormone profile may provide clues about the brain areas of love. For instance, circulating levels of nerve growth factor (NGF) are increased among subjects in love. NGF affects sensory neurons and provides better pain control, which makes it a love painkiller.

THE PLACE OF THE VIRTUAL DISSECTION TABLE SECTRA IN ANATOMY LESSONS OF MEDICAL STUDENTS

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Anatomy is a key discipline not only in the field of medical education, but also in the professional development of a young medical doctor. It is the first discipline for every healthcare student when starting his/her training. In this process many students face psychological and pedagogical challenges – a lot of information, difficult knowledge reproduction and systematization, first dissection hall and working with corpses. This is an integral part of the classical anatomy training methods – dissection practice. The advances in technology have allowed the development of new and interactive methods, such as the medical table for virtual dissection Sectra. Different points of view can be demonstrated using some instruments in the 3D dissection of a real human body.

Our aim was to evaluate the students' opinion on the new innovative interactive method provided by the virtual dissection table Sectra in dissection practice. During the second semester of practical training in cadaver dissection cycle, we performed a pilot practical exercise using the medical table for virtual dissection Sectra. An anonymous questionnaire was given to 50 students to evaluate their impressions and opinions concerning the place of the dissection table in their anatomy education. The students point the classical method for dissections as the main and most effective one, although accompanied by some obstacles. On the other hand they think that the innovative method is interesting and useful. Most of the students' point that combining the two methods would be the best way to help them understand the information.

The classical dissection method has kept its relevance and importance. On the other hand the innovative methods are gaining more popularity and support because of the times we live in. The Sectra table gives us the unique opportunity to provide basic knowledge of the body, adding more opportunities to the classical method. This combination gets the highest evaluation by students and instructors concerning effectiveness and success rate.

THE CORRELATION BETWEEN SYNTAX SCORE AND THE SEVERITY OF CAROTID ARTERY DISEASE

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Coronary and carotid artery disease are both known to be specific manifestations of atherosclerosis. The relationship between carotid atherosclerotic disease and coronary artery disease (CAD) has been shown in previous reports. However, the association between complex CAD assessed by the Syntax Score (SxScore) and the severity of carotid arterystenosis (CAS) has not been fully investigated. The objective of this study was to analyze the correlation between the severity of CAS and CAD complexity evaluated by SxScore.

Materials and Methods: A total of 45 patients with simultaneously carotid and coronary angiography performed were included in our study. SxScore, a marker of CAD complexity, was determined by a special computer software. The patients were divided into two groups according to the classification of SxScore: low SxScore (n=33, SxScore<22) and intermediate-to-high SxScore (n=12, SxScore≥22). The severity of CAS was assessed by digital subtraction angiography (DSA) of six distinct segments of the carotid artery included the left and right common, internal and external carotid arteries. Spearman's correlation coefficients were used to determine the correlations between the SxScore and the CAS score.

Results: The CAS severity score was significantly higher in patients from the intermediate-to-high SxScore group than in those from the low SxScore group (3/4/4.75 vs. 1/2/3, p=0.001). There was also a significant correlation between the SxScore and the CAS severity score(r=0.47, p=0.001).

We revealed that there was a proportional increase in the severity of CAS to CAD complexity using SxScore. It may suggest that complex multivascular atherosclerotic disease is the systemic nature of atherosclerosis.

ASSESSMENT OF SEX DIFFERENCES IN SIZE AND SHAPE OF FORAMEN MAGNUM ON CT SCANS OF BULGARIAN ADULTS

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The aim of the study is to assess the sex differences in size and shape of the foramen magnum, to develop discriminant functions based on the measurements of the foramen magnum and to establish the most reliable variables for sex determination in Bulgarian adults.

Computed tomography scans of the heads of 120 Bulgarian adults (52 males and 68 females) were used in the study. The segmentation of the skulls was performed with the software InVesalius. The length, breadth, and circumference of the foramen were measured digitally based on the 3D coordinates of definite landmarks and semi-landmarks. The area of the foramen magnum was calculated using the Radinsky and Teixeria formulae, based on the dimensions of the foramen length and breadth. The shape of foramen magnum was classified as narrow,

medium wide or wide on the basis of the breadth/length index values. The significance of the sex differences in the measurements and shape of foramen magnum was assessed by independent sample t-test and chi-square test. Univariate and multivariate discriminate function analyses were conducted. The leave-one-out classification method was used as a cross-validation procedure.

The results demonstrated statistically significant sex differences in the size and shape of the foramen magnum. The area of the foramen magnum is the best discriminating parameter. Generally, the results from the univariate and multivariate discriminant function analysis showed that the measurements of the foramen magnum did not provide high predicting rates for sex estimation among the Bulgarian population.

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APPLICATION OF THE DEBLURRING TECHNIQUE AND SEGMENTATION ON MEDICAL COMPUTED TOMOGRAPHY (CT) IMAGES FOR IMPROVED VISUALIZATION OF THE CRANIAL SUTURES

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Introduction: **The investigation of the cranial suture morphology and the changes occurring during the obliteration is an important precondition for understanding suture maturation and its relation to ageing. The μCT-imaging of dry skulls with known age-at-death is a powerful tool in this direction, but this technology is still expensive, difficult to access and entirely inapplicable on living individuals. On the other hand, the diagnostic imaging of patients accumulates large amount of volumetric images, which could be used for scientific researches. In medical imaging, the generated data is typically artificially blurred for denoising purposes. The standard Gaussian blur assigns a weighted average value for the gray-scale intensity of each pixel, based on the initial intensities of the pixel and its neighbors. Unfortunately, together with the noise reduction, such a procedure decreases the contrast on image contours and significantly complicates the study of cranial sutures.**

Aim: **This study aims to apply theoretical and experimental scientific techniques for optimal extraction of useful data for the degree of suture obliteration, from medical tomographic images.**

Materials: **Volumetric images obtained from diagnostic imaging of patients with multislice CT system Toshiba Aquillion64 were used.**

Methods: **We developed a deblurring technique, based on the penalized total variation optimization. We also segmented the output image, using an appropriate threshold in order to separate the suture (region of interest) from the background.**

Results: **After processing the images, the contact between the adjacent cranial bones is clearer and the degree of suture closure could be interpreted more accurately.**

Acknowledgements: **This study was supported by the Bulgarian National Science Fund, grant DN01/15-20.12.2016.**

CORRELATIONS BETWEEN SOME ANTHROPOMETRIC PARAMETERS, THE LIPID PROFILE AND GLYCATED HEMOGLOBIN IN BULGARIAN MEN WITH TYPE 2 DIABETES MELLITUS

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Aim: **To investigate the correlations between the lipid profile indices, as well as glycated hemoglobin levels, and some anthropometric parameters of Bulgarian male patients with type 2 diabetes mellitus (T2DM). The study included 136 males with T2DM, divided into two age groups. The anthropometric parameters were: waist (WC) and hip (HC) circumferences. The calculated indices were: body mass index (BMI) and waist-hip ratio (WHR). The laboratory indices included: total cholesterol (TCH), triglycerides (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), very low-density lipoprotein (VLDL), and glycated hemoglobin (HbA1c).**

Results: **In the present study we found eleven significant correlations between some anthropometric and laboratory parameters in Bulgarian male patients with T2DM. They were: 1st age group 40-60 years: TCH/BMI, TCH/WC, TCH/WHR, HDL/BMI, HDL/WC, and LDL/HC; 2nd age group 61-80 years: TG/BMI, VLDL/BMI, HbA1c/BMI, HbA1c/WC, and HbA1c/HC. The levels of triglycerides and VLDL were significantly higher in the group of 40-60 year-old men, than in the second male group.**

Conclusion: **The study showed that the correlations between the anthropometric parameters with lipid profile indices and the glycated hemoglobin values in the different age and gender groups of T2DM patients could predict the prognosis or the susceptibility of persons to the risk of disease complications. The multidisciplinary approach to T2DM requires the monitoring of the patient's lipid profile and glycated hemoglobin levels, together with the anthropometric parameters.**

THE SKELETON OF BOROV DOL – MYSTERY AND REALITY

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Borov dol is a narrow valley situated between the hills Patrahilets and Kupelaritsa, west of the road Stara Zagora-Kazanlak. Until 1979, a 2-meter high monastery remnant, part of monastic complex named Boruj Mala Sveta Gora stood in this location. At the time of archeological excavations, which began in 2014, the area of the monastery has revealed a grave with a preserved skeleton. Bones were in an anatomical order, so it could be assumed that it was a single primary funeral. The skeleton was in a stretched position lying on the back. The arms were parallel to the body, but the right forearm was placed in front of the body. The left forearm, feet and part of the cranial bones were missing. The skull was turned to the right, the lower jaw was in its normal position, almost in the occlusion position, being slightly distant (1-2 cm) from the base of the skull. Perhaps this departure was due to shifting terrestrial layers or has occurred during discovery of the skeleton and its cleansing of the earth. The chest and upper thoracic vertebrae were curved to the right (the spine was curved around its axis), but it was not clear whether the corpse was placed in this position or it occurred after the burial. This was a male individual, probably

a representative of the Caucasian race with a height of 173-175 cm and a presumed age of about 50 years. He was of an average to tall stature, combined with a small face and a narrow skull. Our hypothesis is that he was a monk (probably a left-hander), accused of vampirism, so the feet and his left forearm were cut off.

AGE-RELATED CHANGES IN BODY COMPOSITION IN BULGARIAN WOMEN BETWEEN 19 AND 89 YEARS OF AGE

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Progressive changes in body composition occur with aging. The aim of the study was to analyze body composition and to assess the changes that occur with age in Bulgarian women. A cross-sectional study of 123 apparently healthy women aged 19-89 years was conducted. Body composition was measured using a direct segmental multi-frequency bioelectrical impedance analyzer (InBody 170). The body fat mass (BFM) of the participants increased with age and reached its peak in 60-69-years-old women. The body fat percentage (BFP) and visceral fat (VF) also increased with age and reached the highest values in women over 70 years. The opposite tendency was observed for fat-free mass (FFM). Its values decreased in elderly women. The results suggested that in women aged 19-89 years there a progressive increase of mean BFM, PBF and VF and reduction of FFM were noted.

ANTHROPOMETRIC UPPER LIMB ASYMMETRY IN 12-YEAR-OLD TENNIS PLAYERS

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The process of training of tennis players creates anatomical differences between dominant and non-dominant upper limbs and leads to an asymmetry in the muscle mass distribution. The aim of the study is to estimate the degree of muscle mass asymmetry between the dominant and non-dominant upper limbs in young tennis players, using multi-frequency bioelectrical impedance measurements. The study sample included 14 male tennis players and 11 school children aged 12 years. Segmental analysis of body composition was conducted by a bioelectrical impedance analyzer (model: InBody 170). The athletes had a higher muscle mass of the dominant upper limb compared to the non-dominant. The non-athlete boys were characterized by lower asymmetry coefficient level of the muscle mass of the upper limbs compared to the tennis players ($p<0.05$). No significant relationship between the asymmetry coefficients of the upper limbs, mean age and years of training was found in tennis players.

AESTHETIC ANALYSIS OF FACIAL ANATOMY

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The anatomy of the face is influenced by sex, race and age. It is the most variable part of the human body. The increase in our knowledge about aesthetic anatomy has brought an evolution in the form of new techniques for aesthetic surgery and medical aesthetics.

Our knowledge of the layers and structures of aesthetic facial anatomy is of crucial importance when performing aesthetic surgery.

In aesthetic medicine, the key to success is following a proven process of patient assessment and the development of an individualized injection strategy, in addition to proper injection technique. Underlying this process it is a foundation based on an understanding of facial anatomy (bone structure, nerve and vascular system, fat pads, and muscles) and how it changes with aging, as well as how to use neuromodulators and soft tissue fillers alone, and in combination, to obtain aesthetic and natural results. Safe and effective injection relies on clear knowledge and understanding of the facial anatomy.

SOME VARIATIONS OF THE LIVER BLOOD SUPPLY

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In addition to the classic textbook descriptions of the hepatobiliary arterial system in a human, a number of various arterial patterns can be identified in nearly 40% of people. The knowledge of these aberrant arteries might be of considerable importance for liver resections, liver transplants, and some laparoscopic procedures.

Two interesting dissection cases of an aberrant hepatic arterial system are reported here. In the first case, an unusually small proper hepatic artery was identified supplying the left lobe of the liver. An accessory left hepatic artery was observed arising from the left gastric artery. An aberrant, large in diameter, hepatic artery was also found starting from the proximal part of the superior mesenteric artery and replacing the right hepatic artery. From its origin the aberrant artery ascended right behind and slightly lateral to the hepatic portal vein. In the second case, during the dissection of the celiac trunk branches, a similar pattern was considered at first. There was a small left hepatic artery branching from the common hepatic, an accessory left hepatic artery - from the left gastric, and a large in size arterial vessel behind and lateral to the portal vein. The further dissection, however, revealed that this aberrant vessel started from the celiac trunk and then it passed behind the portal vein and ascended to supply the right lobe of the liver.

CREATING 3D PRINTED PLASTIC MODELS OF THE GREAT SAPHENOUS VEIN SYSTEM USING ANGIOGRAPHIC AND TOMOGRAPHIC STUDIES IN NORM AND PATHOLOGY

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Aim: **Blood vessels forming the venous system of the lower limbs, and more precisely the superficially located great saphenous vein, are of great importance for the blood flow. As a result of various conditions, varicose veins emerge, increasing the risk of thrombus formation. The detailed study of the morphology of these vessels in individuals that suffer from varicose veins is critical for evaluation of the risk for thrombus formation. The objective of this study is to complement angiographic diagnostics of varicose structures by building three-dimensional plastic model available for visual inspection. Further, fluid dynamics in varicose vessels will be tested against controls.**

Materials and Methods: **We propose a method for developing a three-dimensional plastic model of the venous system, which describes its volume features into great details. First, we reconstructed digitally the three-dimensional model of the vein from raw DICOM angiographic data using the Slicer 3D software. Second, the model was converted into Standard Tessellation Language (STL). Third, the STL data were used by a 3D printer to build the plastic model. As a result, we obtained a detailed and reliable model of the morphology of the great saphenous vein system.**

Conclusion: **We reliably isolated targeted blood vessels from an angiographic study and subjected virtual data to 3D printing. Corresponding 3D prints were used both for visual and/or tactile evaluation of complex three dimensional vessel morphology and further simulation of fluid flow in normal and varicose veins. We derived qualitative and quantitative data accounting for impaired blood flow in varicose structures in the lower limbs.**

AORTIC ARCH ANEURYSM REPRESENTED BY 3D PRINTING AND SIMULATION OF FLUID MOVEMENT THROUGH IT

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Aim: **By definition, an aneurysm is a localized dilation of an artery with a diameter at least fifty percent greater than normal size. This often occurs at the human aorta in thoracic, abdominal and arch regions. Relatively more rarely, the aneurysm affects the arch region closer to the semilunar valve. However, it is a condition of importance because an ascending aortic aneurysm may be fatal due to the risk of dissection or rupture as a result of e blood pressure. Therefore, we have decided to model the fluid dynamics and compare it to a normal condition. The main objective of the study is to build realistic 3D model of heart with and without ascending aortic aneurysm and to successively simulate fluid movement there.**

Materials and Methods: Two flexible plastic hearts in norm and pathology were digitally rendered and 3D printed. Those were attached to a blood flow simulator made by silicone tubes. Fluid pressure and particle velocity were measured accordingly. The numerical simulation of fluid provides information about the severity of the impairment of circulatory flow in the arch caused by vortices.

Results: Precise 3D printed models, based on real arch aneurysm data were subjected to test and showed increased turbulence in pathology as compared to normal conditions. Additionally, a concept of proof for numerical modeling was established.

Conclusion: In conclusion, for the first time in Bulgaria such innovative approach for 3D model vascular analysis had been established and verified. However, further investigation is needed to prove whether 3D modeling of vascular disease from a patient scan in combination with fluid blood flow simulation can serve as an alternative method for diagnosing and assessing the severity of vascular diseases.

RADIAL ARTERY VARIATIONS IN PATIENTS UNDERGOING TRANSRADIAL HEART CATHETERIZATION

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Nowadays, the transradial cardiac catheterization is performed regularly. This is a result of the lower incidence of complications in comparison to the femoral approach. The positive characteristics of the transradial approach are due to the fact that the radial artery is located just beneath the skin and is easy to access for hemostasis. This way, the common complications of hematoma, pseudoaneurysm and arteriovenous fistulas of the femoral approach could be avoided. Moreover, if the above complications occur during the transradial approach, surgery is not needed and the treatment is usually non-operative. The other advantage of the transradial approach is double blood irrigation of the hand, which prevents hand ischemia after radial artery thrombosis or spasm. Although the transradial approach has the advantage of reduced local complications, it is associated with specific technical challenges and has a relatively high incidence of catheterization failure. Commonly, the procedure challenges are due to a failure to puncture the artery, anatomic variations of the radial artery and radial artery spasm. The anatomical variations are the second most frequent factor impeding transradial catheterization. Therefore, the precise anatomical information about the variations of the radial artery should be kept in mind and the knowledge of interventional cardiologists will be helpful in performing the transradial coronary procedure during left heart catheterization.

RENAL ARTERIAL PATTERN VARIETIES OBSERVED IN GROSS ANATOMY LAB

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Variations of the renal vascular pattern, like all anatomy variations, are divided into three categories by macroscopic criteria: morphometric parameters, consistency, and spatial distribution. During a short period of one academic semester, while completing routine dissections, we found rare varieties in the above-mentioned vessels in 2 out of 13 cadavers. Double renal arteries on the left and earlier point of division of the opposite artery were observed in a 70-year-old female cadaver. Five variations in 75-year-old male cadaver were found. One of them was a rare right testicular vein that empties in the right renal vein. The right renal artery was atypical with early branching into its hilar and segmental branches. The left renal artery had three extrahilar branches and one accessory renal artery starting separately inferior to it. Another spatial variation was the nearly horizontal position of the right kidney. The detailed anatomy of this region, including the possible variations, should be considered by clinicians and especially by surgeons when performing precise invasive and radiological procedures.

FUNCTIONAL BEVERAGES BASED ON ARONIA MELANOCARPA AFFECT AGE-RELATED AORTIC WALL RESTRUCTURING IN RATS

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The morphological alterations of the wall of the large arteries of elastic type, which also include the aorta, are a result of age-related restructuring and create pathophysiological prerequisites for worsening of their function and the occurrence of pathological changes. They lay at the core of many diseases, such as hypertension, dissection, aortic aneurysm and rupture of the aorta, etc. The application of natural antioxidants is one of the strategies for slowing down the vascular aging process. *Aronia melanocarpa* juice is a rich source of polyphenols and exhibits extremely high antioxidant activity. Enriching it with pectin and herbal extracts potentiates its effects.

The aim of this experimental study was to determine the impact of various functional beverages based on *Aronia melanocarpa* on the age-related structural alterations in the aortic wall in rats.

Methods: A model of aging male rats was used (12 months old). It was supplemented for 3 months with 100% *Aronia melanocarpa* juice (A), *Aronia melanocarpa* juice enriched with 1% pectin (A+P) and *Aronia melanocarpa* juice enriched with herbal extracts (A+I). At the end of the experiment a histochemical and morphometric examination of the wall of the thoracic aorta was performed. The following parameters were measured and processed statistically: thickness of tunica media, number and thickness of the elastic membranes, and width of intermembrane spaces.

Results: The comparative analysis between the groups established that in the animals supplemented with 100% Aronia juice the elastic membranes of the aortic tunica media were the thickest and the intermembrane space of

the group A+I was the narrowest. The collected data suggests that *Aronia melanocarpa*-based functional beverages affect some of the age-related alterations in the vascular wall and may be recommended as a prophylactic means to healthy aging.

THE EVALUATION OF NECK MOVEMENTS IN YOUNG ADULTS USING THREE-DIMENSIONAL ULTRASOUND ANALYSIS

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There are differences between the ranges of joint movements even though they are quite similar among the genders. For this reason, a normative database on each gender group can be used to provide more reliable data on whether or not there is limitation of movement of the joint in any person or patient, or whether the existing limitation has reached normal values by treatment.

Restrictions on cervical region mobility have adversely affected people's daily life activities, as well as causing secondary health problems in the long run. The determination of the normal values of the range of motion of this region is now accurately measured by three-dimensional motion analysis systems. The first goal of our study was to determine whether there was a meaningful difference in the cervical region movement scores between male and female individuals and secondly to determine whether to publish the data obtained intending to contribute to the national database.

A hundred healthy young adults, 50 men and 50 women between the ages of 18-26 have voluntarily taken part in our study. The measurements were made in the Motion Analysis Laboratory of the Anatomy Department of Basic Medical Sciences of Trakya University with Zebris CMS 20P device and compatible WinSpine computer program.

As a result of our study, flexion p=0.206, extension p=0.418, left rotation p=0.233, right rotation p=0.725, left lateral flexion p=0.714 and right lateral flexion p=0.390 were found in the left and right lateral flexion of the male and female subjects, respectively. No significant difference was detected in any of the movements. In both male and female subjects, we found that mean flexion was 66.26°, extension was 73.68°, right rotation was 71.03°, left rotation was 73.18°, right lateral flexion was 40.69° and left lateral flexion was 41.57°. We believe that these data will contribute to the creation of a national database.

EXPRESSION OF GLYCOGEN SYNTHASE AND GLYCOGEN CONTENT IN SKELETAL MUSCLES AFTER ADMINISTRATION OF ANTIANDROGENS IN ENDURANCE TRAINING OF RATS

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Depletion of muscle glycogen is an accurate marker of the onset of exhaustion in aerobic physical exercise. Glycogen synthase (GS) catalyses the processes of addition of α-1,4 glucose units to the growing glycogen molecule. The type and utilisation of energy substrates used by muscles depend on the hormonal status and physical activity. There is a lack of data about the influence of androgens through their androgen receptor (AR) on glycogen content and GS in aerobic exercise of skeletal muscles.

The aim of the study was to investigate the effect of endurance training and the AR blocker on the glycogen content and expression of GS in skeletal muscles.

Wistar rats were allocated in trained and non-trained groups. The trained rats were subjected to submaximal training. Half of the trained and non-trained rats received Flutamide for 8 weeks. An immunohistochemical study for GS was carried out, as well as histological testing of glycogen through PAS-reaction, followed by morphometric and statistical analysis.

Training increased glycogen content and immunoexpression of GS in soleus (Sol) and extensor digitorum longus (EDL). AR blocking decreased glycogen content in Sol and GS immunoexpression in EDL of trained animals.

The increased expression of GS found in trained rats reveals the role of the enzyme in adaptation processes in endurance training. The lowered glycogen content in Sol after AR blocking in trained rats proves the participation of androgens through AR in adaptation to exercise. The lowered expression of GS after administration of antiandrogen in EDL after endurance training can be explained by differences in the AR content in the different types of skeletal muscles, which determines their response to androgens.

THE EXTENSOR MEDII PROPRIUS MUSCLE

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The extensor indicis is a deep muscle of the posterior group of the forearm, which originates from the interosseus membrane and the distal part of the body of the ulna. Its tendon passes under the fourth compartment of the extensor retinaculum and projects into the distal aponeurosis of the index finger. In the literature, many studies have discussed the clinical significance of the variations of this muscle in the region of plastic reconstruction of the hand and upper limb surgery.

The present case report presents an additional muscle, which was observed in the posterior forearm region during a routine anatomical dissection of the left upper limb of a 69-year-old female cadaver. The muscle originated from the distal part of the extensor indicis muscle and its bundles ran parallel in distal direction. The final ten-

don passed through the fourth extensor compartment along with the extensor indicis and the extensor digitorum and inserted into the dorsal aspect of the capsule of the metacarpophalangeal joint of the middle finger. The dissection revealed that this muscle was innervated by the radial nerve.

Numerous variations of the extensor indicis muscle have been described – absence, extremely distal origin, the presence of two heads or complete duplication of the muscle, the existence of extensor indicis brevis muscle, etc. The reported incidence of the extensor medii proprius muscle in cadaveric dissections ranges from 0% to 12%. Knowledge of the described muscle is clinically important, because it may be injured by inappropriate dissection or misdiagnosed as tumor-mimicking lesion. Furthermore, the tendon of this additional muscle may be used as a flap in reconstructive hand surgery.

ARTISTIC ANATOMY AFFECTING THE AESTHETIC PERCEPTION OF FACE MORPHOMETRY

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Throughout history, artistic anatomy has been a branch of science that studies the outer shape of the body, body projection and movements. Artistic anatomy has existed in art history and art theory. Examination of the outer body shapes and creating works of art on this basis requires the measurement of various lengths in the body and dividing them. As a consequence various equations and proportions are set forth. All the artists involved with fine arts like painting and sculpture are talking about an optimum ratio of beauty (canons). However, no universal criteria have been established.

Also, it is believed that there is a relationship in terms of facial beauty among the ratios related to nose. Some suggest this to be the perception of beauty and this balance is linked to the golden ratio. At this point, some people mention the golden ratio, which has been applied for centuries in physics and mathematics. The ratio, which was used in the works of artists, is mentioned frequently in the field of plastic and reconstructive surgery today.

These ratios were handled are the subject of our study. The effects of these ratios were determined on people of present times. For this purpose, first a questionnaire was prepared. It contained two parts. The first part contained demographic information and the second part measured aesthetic perception. As part of the aesthetic perception survey, the questionnaire included measurements in three sections. Each section contained four pictures with a total of 12 pictures. These pictures were prepared with the help of professional artists. They included, for example, changing the width of the nose. Second, 384 volunteers were chosen for our study. These people were between 18 and 65 years of age. The volunteers did not have any communication problems and filled out our questionnaire.

Evaluating the resulting data, we noticed that the proportions of the nose had an effect on perception of the facial aesthetics. As it is known, according to some people, these proportions represent beauty. Are these proportion really representative of beauty according to the people today? This issue was examined. In this way, the effect of this factor was established, as well as the proportions determining beauty according to the interviewed individuals. The study found that the following were relevant: nose-index - 55.2%, naso-orbital index - 79.4%, naso-oral index - 79.4%. The results of this study have shown that these proportions represent beauty. Based on the obtained results we believe that we have objective information about aesthetic perception. Science, art and society were given more information about these ratios. We have provided knowledge for artists and surgeons. Using it we could contribute to more successful operations, especially in the field of plastic and reconstructive surgery.

EMBALMING TECHNIQUES OF THE RENAISSANCE PERIOD

M. Karahan, D. Donmez, S. Cikmaz

Trakya University, Turkey

Cadaver dissection is very important for anatomy training. However, another other thing that is just as important is preserving the cadaver for a long time without decomposition. Therefore, embalming techniques have been developed. The purpose of this study is to investigate the embalming techniques of anatomists during the Renaissance.

It was forbidden to use cadavers in medical schools in the Dark Ages. The Renaissance was a period of freedom in medicine and anatomy as well as in other fields. In this period, anatomists developed various embalming techniques.

Leonardo da Vinci (1452-1519) used a mixture of turpentine, lavender, vermilion, wine, rosin, sodium nitrate and potassium nitrate in the embalming process. He also injected wax into ventricles. Jacobus Berengar (1470-1550) injected hot water into veins. Bartholomeo Eustachius (1520-1574) used hot ink. Frederic Rusch (1638-1731), a German anatomist, was the first person who embalmed a cadaver by injecting a chemical preservative solution into the bloodstream. However, his technique is unknown. Dr. William Hunter (1718-1783) was the first person to perform arterial injection to prevent decomposition of cadavers. Guiseppe Tranchina (1797-1837) successfully used arsenic solution for arterial injection. His technique was the first procedure ever performed without eviscerating the body. In 1876, the German chemist August Wilhelm von Hofmann discovered formaldehyde, the basis of modern embalming. Formaldehyde, ethanol and methanol are still the basic elements of modern embalming.

Researchers are still investigating methods of embalming using more effective but less toxic liquids.

COLORED SILICONE INJECTION IN CADAVER STUDIES

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The anatomy of the circulatory system is one of main subjects of human anatomy and requires much time to learn. It is vital to know the anatomical structure of the veins during surgical operations. The ideal place to learn anatomy is an anatomy laboratory. It is easier to see vascular structures in materials injected with colored silicone.

The process of filling vessels with colored, self-hardening silicone in cadavers is a 6-step process. First, the great veins in the cadaver are caught. They are irrigated. Silicone is colored with blue and red dyes and injected into the cadaver. After the hardening of the silicone in vascular system, it can easily be examined during dissections.

Filling vessels with colored silicone in cadavers, allows the easy selection of arterial and venous vessels. This method contributes to the better understanding of the anatomy of the circulatory system, which has many variations. This way, cadaver veins gain tonus as in living humans. Medical students can learn the anatomy of the circulatory system more comprehensively. The use of silicone-injected cadavers as educational materials in surgical clinics may contribute to the practical aspect of medicine.

RELATIONSHIP BETWEEN PROSTATE VOLUME AND SOME ANTHROPOMETRIC MEASUREMENTS

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Prostate volume measurements are frequently used to diagnose prostate abnormalities. Knowing the normal values of the prostate volume helps to diagnose prostate pathologies. The aim of this study is to investigate the relationship among prostate volume, interspinal distance and symphysis pubis height.

Thirty-nine volunteers who were patients of the Urology Clinic at the Faculty of Medicine of Trakya University participated in this study. Volunteers did not have any prostate diseases. After demographic data, such as age, height, and weight of the volunteers were recorded, the right and left spina iliaca anterior superior were marked on the skin to measure the interspinal distance. The distance between the two points was measured by using a pelvimeter. Likewise, the upper and lower points of the pubic symphysis were marked and the distance between them was measured using a caliper. The transabdominal screening method using Esaote brand MyLab ™ 40 model ultrasonic device was applied to measure prostate volume.

Mean values of age, height, weight, bispinal distance, pubic symphysis height and prostate volume of the volunteers were 42.35 - 174.46 cm - 78.69 kg - 28.10 cm - 6.21 cm - 24.53 cm, respectively. There was a significant correlation between prostate volume and pubic symphysis ($p<0.05$). Similarly, there was a significant relationship between prostate volume and bispinal distance ($p<0.05$). Since prostate volume is associated with these morphometric measurements, it can give clues to clinicians while examining patients. We suggest expanding this study with more participants to ensure more accurate results.

WHAT CAN THE GOLDEN RATIO TELL US ABOUT FACIAL BEAUTY?

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You could see the Golden Ratio in many kinds of art like music, painting, architecture, and sculpture. The Golden Ratio is widely known in art and also well known in the field of plastic surgery. The Golden Ratio appears in the human face, as demonstrated in a many studies. Stephen Marquardt focuses on human facial beauty in his work. Dr. Marquardt has conducted surveys to define the differences in beauty between cultures. He has also analyzed the human face from ancient times to modern days. The definition of facial beauty is based on dynamic assumptions. It should not be forgotten that it varies depending on various factors. Many structures such as nose, eyes, eyebrows, cheeks, chin, and lips need to be evaluated to describe facial beauty. All these studies show us which is the Golden Ratio and the importance of this marker in regard to beauty. It is also conspicuous that the Golden Ratio will be a spotlight with a gradual increase in significance.

PRESENCE OF BRONCHIAL MAST CELLS IN THE RAT LUNG

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The aim of this investigation is to present the proportion of serotonin positive mast cells in the different layers of bronchial tree. According to Directive 2010/63/EU of the European Parliament, after anesthesia, 6 male adult Wistar rats were perfused with 5% paraformaldehyde in phosphate-buffered saline. Material from the lungs was removed and processed in accordance with the histological requirements for immunohistochemistry. Six µm sections were treated with Monoclonal Mouse Anti-Human Serotonin, incubated for one hour with DAKO-REALTM En-VisionTM detection system and visualized with diaminobenzidine. Demonstration of mast cells was done with toluidine blue staining on the same sections. For statistical analysis GraphPad Prism 6 for Windows was used. Most serotonin positive mast cells were observed in the tunica adventitia and tunica muscularis of the bronchi but less mast cells were seen in the bronchial lamina propria. The findings were similar in the bronchioles. Comparing the bronchi and the bronchioles, it was observed that the number of serotonin positive mast cells was higher in the larger ones.

NEW TRENDS IN S10 PLASTINATION TECHNIQUE

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Trakia University, Bulgaria

According to the original S10 methodology, the processing of biological material takes place at low temperature (-30°C) and vacuum (10-3-10-6 Torr). From the creation of the method until today, the S10 plastination technique has changed significantly.

Injection plastination by Shawnda et al. (2013) is a new economical method for producing of elastic, life-like and safe anatomical preparations. The impregnation is carried out at room temperature, at which Biodur S10 is a liquid, and normal atmospheric pressure.

The Brazil POLISIL® Silicones products allow impregnation to take place at room temperature. The final products are dry, with reliable structures, odourless, durable, with good flexibility and preserve the anatomical structures.

A modified room temperature plastination technique by Ottone et al. (2016) is used in South America. Three-dimensional reconstructions were obtained from plastinated specimens. Further three-dimensional and two-dimensional cuts were achieved.

According to Starchik (2016) the average shrinkage for tissue cores plastinated by the room-temperature technique was 1.5 times less than the cold-temperature method ($p < 0.05$). The total duration of impregnation and curing stages of core samples for cold-temperature plastination proved to be 1.54 times longer than those of the room-temperature technique.

Conclusion: Impregnation at room temperature and low pressure is a promising method for plastering of anatomical objects. It is cheaper, faster and more economical.

USING ALTERNATE LIGHT SOURCE FOR SHEET PLASTINATION

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UV rays are the most active part of the light spectrum. They are used for curing in P40 plastination technology. The UV rays are divided into three sub-spectra, which also affect the human body: UVA - pigment-forming or long-wave (400-320 nm); UVB - erythrogenic, vitamin D forming or medium (320-280 nm); UVC - bactericidal or shortwave (280-100 nm). The sources of UV rays are two types of luminescence: non-selective mercury-quartz lamp, emitting all the spectra of light but most of all UV rays; and selective - bactericidal lamps, emitting at a fixed frequency of about 254 nanometers.

An alternative source of UV radiation is a light-emitting diode (LED) lamp where the light source is a light-emitting diode, which emits at a wavelength of 365-395 nm. LED is a semiconductor crystal that emits energy in the form of visible, infrared and ultraviolet light, with little electrical power.

The advantages of this alternative source are low power, resistance to shocks and vibrations, and low level of exposure to the human body. The disadvantage of LED is the narrow field of irradiation (no more than 30 cm), which is not an obstacle, as it rarely exceeds it in the sheet plastination. With the future development of LED technologies, this alternative source of UV rays will be very relevant in the preparation of brain slices.

TRIGEMINAL ARTERY AND ITS CLINICAL IMPORTANCE

D. Donmez, M. Karahan, A. Yilmaz

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The trigeminal artery can be seen during embryonic development. This artery supplies the basilar artery. The trigeminal artery involutes after the posterior communicating artery develops. In some cases, the artery becomes permanent and causes medical complications, especially intracranial aneurysms.

The trigeminal artery arises from the internal carotid artery when the embryo is 6 weeks old. It is responsible for the supply of this region before other arteries develop. It involutes when the posterior communicating artery arises from the internal carotid artery. Normally, the artery's length of existence is 7-10 days. Sometimes this artery continues to exist in adulthood. In this case this structure is named persistent trigeminal artery (PTA).

The clinical conditions associated with the trigeminal artery are as follows: ectasia and fenestration of the anterior communicating artery (ACA), infraoptic part of the A1 segment of ACA, occluded internal carotid artery (ICA), absence of the common carotid artery and both ICAs, hypoplastic basilar artery (BA) and vertebral artery (VA), bilateral occlusion of VA, primitive otic artery and PHACE syndrome. There are many reports about aneurysms being a result of the presence of PTA. The prevalence is approximately 3%. In patients with PTA, occlusion of PTA leading to a decreased blood flow in the BA can be found. Carotid-cavernous fistula and PTA-cavernous sinus fistula can be idiopathic or traumatic. In this context the Klippel-Feil syndrome, Moyamoya disease as well as infratentorial and supratentorial AVMs have been reported. PTA may be associated with cerebral syndromes and disorders. It has clinical importance that should be considered during clinical examination.

HYPOGLOSSAL ARTERY AND ITS CLINICAL IMPORTANCE

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The hypoglossal artery is one of the carotid-basilar system anastomoses found in the embryonic period. It can be permanent in adulthood due to the failure of involuting vascular structures such as trigeminal, otic and intersegmental arteries. Batujeff was the first one to describe this artery. Oertel suggested the name "hypoglossal artery" because the artery was following the hypoglossal nerve.

When we examine its anatomical and clinical aspects, there are two forms of the hypoglossal artery: the embryonic, or primitive form, is named primitive hypoglossal artery (PHA) and the persistent fetal, or adult form, is named persistent primitive hypoglossal artery (PPHA). When the embryo is 4 mm, PHA emerges as one of the presegmental branches of the dorsal aorta and serves as an anastomosis between the primitive internal carotid artery and the bilateral longitudinal neural arteries. It disappears the embryo reaches a size between 5 and 6-mm, or between 7 and 12 mm, i.e. on the 29th day of gestation. Matsamura and Lindgren are the first to identify PPHA radiologically. There are several hypotheses about the topography of PPHA.

In the presence of PPHA, various clinical associations such as, complications of head and neck surgery, aneurysms, subarachnoid hemorrhage and several syndromes can be observed.

DA VINCI'S ANATOMICAL MISTAKES

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Leonardo da Vinci is an important philosopher, astronomer, architect, engineer, inventor, mathematician, anatomist, musician, sculptor, botanist, geologist, cartographer, writer and painter who lived in the Renaissance period. In addition to his numerous skills, he has made important contributions to anatomy, which are the cornerstones of medicine. Using our modern anatomical knowledge, we can determine that most of da Vinci's descriptions of human anatomy are wrong. For example, he described four umbilical arteries. In the plexus brachialis drawings, it is seen that the thoracic spinal nerve does not participate in its formation. The seminal vesicle was shown incorrectly. He had an idea that semen moves into penis from the spinal cord, so he described the spinal cord extending into the penis. The spleen has an artery, which comes from the liver.

Nevertheless, da Vinci made important discoveries and observable predictions about the facts, which had not been discovered previously. Unlike his contemporaries, he was the only one who carried out the work on the human body so meticulously. His scientific correctness added to Galen's anatomical teachings.

ANATOMICAL PECULIARITIES AND MORPHOMETRIC CHARACTERISTICS OF THE INTRAMURAL PART OF THE PORCINE URETER

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The intramural part (located within the wall of urinary bladder) of the porcine ureter from 100 (50 male and 50 female) six months old Bulgarian White x Landrace pigs, with weight between 95 and 105 kg, slaughtered for meat consumption in accordance with Bulgarian legislation, were studied after silicone filling, radiography and corrosion casts. It was established that the intramural part showed a well expressed curved course (almost 90°) with laterally oriented arch and distension just before transforming into ureteral columns. The statistical data (presented as mean ± SD) of the studied morphometric parameters on silicone replicas - diameter and length of both sides of this part of the ureter and the distance between the two ureteral orifices (ostia ureterica), as well, showed slightly higher values in females compared to males, with no statistical significance (P>0.05, one-way ANOVA). Similarly, the diameter and length of the right ureters were with slightly higher values that these of the left intramural parts. Also, an asymmetry between ureteral ostia was observed – 15.4% in males and 38.5% in females, with different position toward each other. Ureteral ectopy was not observed in all animals studied. The original data obtained present a species-specific feature and could be useful also in medico-biological studies related to humans and probably in xenotransplantation, as well.

A NEW COMBINATION: OBESITY SURGERY AND ACUPUNCTURE VERSUS THE DISEASE OF THIS CENTURY - OBESITY

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Obesity is the most common health problem of this century. The causes of overweight and obesity are complex. Over 1 million people on earth are currently obese. More importantly, 10% of them are children. Obstructive sleep apnea syndrome (OSAS), certain corporal changes like arthritis, or psychological effects stemming from the change of the outlook of the body are some of the problems.

Studies are continuously conducted, focusing on the way obesity affects the quality of life.

Obesity surgery is the most frequently used approach. The literature sources we have examined indicated very high success rates. However, the most important problem concerns postoperative complications. Another way to treat obesity is acupuncture, which is linked to a lower incidence of complications. It is cheaper and an easier approach to apply. Of course, there are a lot of studies about acupuncture effectiveness in obesity treatment. But it is not enough when used on its own.

In our opinion, acupuncture could be combined with obesity surgery when treating obesity. This could reduce the complications from both the preoperative and postoperative period. It could be successful in creating new life standards and, of course, it may improve the outcome of the surgery.

TOPOGRAPHIC ANATOMY OF ACUPUNCTURE POINTS ON THE HEAD-NECK AREA

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Acupuncture is a treatment method which aims to treat diseases by pricking needles on specific skin points. These acupuncture points are located on linear meridians. These points are stimulated by using needle, pressure, light, ultrasound and power current. The treatment relies on the principle of affecting the points.

The meridians which include these points are linear lines where energy flow occurs. The meridians of traditional Chinese medicine are defined as follows: Conception Vessel Meridian (CV), Extra Acupuncture Points (EX); Gall Bladder Meridian (GB), Governing Vessel Meridian (GV), Heart Meridian (HT), Kidney Meridian (KD), Large Intestine Meridian (LI), Lung Meridian (LU), Liver Meridian (LV), Pericardium Meridian (PC), Small Intestine Meridian (SI), Spleen Meridian (ST), Stomach Meridian (ST), Triple Heater Meridian (TH), and Urinary Bladder Meridian (UB).

The head and neck region contains a lot of these meridians. It has a potential function of expressing our emotional states. Nerves and vessels are in a close relationship with the skin. Because of that it necessitates more careful treatment during the interventional procedures.

The specialist must be especially careful about the Stomach Meridian because of the large intestine points near the foramens. Also, attention must be paid while applying acupuncture to the Triple Heater Meridian, because of the ear section.

THE SECRET ENERGY CURRENT OF THE HUMAN BODY: ACUPUNCTURE MERIDIANS

A. Yilmaz, M. Donmez

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The meridians we often hear about are lines which carry energy to various directions. They are composed of non-visible acupuncture points below the skin in a single file and in a successive arrangement. This is the map of our body. The classical meridian classification is as follows: 1) Main Meridian (12); 2) Branch Meridian (numerous); 3) Connecting (Luo) Meridian (15); 4) Diverging Meridian (12); 5)Muscle Meridian (12); 6) Extra Meridian (8).

Every group of 12 meridians has a Luo point. The energy current between the meridians occurs due to the Luo points. The meridians composed by Luo points generally exist superficially. They are used in superficial diseases. The meridian that the energy circulation starts from the Lung Meridian. It is not enough to know the acupunc-

ture points for treatment. The relationship between the points and meridians, the energy current and fluctuations during the day, have to be well known. In addition, it must not be forgotten that acupuncture is an alternative and it is not competing with traditional medicine. It is a subsidiary to medicine. Another point is that acupuncture has to be applied only by medical doctors.

TOPOGRAPHIC ANATOMY OF ACUPUNCTURE POINTS ON THE UPPER EXTREMITIES

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The upper limbs are associated with the lateral aspect of the lower portion of the neck. They are used for support, stability and locomotion, the upper limbs are highly mobile in order to be capable of positioning the hand in the space around. However, they are far away from the organs that have vital importance. The nerves and the vessels on the upper extremities are protected by powerful muscles. The specialists have to be careful about pricking needles on the wrist, elbow and shoulder. Here are the meridians on the upper extremity: Heart Meridian (HT), Large Intestine Meridian (LI), Lung Meridian (LU), Pericardium Meridian (PC), Small Intestine Meridian (SI), Triple Heater Meridian (TH), Urinary Bladder Meridian (UB). Because of being closer to n. medianus, and a. ulnaris, the specialist has to be more careful when working with the HT and PC meridians.

OTIC ARTERY AND ITS CLINICAL IMPORTANCE

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Three primitive arteries - trigeminal, otic, and hypoglossal - are named according to their close relationship with the 5th, 8th, and 12th cranial nerves. While the incidence of permanent trigeminal artery ranges from 0.06% to 0.6%, the persistent primitive otic artery has been documented on fewer occasions.

When the human embryo is 4 mm in length, these arteries serve as transitional anastomoses between the primitive internal carotid arteries and the bilateral longitudinal neural arterial plexus.

The primitive otic artery originates from the primitive internal carotid artery and joins the basilar artery. It regresses at the 30th day of embryogenesis. Persistent primitive otic artery remains in adulthood and it is also called "acoustic artery". It originates from the petrous part or the C2 part of the internal carotid artery. Sometimes, as a part of another pattern, the persistent primitive otic artery arises from the persistent primitive hypoglossal artery at cervical level.

Hypoplasia or aplasia of the vertebral and posterior communicating arteries can be seen with the presence of persistent primitive otic artery. Also an angiomatous malformation, the occluded unilateral internal carotid artery and bilateral vertebral arteries are visualized in some cases.

Knowledge about persistent carotid-vertebrobasilar anastomoses between the anterior and posterior head circulation can be of vital significance during invasive procedures like angiography and surgical operations.

HISTORY OF SINUS CAVERNOSUS

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Sinus cavernosus has been an interesting structure for researchers from past to present. The purpose of this study is to explain how our knowledge about sinus cavernosus has changed since the discovery of this structure.

Claudius Galen (130-200) was the first person to describe the anatomical entity of the sinus cavernosus. Galen described the parasellar carotid artery as "a parasellar carotid retia bathed in venous blood". This definition was accepted for 15 centuries. Raymond de Vieussens (1641-1715) described a pair of dural sinuses on both sides of sella turcica. He showed that sinuses were connected via anastomoses. He named this area a "receptacle". Humphrey Ridley (1653-1708) described the connection of sinuses in his book "The Anatomy of the Brain Containing its Mechanism and Physiology". He called this structure the "Ridley Sinus". For the first time in 1732, Jacques Benigne Winslow noticed trabeculae in the sinus cavernosus. He stated that internal structure of sinus cavernosus is similar to corpus cavernosum penis. The term "sinus cavernosus" has been used since then. In 1933, Campbell reported that the trabeculae in sinus cavernosus slowed down the blood flow. Hollinshead supported this view and stated that this trabecular structure facilitated thrombus formation. Bonnet claimed that the trabeculae are actually sections of numerous small vessels. Balo could not find trabeculae in his histological study. Bedford defended the existence of the trabecular structure in his work. Parkinson's microanatomical studies gave opportunity to explain sinus cavernosus anatomy in a more detailed aspect.

Owning to technological developments and the progress of dissection techniques we now are able to understand the anatomical structure of sinus cavernosus. Future studies may increase our knowledge about it.

PROSTATE GLANDULAR PARENCHYMA OF MEN AGED 36-60 YEARS

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Aim: The aim of this paper was to analyze quantitatively the epithelium and lumen in all lobules of normal human prostates.

Materials and Methods: Samples of the prostate gland from all lobules were obtained during necropsy of prostates of 19 adult men aged 36-60 years without clinical symptoms of lesion of the urinary apparatus. The following variables were measured: number, length, width, area of acini, total gland area, area of the lumen and epithelial height using computerized morphometric techniques.

Results: The anteromedial lobule was characterized by the large size of the lumen of the alveoli: the length was 51.3±4.6 μm, the width was 102.2 ± 11 μm, the area was 3677±388 μm^2 and the epithelial height was 16.9±1.1 μm ($p<0.05$). In the superomedial lobule respectively, the sizes of the alveoli were: length - 68.4±10.1 μm, width - 51.3±4.6 μm, area – 48372±4633388 μm^2, and epithelial height - 16.9±1.1 μm.

The sizes of the inferolateral lobule and the posterolateral lobule did not differ significantly from each other ($p>0.05$): width - 28.4 and 36.9 μm, length - 60.9±8.7 and 60.4±12.1 mm, area of the alveoli - 1718±356 and 1650±08 μm^2, epithelial height 15.8±1.4 and 17.0±0.6 μm.

Conclusion: There was a significant difference in the number, length, width of acini, total acinar area, area of the lumen, epithelial height of acini between different lobules of the prostate.

FORAMEN STERNALE

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The sternum is a flat bone located on midline in front of the rib cage. It has three parts called manubrium sterni, corpus sterni and processus xiphoideus. Round or oval defects in the sternum, developed during the formation of the bone in the embryological period are called foramen sternale. This common variation of the sternum was first described in the 17th century.

Variations in the sternum are usually noticed on imaging studies or at autopsies. Foramen sternale should be distinguished from osteolytic, primary neoplastic, and metastatic lesions. In addition, foramen sternale can be misdiagnosed as a bullet injury in autopsy. It is also important that the physician should have knowledge about this variation before invasive procedures such as sternum bone marrow aspiration, sternotomy for cardiac surgery or acupuncture.

It is clinically necessary to distinguish the pathological conditions of variations in the human skeleton. Knowledge of the physicians about foramen sternale, which is a common variant of sternum, can help to prevent wrong diagnoses and treatments.

BIOARTIFICIAL ORGANS

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Organ transplantation has become an essential treatment option in a wide variety of clinical conditions. However, millions of patients are waiting for lifesaving organs and the rise of chronic diseases only increases the demand. Also, allo- and xenotransplantations carry the risk of severe complications like rejection of the transplanted organ, side effects of the immunosuppressant medications and the risk of acquiring infections from the donors. Bioartificial organs combine the physical aspects of implantable prostheses with the biological advantages of organ transplantation. Enclosing live cells in a permissive, synthetic scaffold ensures immunoisolation, respectively avoids rejection by an immunologically incompatible host while the space limitation of the closed polymer

capsule prevents overgrowth of the transplanted material. Currently, several types of bioartificial organs are being actively developed, including kidney, pancreas, dopamine-secreting brain implants, ovaries, etc., giving hope to millions of patients, waiting for organ transplants.

PROLONGED MICROGLIAL ACTIVATION FOLLOWING INTRACEREBRAL TRANSPLANTATION IN A PARKINSON'S DISEASE MODEL

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Intracerebral dopaminergic transplantation has been accepted as a viable treatment option of Parkinson's disease. However, despite the abundant data regarding the fate of the transplanted neurons, little is known of the glial reaction following transplantation.

In the present study, we investigate the reaction of host microglial cells to intracerebral transplantation of embryonic dopaminergic cells in a rodent model of Parkinson's disease. By means of immunohistochemistry, we compare the characteristics of the microglial reaction in different zones surrounding the intrastriatal grafts, with those in areas of mechanical influence by the transplantation instrument only at two distinct time points.

Data shows that activated microglia is present around grafts, as well as around areas of mechanical influence. Activated ameboid microglial cells are present along the graft-host interface on day 28 following transplantation. This finding stands out against the reaction around areas of mechanical influence only, in which on day 28 the microglial cells tend to have a more quiescent morphology. Surprisingly, the quantitative characteristics of microglial recruitment are significantly lower around grafts, compared to the respective zones around areas of mechanical influence.

Our findings of fewer, but more active cells around grafts, are suggestive of a prolonged activation of host microglial cells by transplanted tissue. Because no graft rejection is present, this can be explained with modification of microglial reaction by grafted cells. An equilibrium between neuroprotective and proinflammatory microglia exists around grafts, and it is crucial for the synaptic integration of transplanted cells. Further clarification of mechanisms of microglial activation following transplantation could potentially contribute to the development of novel neuroprotective strategies.

CO-LOCALIZATION OF TRANSCRIPTION FACTORS ZBTB20 AND SOX2 IN GERMINATIVE ZONES OF A HUMAN FETAL TELENCEPHALON

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We are presenting data about the co-localization of Zbtb20 and Sox2 during gestation week 20 in the germinative zones of a human fetal telencephalon. We investigated the ventricular (VZ), internal and external subventricular zones (iSVZ and oSVZ) in the frontal lobe by immunofluorescence confocal microscopy. We determined the proportion of double positive cells to all Zbtb20+ cells and compared it to different zones. The analysis demonstrated statistically significant difference between VZ and oSVZ. In oSVZ, we identified populations of Zbtb20+ cells different than Sox2 expressing radial glial progenitors.

FIBROBLASTIC CELL SUBPOPULATIONS ROLE DURING HEPATIC DAMEGE

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Accumulation of extracellular matrix observed in fibrosis and cirrhosis is due to the activation of fibroblasts, which acquire a myofibroblastic phenotype. Myofibroblasts are absent from normal liver. They are produced by the activation of precursor cells, such as hepatic stellate cells and portal fibroblasts. These fibrogenic cells are distributed differently in the hepatic lobule: the hepatic stellate cells resemble pericytes and are located along the sinusoids, in the Disse space between the endothelium and the hepatocytes, whereas the portal fibroblasts are embedded in the portal tract connective tissue around portal structures (vessels and biliary structures). Differences have been reported between these two fibrogenic cell populations, in the mechanisms leading to myofibroblastic differentiation, activation and "deactivation", but confirmation is required. Second-layer cells surrounding centrolobular veins, fibroblasts present in the Glisson capsule surrounding the liver, and vascular smooth muscle cells may also express a myofibroblastic phenotype and may be involved in fibrogenesis. It is now widely accepted that the various types of lesion (e.g., lesions caused by alcohol abuse and viral hepatitis) leading to liver fibrosis involve specific fibrogenic cell subpopulations. The biological and biochemical characterisation of these cells is thus essential if we are to understand the mechanisms underlying the progressive development of excessive scarring in the liver. These cells also differ in proliferative and apoptotic capacity, at least in vitro. All this information is required for the development of treatments specifically and efficiently targeting the cells responsible for the development of fibrosis/cirrhosis.

MORPHOLOGICAL BASIS OF THE AGING ERYTHROCYTES DEFORMABILITY

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Red blood cells are flexible and oval biconcave disks, they lack a cell nucleus and their disk diameter is 7–10 μm. Approximately 2.4 million new erythrocytes are produced per second and circulate for about 100–120 days in the body. Red blood cells are cells present in blood in order to transport oxygen, the aging red blood cell undergoes changes making it susceptible to selective recognition by macrophages and subsequent phagocytosis.

The cytoplasm of erythrocytes is rich in hemoglobin, an iron-containing biomolecule that can bind oxygen and is responsible for the red colour of the cells. Immature red blood cells are lacking the red hemoglobin pigment so these cells are often shades of grayish blue, only mature cells are red.A glycophorin is a sialoglycoprotein of the membrane of a red blood cell, that contains N-acetylneuraminic acid. Ions such as Na^+ and Ca^{2+} can diffuse rapidly through it and can be for 60% it`s negative charge of the plasma membrane. Typical human red blood cell has a diameter of approximately 6.2–8.2 μm, a thickness - 2 μm, circumference – 76-110 μm, speed no more than 2 cm/sec that is enough to transport oxygen from hemoglobin toward myoglobin. Listed features are changed depending on the lifespan of red blood cells:

1. **Decreases the percentage of hemoglobin content, within the part of it is broken down.**
2. **Changes occurs in the activities electronic change in oxidation and restoration of Fe.**
3. **As erythrocyte ages, it undergoes changes in its plasma membrane, in particular sialic acid activity.**
4. **Erythrocyte membrane becomes inflexible, less elastic and rough.**
5. **Worn-out red blood cells (100-120 day) have a limited functional significance.**

As a result of this research, both functional and physical indicators are strictly differentiated regarding to human age:

1. **The length of erythrocyte life in the elderly (70-75) is twice longer than in younger people (25-30).**
2. **Red blood cells are remarkably deformable in younger than in elderly people.**
3. **Because of decreased deformability of the red blood cell, they have trouble to squeeze through capillaries which is the reason of hemodynamics local violations.**

LIVER STELLATE CELLS: MAGNIFICANT CHARACTERISTICS IN HUMAN CELL BIOLOGY

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Hepatic stellate cells (HSCs), a mesenchymal cell type in hepatic parenchyma, have unique features with respect to their cellular origin, morphology, and function. Normal, quiescent HSCs function as major vitamin A-storing cells containing over 80% of total vitamin A in the body to maintain vitamin A homeostasis. HSCs are located between parenchymal cell plates and sinusoidal endothelial cells, and extend well-developed, long processes surrounding sinusoids in vivo as pericytes. However, HSCs are known to be 'activated' or 'transdifferentiated' to myofibroblast-like phenotype lacking cytoplasmic lipid droplets and long processes in pathological conditions such as liver fibrosis and cirrhosis, as well as merely during cell culture after isolation. HSCs are the predominant cell type producing extracellular matrix (ECM) components as well as ECM degrading metalloproteases in hepatic parenchyma, indicating that they play a pivotal role in ECM remodeling in both normal and pathological conditions. Recent findings have suggested that HSCs have a neural crest origin from their gene expression pattern similar to neural cell type and/or smooth muscle cells and myofibroblasts. The morphology and function of HSCs are regulated by ECM components as well as by cytokines and growth factors in vivo and in vitro. Liver regeneration after partial hepatectomy might be an invaluable model to clarify the HSC function in elaborate organization of liver tissue by cell-cell and cell-ECM interaction and by growth factor and cytokine regulation.

UNIQUE PROTECTIVE FUNCTION OF KUPFFER CELLS

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Kupffer cells

The most important function of Kupffer cells is defense against infections and tumor cells. Kupffer cells act as antigen presenting cells and as effector cells that act directly by phagocytosis, or indirectly by activation of other cells, e.g. NK cells. Though Kupffer cells are constantly acting as scavengers, they can be activated through different pathways. Firstly, soluble mediators can trigger their activation. IFN-γ is the prototypical macrophage activating factor. It is central to the development of Th1-dominated immune responses, and it affects not only macrophages in an autocrine fashion, but other immune cells as well.One of the key events during innate immune reactions is the production of IL-12, mainly by macrophages. IL-12 induces NK cells to rapidly secrete IFN-γ, which then in turn activates macrophages early in the immune response. It also induces IFN-γ production by T cells. IL-18, which is produced by Kupffer cells and other cell types, is also involved in enhancing IFN-γ production by T cells. Macrophages also secrete IFN-γ upon stimulation with IL-12 and IL-18 together. It is known that IFN-γ is a possible reducer of metastasis of colon cancer in the liver. Macrophages can also be activated by direct interaction with micro-organisms or bacterial products such as lipopoly-saccharide, glucan, muramyl dipeptide and lipid A[52]. A pivotal role for IFN-γ in the clearance of various intracellular pathogens has been amply demonstrated. It

has been described that macrophages release the cytotoxic radical, NO. *In vitro* **studies suggested that NO induces mitochondrial dysfunction in tumor cells followed by membrane barrier dysfunction in the liver sinusoid. Another important cytotoxic factor released by activated macrophages is tumor necrosis factor alpha (TNF-α), which is produced in both soluble and membrane-bound forms. After binding to its receptor, apoptosis can be induced in the target cell.Cytotoxicity of macrophages can be classified into antibody-dependent and antibody-independent cell-mediated cytotoxicity. Both pathways are contact dependent and induce tumor cell death after a number of hours. Antibody-dependent cell-mediated cytotoxicity is based on the recognition of an antibody-coated target by Fc receptors on the effector cells. Upon cross-linking of the Fc receptor, secretion of cytotoxic mediators occurs. Secretion of reactive oxygen species, IL-1 and TNF-α are probably involved. Antibody-independent cell-mediated cytotoxicity involves binding to the macrophage followed by translocation of the lysosomal organelles to the target. Moreover, cytotoxicity towards tumor targets involves cytolysis and phagocytosis.In certain pathophysiologic conditions, apoptosis is chaotic and non-selective, may be massive and occurs persistently over an extended period of time. A large number of apoptotic bodies produced are phagocytosed by Kupffer cells. It has recently been reported that engulfment of apoptotic bodies results in the generation of death ligands, such as FasL and TNF-α on the membrane of the Kupffer cells, but engulfment of latex beads does not produce a similar response. This means that uptake of apoptotic bodies induces an additional immunologic response involving liver inflammation and fibrosis.**

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