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ABSTRACTS

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SCIENTIFIC PROGRAM

Tuesday, 8 September 2009

17:30-19:00: Opening of the Reception Desk of the XXIV Congress of the Spanish Society of Anatomy.

Congress Venue at Palacio Euskalduna.

- Registration. Sign-in and welcome package at the *Palacio Euskalduna.*
- Hanging of poster presentations. *Hall at the Palacio Euskalduna* .

Wednesday, 9 September 2009

8:00-8:30:

- Registration. Sign-in and welcome package. *Palacio Euskalduna.*
- Hanging of poster presentations. *Hall at the Palacio Euskalduna.*
- Delivery of electronic files for oral sessions and special lectures. Specific room where the presentation will take place (please *hand over the files at least 30 minutes before oral sessions start).*

8:30-9:00:

- Wellcome greetings and Presentation of the Congress (Sala A3)

9:00-11:00: Parallel Oral Sessions.

- Clinical Anatomy. *Sala A3.*
  Oral communications AC-0-1 to AC-0-8.
- Neuroanatomy. *Sala A2.*
  Oral communications N-0-1 to N-0-8.

11:00-11:30: Coffee break.

11:30-12:30: Poster Sessions. *Hall at the Palacio Euskalduna.*

- All posters must be exhibited.

12:30-14:00: Opening Ceremony of the Congress. *Sala A3.*

Opening Lecture:

- Title: *"**La construcción del cuerpo humano**"*
- Speaker: *Prof. Dr. Juan Luis Arsuaga*, Madrid, Spain.

16:00-17:30: Visit to the Guggenheim Museum.

Thursday, 10 September 2009

8:30-9:00:

- Delivery of electronic files for oral sessions and special lectures. Specific room where the presentation will take place (please *hand over the files at least 30 minutes before oral sessions start).*
- Hanging of poster presentations. *Hall at the Palacio Euskalduna.*

9:00-11:00: Parallel Oral Sessions.

- Neuroanatomy. *Sala A3.*
  Oral communications N-0-9 to N-0-17.
- Clinical Anatomy. *Sala A2.*
  Oral communications AC-0-9 to AC-0-16.

11:00-11:30: Coffee break / Poster Sessions.

11:30-12:30: Parallel Oral Sessions.

- Teaching in Anatomy. *Sala A3.*
  Oral communications D-0-1 to D-0-5.
- Clinical Anatomy. *Sala A2.*
  Oral communications AC-0-17 to AC-0-21.

12:30-13:30: Plenary Lecture. *Sala A3.*.

- Title: ***"Axotomy-induced neuronal plasticity and regeneration in the peripheral nervous system"***
- Speaker: *Prof. Dr. Lars Klimaschewski,* Innsbruck, Austria.

15:30-17:30: Transport and visit to the Forest of Life (Bosque de la Vida), Universidad del País Vasco, Leioa.

Friday, 11 September 2009

8:30-9:00:

- Delivery of electronic files for oral sessions and special lectures. Specific room where the presentation will take place (please *hand over the files at least 30 minutes before oral sessions start).*

9:00-10:00: Parallel Oral Sessions.

- Teaching in Anatomy. *Sala A3.*
  Oral communications D-0-6 to D-0-10.
- Embryology. *Sala A2.*
  Oral communications E-0-1 to E-0-4.

10:00-11:00: Plenary Lecture. *Sala A3.*

- Title: ***"Surgical and radiological anatomy: new perspectives"***
- Speaker: *Prof. Dr. Raffaele di Caro,* Padova, Italy.

11:00-11:30: Coffee break / Poster Sessions.

11:30-12:30: Oral Session.

- Teaching in Anatomy. *Sala A3.*
  Oral communications D-0-11 to D-0-15.

12:30-14:00: Closing Lecture. *Sala A3.*

- Title: ***"Estructura y Dinámica de la Memoria en la Corteza Cerebral"***
- Speaker: *Prof. Dr. Joaquín Fuster,* Los Angeles, USA.

15:30-16:00: Poster taking down. *Hall at the Palacio Euskalduna.*

16:00-17:30: General Assembly of the Spanish Society of Anatomy. *Sala A3.*

18:30: Transport to the Valle de Atxondo.

20:30: Closing Dinner. Award of the Enrique Martínez Prize.

- *Mendi Goikoa Jatexea (Valle de Atxondo)*

ORAL COMMUNICATIONS

AC-O-01 – AC-O-021 Clinical Anatomy

AC-O-01 3D CLINICAL ANATOMY PODCASTS –COLLES' FRACTURE– AN INTEGRATED METHOD OF TEACHING CLINICAL ANATOMY

Abrahams, P.[1], Roebuck, J.[1], Gogalniceanu, P.[2], Fletcher, A.[1], Barrientos-Cordova, J.[3], Lord, G.[3]

[1]Institute of Clinical Education, Warwick Medical School, Warwick University; [2]Vascular Surgery Unit, University College London Hospital; [3]Primal Pictures, London.

Clinically integrated anatomy teaching requires cadaveric material, radiological images and simulators. Unfortunately, it is difficult for students to access all of these resources at once in a non-academic environment.

Aims and Method: To design a software package that would integrate these teaching tools, whilst at the same time being portable, affordable and readily accessible world wide. **Results:** A series of anatomy podcasts compatible with multimedia MPEG-4 players were designed, taking advantage of high resolution digital imaging and three dimensional animations combined with narrative and visual explanations. We present a sample 3 minute podcast on the clinical anatomy relevant to Colles' fracture. This 500 word multimedia package was created incorporating three dimensional simulations, clinical images, plain radiographs, and computed tomography (CT). These were complemented by audio and on-screen text explanation, as well as digital highlighting techniques. Clinical integration was achieved by linking regional anatomy, to pathology and radiology in a clinical context, and in particular focusing on surgical diagnostic and operative procedures.

Conclusion: Anatomy Podcasts allow integration of basic and clinical sciences utilising an extensive variety of anatomical imaging. They facilitate anatomical education outside the dissection room in a highly portable and accessible format. Current studies are assessing the validity and benefits of Podcasts as a teaching method and it is hoped that the definitive results on this medical education project will be available by the time of this meeting.

AC-O-02 ANATOMO-CLINICAL STUDY OF THE ANASTOMOSIS BETWEEN THE MUSCULOCUTANEOUS AND MEDIAN NERVES

De la Cuadra, C.*[1], Peces, M.D.[2], Ruiz, J.R.[3], Sanz, L.A.[3], Rodríguez-Vázquez, J.F.[1], Mérida, J.R.[1]

[1]Departamento de Anatomía y Embriología Humana II. Facultad de Medicina. U. Complutense. Madrid. Spain. [2]Departamento de Anatomía y Embriología Humana I. Facultad de Medicina. U. Complutense. Madrid. Spain. [3]Servicio de Traumatología. Mutua de Accidentes del Trabajo FREMAP. Madrid. Spain.

The variations of the brachial plexus and its branches have been widely documented. We have studied the anastomosis between the nerve musculocutaneous and the median nerve.

For the anatomical study, during the last three academic years (2005-2008), there was a dissection of the upper extremities of 24 cadavers. We analyzed the disposition of the brachial plexus. The cadavers (15 female and 9 male) were fixed in formalin at 10 %. The ages of the cadavers ranged from 69 to 95.

For the clinical study, during the months of May through September 2008, we have studied 35 patients from the Co Accidents "FREMAP". These patients needed anesthesia by axillary block for surgical treatment of pathology of the upper limb. The anesthetic process was guided by ultrasound using the apparatus Siemens Acuson X150 ultrasound, high frequency waves between 5-10MHz.

Of the 24 cases studied, we observed the anastomosis between the musculocutaneous nerve and median nerve in eleven cases. The anastomosis of the musculocutaneous nerve to the median nerve was located preferably at axillary level in nine cases, and brachial level in three cases (one case showed double anastomosis, axillary and brachial).

The variations described are vulnerable to injury in the surgical access of the arm. They are also important in the anesthetic blockades through neurostimulation, avoiding repeated punctures to a total or partial nerve inadvertently blocked by a prior injection. The use of the ultrasounds to identify the median and the musculocutaneous nerves reduces in the anesthesia procedures the residual neuritis post-puncture.

AC-O-03 UPPER EXTREMITY PERIPHERAL NERVE BLOCKS, ULTRASOUND – GUIDED

Sánchez del Campo, F.[1], Sánchez Ferrer, M.*[1], Hernando, J.[2], Garramone, D .[2], Roqués, V.[3], Tejada, S.[2], Almenar, V.[1], Terol, F.[1]

[1]Departamento de Histología y Anatomía. Universidad Miguel Hernández (Alicante). [2]Servicio de Anestesia. Hospital Clínico Universitario de San Juan (Alicante). [3]Servicio de Anestesia. Hospital Virgen de la Arrixaca (Murcia).

Background: In recent years there has been a growing interest in the practice of regional anesthesia and, in particular, peripheral nerve blocks for surgical anesthesia and postoperative analgesia (effective analgesia with few side effects). The success of nerve blocks needs the knowledge of the anatomy of plexus and peripheral nerves.

Current methods of nerve localization (paresthesia and nerve stimulation) are essentially "blind" procedures with systemic and neurological complications.

Guidance for nerve localization holds the promise of improving block success and decreasing complications. Ultrasonography seems to be the one most suitable for regional anesthesia, the advantage of ultrasound technology is the ability to provide anatomic examination of the neu-

ral structures, and visualize the needle and local anesthetic spread in real-time.

Objectives: To describe the different anatomical references to locate the peripheral nerves of brachial plexus on the elbow-forearm and wrist, ultrasound-guided. We will show photos and videos of the nerves radial, medium and ulnar, ultrasound guided.

Methods: We use portable ultrasound machine and high-frequency linear probes, in the range of 10 to 15 MHz, are best suited for imaging the brachial plexus in most locations.

The patient is in the supine position with the arm abducted.

Forearm, in the fold of the elbow: The ***medial nerve*** is located medial to the brachial artery and to the tendon of the brachial biceps. The ***radial nerve*** is located one centimeter lateral to the tendon of the brachial biceps. The ***ulnar nerve*** is identified in the channel epitrocleo-olecraniano.

The wrist is best kept in a slight dorsiflexion: The ***medial nerve*** is located between the tendons of the palmaris longus and the flexor carpi radialis.

The superficial branch of the ***radial nerve*** runs along the medial zone of the brachioradialis muscle. Just above the styloid process of the radius, it gives digital branches. Several of its branches pass superficially over the anatomic "snuff box".

The ***ulnar nerve*** passes between the ulnar artery and tendon of the flexor carpi ulnaris. The tendon of the flexor carpi ulnaris is superficial to the ulnar nerve.

Conclusions: The technique of blocking branches of the ulnar, medial, and radial nerves at the level of the forearm and the wrist, is simple to perform, essentially devoid of systemic and neurological complications using ultrasound guidance, and highly effective for a variety of procedures on the hand and fingers.

This basic peripheral nerve block technique and the knowledge of ultrasound in regional anesthesia should be in the armamentarium of every anesthesiologist.

AC-O-04 CONFIDENCE ON CADAVERIC OR SURGICAL SAMPLES FOR PERFORM ANATOMICAL STUDIES. VARIABILITY OF TOPOGRAPHICAL RELATIONS OF THE RECURRENT LARYNGEAL NERVE IN THE NECK

Viejo, F. *[1], Maranillo, E., Asgharpour, E.[2], Vázquez, T.[1], Valderrama, F.[1], Peña, A.[1], Parkin, I.[3], Sañudo, J.R.[1]

[1]Human Anatomy and Embryology I. Complutense University of Madrid (Spain). [2]ENT Dept. Getafe Hospital Madrid (Spain). [3]Cuschieri Surgical Skills Centre. Ninewells Hospital. Dundee. UK.

Introduction: One of the structures that has promoted more anatomical studies in the neck is the recurrent laryngeal (RLN). The aim of more of that studies is to stablish an accurate topographical relations with neigbouring structures like the inferior thyroid artery (ITA), tracheoesophageal groove, (TEG), Berry's ligament (BL) or extralaryngeal division for avoid malpractice during thyroid surgery. However, in spite that there are many studies published continuing to be important statistical differences between results. Some studies were published using cadaveric and another surgical samples. The aim of this work is to study the above-mentioned parametres in two samples (cadaveric and surgical) at the same time that do a metanalysis based on a deep review of the literature.

Methods: We have studied two samples: cadaveric (143 human cadavers from the University of Cambridge, U.K.) and surgical (57 heminecks, belonging to 47 patiens who suffered of total or hemi-thyroidectomies, Hospital of Móstoles, Madrid, and Hospital Universitario of Getafe, Madrid.

The parametres evaluated were the relations of the RLN with: 1, TEG; 2, ITA; 3, BL and 4, extralaryngeal division. Comparations between results of samples were made using Fisher's exact test.

Results: Results of two parametres were similar in our two samples (relation with Berry's ligament and extralaryngeal division) but the other two were different. The RLN in the cadaveric sample was located in front of the TEG in 41,6% of cases, but in the surgical sample it was more frequent located posterior or deeply into the REG (43,9%). The RLN in the cadaveric sample was more frequent located in front of the ITA (35,8%), in the surgical sample the most frequent relation observed was the nerve passing between branches of the artery (54,4%).

Conclusions: Different results obtained could be do related with the observer, type of sample, definition of parametres.

Supported by a Spanish Government Grant F.I.S. nº: 06/0276

AC-O-05 THE APPLICATION OF PIEZOSURGERY TO THE VERTEBRAL COLUMN

Doñate-Oliver, F.*[1], Álvarez Irusteta, E.[2], Bilbao Soto, A.[1], Monjil Escudero, R.[1]

[1]Departamento de Neurociencias, UPV/EHU. [2]Hospital de Basurto (Bilbao)

The Piezosurgery technique, consisting of a cutting system which works by means of controlled ultrasonic oscillations, has been used by dentists and maxillofacial surgeons since it was created by Professor Tomaso Vercelloti ten years ago. Here, we report the application of this technique to surgery of the vertebral column.

This technique has been developed to provide improved precision and security with respect to the traditional bone cutting techniques. We at the Department of Neuroscience of the University of the Basque Country have performed several tests (both macro and microscopical) on pig and rabbit spine, as well as on the human corpse, which demonstrate that this cutting system does not injure nervous tissue or soft tissue adjacent to bone. These results and the safety of this technique were further confirmed by performing decompressive surgery of the spinal cord in several patients.

The Piezosurgery technique, experiments and testing which we carried out are described in full detail herein. The various surgical steps (cervical decompressive laminoplasty) using this technique are presented in a video. On the basis of these results, we consider this technique to be a novel tool with important clinical applications for both orthopedic surgeons and neurosurgeons.

AC-O-06 MAXILLARY ARTERY PERFORATING INFERIOR ALVEOLAR NERVE: AN ANATOMICAL STUDY

Mujahid Khan, M.

Departamento de Anatomía. King Saud University. Riyadh. Saudi Arabia

The infratemporal fossa is a clinically significant anatomical area for the delivery of local anesthesia in the practice of dentistry and maxillofacial surgery. A study of the infratemporal fossae in Caucasian cadavers was conducted and the topographical relations of the inferior alveolar nerve with the maxillary artery were studied. In 3 out of 50 fossae dissected, the maxillary artery passed through the inferior alveolar nerve, splitting it into superficial and deep divisions. Entrapment of the maxillary artery may cause numbness or headache and may interfere with injection of local anesthesia in the infratemporal fossa.

AC-O-07 THIEL MODIFICATION METHOD

Sánchez del Campo, F., Sánchez Ferrer, M., Palau, F., Almenar Garcia, V., Terol Calpena F.

Departamento de Histologia y Anatomía. Facultad de Medicina. Universidad Miguel Hernández (Alicante).

The Thiel modification method for corpse preservation is the most similar to fresh or frozen corpses as it gets similar colour, elasticity and consistency and it eliminates the risk of infection by biological materials. Besides the same piece can be used more than once.

These characteristics are important for their use in investigation and technical training such as arthroscopy, laparoscopy, endoscopy or other minimally invasive techniques. However this technique present a problem, it does not produce a tisular fixation which is important to study the tissues under the light microscope.

We used wistar rats and guinea pig carcass. We made three groups: one group we perfunded with formol, the second one with Thiel classical method and the last one is our experimental group. First we cleaned the vascular system with citratic saline solution, then we perfunded with formol at 10% and finally we perfunded with Thiel method. We did not make the selective perfusion endotracheal and longitudinal sinus perfusion posterior. We did not make brain extraction after twenty–four hours, which is recommended in the original Thiel method.

The results obtained showed that with this technique the tissues are the same colour, prestancy and elasticity as in the original technique while friability is slightly reduced. Therefore they can be used for surgical technique which is not possible with the classical methods using formol and they can also be study under microscope as in the classical technique.

AC-O-08 ANATOMY OF THE ECTOPIC PARATHYROID TISSUE. SURGICAL REPERCUSSIONS

Sanchez del Valle, F.J. *[1], Sanchez del Campo, F.J.[1], Romero, M.[2], Arroyo, A.[2]

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Embryological parathyroid tissue is a rare finding in normal people, but in case of renal hyperparathyroidism is possible an embryological parathyroid rests proliferation under the ongoing stimulus of renal failure after surgery, causing recurrent hyperparathyroidism. Thymus and superior mediastinum are the most common places of this phenomenon due to their embryological development.

Material and method: We've studied the thymus and superior mediastinum of 51 corpses to perform standard limits of resection. The inclusion criteria were the absence of parathyroid and renal disease, and corpses up to 40 years of age, in order to set up the embryological hypothesis.

The study is divided in three phases:

Thymus and superior mediastinum dissection. Limits:

Upper: Inferior thyroid pole.

Lower: Left brachiocephalic vein.

Right/left: Homolateral Internal jugular vein.

Parathyroid dissection.

We've divided thymus and superior mediastinum into 6 quadrants: upper, middle and lower, and each one in right and left. All of them were processed, embedded in paraffin, sectioning and staining with Hematoxylin and Eosin. A carefuly microscopical exam has been done.

Results: We have found 25% of embryological parathyroid tissue in thymus and superior mediastinum.

No embryological parathyroid tissue below 43mm from the inferior thyroid pole.

Hystological features:

Embryological parathyroid tissue were <2mm of size.

Thymic rests nearest to embryological parathyroid tissue were <1mm distance.

Capsule's absence surrounding parathyroid tissue.

Fibrofatty tissue between embryological parathyroid tissue and Thymic cells.

Conclusions: Embryological parathyroid tissue would increase parathyroid hormone secretion after total parathyroidectomy without autotrasplantation.

Embryological parathyroid tissue is a recurrence's cause of renal hyperparathyroidism.

Cervical thymectomy is neccesary to remove embryological parathyroid tissue .

AC-O-09 MORPHOLOGY OF THE TRIANGLE OF KOCH AND ATRIOVENTRICULAR CONDUCTION AXIS IN PERINATAL HEARTS WITH EBSTEIN ANOMALY

Murillo, M. *[1], Picazo-Angelin, B.[2], Climent, V.[1], Cabrera, J.A.[3], Sánchez-Quintana, D.[1]

[1]Departamento de Anatomía y Biología Celular, Universidad de Extremadura; [2]Hospital Costa del Sol de Marbella. Servicio de Pediatría; [3]Unidad de Arritmias. Hospital Quirón, Universidad Europea de Madrid.

Advances in catheter ablation procedures (cryoablation) have created the need for a better understanding of the morphology of the triangle of Koch and AV conduction system, particularly as how they relate in congenital heart disease. We have studied such relation in Ebstein anomaly (EA). A total of seventeen perinatal heart specimens, eleven with Ebstein anomaly (7 male and 4 female; mean age 10± 3 days after birth), and six controls with structurally normal hearts (4 male, 2 female; range 35 weeks of fetal life to 2 days after birth) were studied. The triangle of Koch, and AV septal junctional area were removed in block and serially sectioned at 10-µm thickness at right angles to the AV annulus. We measured the lengths of the sides delineating the triangle of Koch, and the length of the compact AV node and penetrating His bundle. The rightward and leftward inferior extensions of AV node were also calculated. With the displacement of the insertion of the septal leaflet in EM, the AV junction on the endocardial surface was marked by a small fibro-muscular ridge in 7 specimens (64%). In the thickness of this ridge the AV nodal artery was located in 3 specimens (27%). The diameter of the orifice of the coronary sinus showed a larger size in specimens with EM than in control hearts (3.5±1.2 mm *vs* 2.3±0.7mm). The area of the triangle of Koch in hearts with EM was significantly smaller than that of normal hearts (18.5±4.5mm^2 *vs* 25.5±6.5mm^2, $p<0.05$). However, the AV node and its inferior extensions have a similar length in the Ebstein than in control hearts (1.7±0.5mm *vs* 1.5±0.5mm). This leads to a smaller space within the triangle of Koch in Ebstein anomaly specimens so that the body of the AV node and inferior extensions are displaced and located at the coronary sinus orifice, at the base of the triangle of Koch. Ablationist should be mindful of the potentially damage of the AV node during ablation of atrioventricular nodal reentrant tachycardia specifically when the ablation target is at the level of the base of the triangle of Koch.

AC-O-10 MITRAL VALVE REPAIR INSTEAD OF PROSTHESIS REPLACEMENT. IMPORTANCE OF ANATOMICAL STUDY

Garrido, J.M.[1]*, Esteban, M.[2], Roda, O[3], Abdallah, A.[2], Lara, J.[2], Moya, J.[1], López-Soler, M.[3], Epeldegui, A.[1], Sánchez-Montesinos, I.[3]

[1]Heart Surgery Department, Ramón y Cajal University Hospital, Madrid, Spain; [2]Cardiovascular Surgery Department, Virgen de las Nieves University Hospital, Granada, Spain; [3]Department of Human Anatomy and Embryology, University of Granada, Granada, Spain.

Objectives: The mitral valve repair as treatment of Severe Mitral Regurgitation has important advantages compared with the valvular substitution by prosthesis (mechanical or biological valve). This technique is, without doubt, the best option in this patient to avoid ventricular alteration and for preserving native tissues, including subvalvular structures. Also, the special case of mitral regurgitation in cardiac failure could be managed in this way, improving the phisio-pathologic situation.

Material/Methods: We analyzed the importance of anatomical study before, and during the surgical procedure to obtain enough information to be able to repair the mitral valve and to restore the left ventricular chamber in dilate miocardiopathy patients.

We use ecocardiography, magnetic resonance imaging (prior to the surgery) and surgical exploration to understand the anatomical structures and ventricular/valve physiology and to design the repair technique.

Results: We could verify the concordance with the real anatomy. The different valvular diseases (rheumatic, ischemic, myxomatous, etc.) modify the normal anatomy of the heart in multiples ways. We have to understand these changes to discover the regurgitation mechanism.

Conclusions: The advantages of mitral valve repair, instead of prosthesis replacement, make this technique the better surgical management of severe mitral regurgitation in these patients because prevents the worsening of the Ejection Fraction after surgery and because of these patients don´t need anticoagulation treatment in compare with mechanical prosthesis replacement. Also, the durability of valve repair is longer than with biological prosthesis, and reduces significantly the morbi-mortality in early postoperatory time.

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AC-O-11 LEFT VENTRICLE MORPHOMETRY IN COMPUTERIZED TOMOGRAPHY

Escalona, C. *, Pérez Bellmunt, A., Lorente, M.

Àrea d'Estructura i Funció del Cos Humà, Facultat de Medicina i Ciències de la Salut, Universitat Internacional de Catalunya.

Introduction: Cardiovascular diseases are a first order worldwide problem. There is not any study of the anatomy of the heart explored by Computerized Tomography (CT) in people with no cardiac disease.

Objective: To determine the morphometry of the left ventricle in people with no cardiac disease using CT in the long axis.

Material and method: Contrast-Enhanced Multidetector Computerized Tomography (MDTC) has been used to perform the retrospective study. Subjects are pacients who don't show cardiac disease. Sample consists in 75 subjects. Using long-axis view of the heart, all parameters have been measured in systole and diastole; in addition, septum, wall and inside diameter were measured in three heights (basal, medium and apical).

Results: In systole, LV has next measures: base: 44,62 ± 4,56 mm; length 83,67 ± 7,82 mm; apex 8,56 ± 5,29 mm; angle 82,46º ± 13,23º. In diastole, these measures are 43,71 ± 4,70 mm. for the base, 92,86 ± 9,16 mm. for the length, 3,42 ± 2,21mm. for apex and 83,07º ± 12,06º for the angle. Septum shows thickness in systole of 12,10 ± 3,81 mm. in basal height, 13,09 ± 2,73 mm. in mid height and 11,61 ± 2,29 mm. in apical height. In diastole, measures are 9,78 ± 2,96 mm. basal, 9,86 ± 2,03 mm. mid and 7,96 ± 2,00 mm. apical. Thickness of cardiac wall is 13,16 ± 3,94 mm. in basal height, 10,91 ± 2,89 mm. in mid height and 11,27 ± 3,09 mm. in apical height. In diastole they are 8,90 ± 2,17 mm. basal, 7,95 ± 1,39 mm. mid and 7,74 ± 1,48 mm. apical. Inside diameter of LV in systole is 35,58 ± 7,17 mm. in basal height, 32,97 ± 6,65 mm. in mid height and 18,52 ± 6,70 mm. in apical height. In diastole, they are 42,70 ± 8,06 mm. basal, 40,54±6,95 mm. mid and 40,64 ± 5,54 mm. apical.

Conclusions: Left ventricle has been measured by means of Computerized Tomography in the long axis.

AC-O-12 CAPILLARY SUPPLY TO THE SINUS NODE IN SUBJETS WITH LONG-TERM ATRIAL FIBRILLATION

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Objective: To compare the content and ultrastructure of blood capillaries in the sinus node of normal hearts in sinus rhythm and in hearts with chronic atrial fibrillation.

Background: The ultimate causes for atrial fibrillation are still uncertain. Previous studies hypothesis that ischaemia of atrial tissue, including sinus node, could be involved in the pathogenesis of this arrhythmia. However, a quantitative and qualitative comparison of sinus node capillarity between hearts with sinus rhythm and atrial fibrillation is lacking.

Methods: Sinus node biopsy specimens were obtained from 16 patients in chronic atrial fibrillation undergoing open heart surgery. Control sinus node specimens of normal hearts were obtained at autopsy from 7 subjects. Specimens were processed for immunohistochemistry, light microscopy and transmission electron microscopy analysis with morphometric techniques.

Results: The density of capillaries in sinus node tissue was estimated as 1.06±1.47% for the atrial fibrillation group versus 2.12±2.0% for controls (p<0.0001). Internal capillary diameter averaged 21.60?m in the atrial fibrillation group and 24.20?m in controls (p=0.175), whereas external diameter averaged 32.2?m in the atrial fibrillation group and 37.9?m in controls (p=0.052). Ultrastructural analysis demonstrated scarce and interrupted myoendocardial bridges and abnormal deposits of elastic fibres under the endothelial basal membrane at the level of precapillary sphincters and metaarterioles of atrial fibrillation specimens.

Conclusions: There is a significant reduction in the density and size of capillaries in the sinus node of hearts in chronic atrial fibrillation. Our findings would support a potential association between sinus node tissue ischemia and chronic atrial fibrillation.

AC-O-13 EFFECT ON ENDOTHELIAL FUNCTION OF ATORVASTATIN AT HIGH CONCENTRATIONS

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Introduction: Atherosclerosis is the primary cause of cardiovascular disease in Western countries. In recent years, studies have highlighted the importance of the endothelium in the maintenance of cardiovascular homeostasis, considering endothelial dysfunction to be the initial pathophysiological alteration in atherogenesis. Pharmacological therapy with 3-hydroxy-3-methylglutaryl CoA reductase inhibitors (statins) has proved to be useful in the control of atherosclerotic disease, adding the benefits generated by their so-called pleiotropic effects to their hypolipemiant properties. These effects are based on their production of an increased bioavailability of nitric oxide at endothelial level, increasing eNOS activity.

Objectives: The objective of this study was to evaluate the effect of atorvastatin at high concentrations on the production of nitric oxide by human umbilical vein endothelial cells (HUVEC) cultured *in vitro*.

Material and Methods: HUVEC were cultured in M199 medium supplemented with VEGF and bovine foetal serum.

The HUVEC were treated with increasing micromolar concentrations of atorvastatin for 24 h: Group 1, 0.5 µM atorvastatin; Group 2, 1 µM atorvastatin; Group 3, 10 µM. atorvastatin; Control Group; Mortality Control (Triton-X).

We quantified levels of nitrites (directly related to the concentration of nitric oxide) in each experimental group.

We evaluated cell viability by using the Trypan Blue Exclusion Test.

Results and conclusions: The effect of atorvastatin treatment on the bioavailability of nitric oxide is clearly dose-dependent. The higher the concentrations of atorvastatin, the higher were the levels of nitric oxide produced by the endothelial cells. After 24 h of treatment, no significant reduction in cell viability (by trypan blue staining) was observed in comparison to the control group.

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AC-O-14 SOMATOCHART AND SYSTOLIC BLOOD PRESSURE

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From the classic age it has seen a relationship between anthropometric parameters and the predisposition to develop different pathologies. Nowadays this continues being an object of study.

In our case, we try to analyze the relationship between somatochart, which was built as the model described by Heath and Carter , and the systolic blood pressure, which was measured in the population of a Health Center in Alicante (Spain). It was a random selection, men and women aged between thirthy and sixty-five. We obtained weight, height, limb girths (waist, hip, arm flexed and tensed, calf), bone breaths (biepicondylar of the humerus, bistylod and biepicondylar of the femur). We took the arterial pressure during the same visit.

We built the somatochart of these patients with this data and compared it with the level of systolic blood pressure.

The results obtained showed that those patients whose level of systolic blood pressure was over one hundred and twenty milimeters of mercury, were in the area of the somathochart between X minor or equal -2 (X<= -2) and Y major or equal 2 (Y>= 2). They are endomorphic-mesomorph and mesomorph-endomorph. However, those patients whose systolic blood pressure was under one hundred milimeters of mercury, were in the area between X minor or equal 0 (X<=0) and Y minor or equal 2 (Y <=2). They are mesomorphic-endomorph and balanced endomorph.

AC-O-15 ANATOMICAL EVALUATION OF POTENTIAL RISK TO LEFT PHRENIC NERVE INJURY DURING EPICARDIAL OR ENDOCARDIAL CATHETER ABLATION

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Our study aimed to clarify the spatial relations between the left phrenic nerve (LPN) and key cardiac structures so as to decrease potential risks of phrenic nerve injury during interventions. We examined by gross dissection and histological sections the course and relationship of the left phrenic nerve in 22 human cadavers. Phrenic nerve arises from the fourth cervical ventral ramus, but also the ventral rami of the third and fifth cervical spinal nerves contribute to the formation of the nerve. It is formed at the upper part of the lateral border of scalenus anterior muscle and descends almost vertically across its anterior surface behind the prevertebral fascia. At the root of the neck, it runs anterior to the second part of the subclavian artery from which it is separated by the scalenus anterior muscle (on the left side, the nerve passes anterior to the first part of the subclavian artery). It descends to the thorax and supplies fibrous pericardium, parietal pleura (mediastinal and central part of the diaphragmatic) and diaphragm. When the left phrenic nerve descended on the fibrous pericardium along one of three courses: over the anterior surface of the left ventricle (18%), over the lateral margin of the left ventricle (59%), or postero-inferiorly (23%). The endocardium of the roof of the left atrial appendage was <4 mm from the LPN in 2 specimens (9%). The nerve passed <2.5 mm from the epicardium of the apex of the left atrial appendage in 7 specimens (31%). Regardless of the position of the nerve in relation to the high left ventricular wall, the nerve was <3 mm from the epicardial surface in 8 specimens (36%), and it passed close to the epicardium of the right ventricular outflow tract at <6 mm in 2 specimens (9%). During electrophysiologic interventions, the left phrenic nerve is especially at risk when procedures are deployed in the vicinity of the left atrial appendage and high left ventricular wall.

AC-O-16 PLACING OF THE NEEDLELESS® SLING SYSTEM FOR FEMALE INCONTINENCE IN FEMININE PELVIS

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Introduction. Urinary incontinence (UI) affects between a 11.1% and a 30% of women. It is defined by International Continence Society as the involuntary, objective and demonstrable leakage of urine that drives to a social, economical and hygienic problem for the person who suffers from it. 80% of patients affected of UI reject any treatment, and among those who decide to treat it, less than 1% are disposed to a surgery, despite the number of interventions is increasing progressively. From Burch colposuspension described in 1960, surgical techniques have evoluted to less invasive procedures with suburethral support as Needleless® Sling System for Female Incontinence.

Objective: new techniques and devices used in the treatment of UI are not complication-free. Because of it, the objective of this study is to determine the position of placing of the Needleless® Sling System for Female Incontinence and its relation to obturator nerve and vessels.

Method: 6 cryopreserved specimens were surgery and dissected to study the placing of the sling and the security distance to obturator nerve and vessels.

Results: to place the sling the three layers of vagina were perforated and in all the cases the central zone of the sling encloses the urethral cranial zone. In four of the pelvis, the sling was put up between visceral and parietal pelvic fascia. When comparing the distance between the sling endings and the obturator nerve and vessels, it was observed a mean of 27.8 mm (± 9.4) in the right side, while in the left side it was of 26.5 mm (± 8.8).

Conclusion: data collected suggest that fibrosis occurred secondary to the sling placing may help to urinary continence mechanisms.

AC-O-17 EXPERIMENTAL STUDY OF THE ACIDOSIS INHIBITION EFFECTS INDUCED BY THE STRENUOUS TRAINING ON THE BONE MASS

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Exercise is usually beneficial for the skeleton, although strenuous exercise may be harmfull. Many factors may cause osteopenia in subjects who practice high-performance sports. The best known are hypogonadism and the stress, changes in calciotropic hormone levels and diet, calcium loss in sweat and less studied factors, such as acidosis. We observed the effects of sodium bicarbonate supplement on bone mass in rats on strenuous treadmill training. Sixty female Wistar rats (93 days old; mean initial weight 261±16 g) were studied. One group of 15 rats was killed at the beginning of the experiments (basal control group), while another group of 15 rats was not manipulated (Exer-NaB-). Another group of 15 rats was exercised but did not receive sodium bicarbonate (Exer+NaB-), while the final group of 15 rats exercised and received sodium bicarbonate (Exer+NaB+) at the dose of 0.05 mg/kg/day, administered by esophageal catheter on exercise day. Those rats were sacrified at the end of 11 weeks. Femoral length, weight, and bone mineral content (BMC) and density (BMD) were measured. According to ANOVA with the Tukey-Kramer test, femur length and weight, femur BMC and BMD, and plasma bicarbonate were lower in the basal control and Exer+NaB- groups than in the two others groups ($p<0.005$-0.0001). Only in the Exer+NaB- group was there was a positive association between plasma bicarbonate levels and femur length ($r=0.78$; $p<0.0005$). Our study demonstrates the adverse effects of strenuous exercise on bone, and the usefulness of sodium bicarbonate supplements in preventing and minimized these effects.

AC-O-18 COMPARED RESEARCH IN BODY COMPOSITION BETWEEN PROFESSIONAL DANCERS FROM THE CENTRO NACIONAL DE DANZA DE ESPAÑA AND ELITE GYMNASTS

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Introduction: The dancers are considered as high performance athletes from a morphotype point of view. The question is how to classify these dancers in the diverse specific groups of highly trained sportman, whereas by the specific characteristics of dancing, they will be compared to elite gymnasts.

Material and Methods: The sample is formed by the permanent collective of professional dancers from the Centro Nacional de Danza, measured by the kinanthropometric technique established by the GREC/ISAK. Informed consent was obtained following the Ley Orgánica 15/1999 de 13 de diciembre de Protección de datos de Carácter Personal. Statistical analysis was performed by the software SPSS 11,5 using an ANOVA with a significance of 95% ($p<0,05$).

Results: Male dancers are 176,75cm. tall and 76,58Kg. weight. Fat percentage is 15,21% (obtained from skinfolds bicipital in mm.-5,66-, tricipital,-9,81-, subscapular -12,45 and supraspinal -12,49-), with a fat mass of 11,96Kg. and fat free mass of 64,62Kg. Female dancers are 161,61cm. tall, 58,10Kg. weight; 25,03% of adipose tissue (obtained from

skinfolds bicipital -7,96-, tricipital -14,82-, subescapular -12,33- y supraespinal -12,33-).

Discussion: Compared to elite gymnasts, male dancers are taller and heavier, with significant higher fat percentage (15,21 vs. 6,50), favored by significant differences in fat mass and skinfolds, whereas is striking the bigger muscular mass of dancers compared to athletes (64,62Kg. vs. 63,20Kg.). Female dancers are smaller and lighter (determined by their younger age) which express the significant differences in fat percentage, skinfolds and fat free mass.

Conclusions:

1) Elite gymnasts are very different from professional dancers

2) Dancers are more muscular than high performance gymnasts, with diminished skinfolds and fat percentagje and mass (consequence of lower age in women)

3) Though these obvious differences, the proportionality of the distinctive corporal components compose very similar morphotypes between both groups.

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AC-O-19 REGENERATIVE POTENTIAL OF MESENCHYMAL STEM CELLS IN PATIENTS WITH CHONDRAL INJURIES

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Despite recent advances in surgical and non surgical, treatment of cartilage injuries remains an insurmountable problem due to the intrinsic biology of cartilaginous tissue, which limits their self-renewal ability. The use of autologous chondrocyte transplantation or osteochondral grafting have been shown effective at clinical level. However, the main problem is the limited proliferative capacity of mature chondrocytes. In contrast to adult chondrocytes, mesenchymal progenitor stem cells (MSCs) have a high capacity for self-renewal and differentiation into multiple cell types. To use MSCs in cartilage repair is necessary to develop efficient and well-defined protocols that allow direct differentiation of these stem cells into chondrogenic lineage both *in vitro* and *in vivo*.

In our experiments, we used osteochondral cylinders and cartilage samples obtained from patients with knee osteoarthritis who were operated for prosthetic replacement. After that we isolated and cultured chondrocytes by mechanical dissection and enzyme digestion. On the other hand, we isolated, characterized and cultured MSCs from aspirated fat from subjects undergoing liposuction. Then, we used several methods to induce chondrocytic differentiation, including culture with selective media, bi- or tri-dimensional MSCs-chondrocyte co-culture and scaffold. Moreover, we induced differentiation by reversibly permeabilizing MSC and exposing them to chondrocyte extracts. After three weeks, we analyzed the degree of chondrocytic differentiation using flow cytometry, immunocytochemistry, histological techniques and transmission electron microscopy.

Our results show the ability of MSCs obtained from lipoaspirates to be differentiated into chondrocytes by factors released into the culture medium from chondrocytes obtained from cartilage samples of patients. We found changes in the patterns of expression of MSCs surface markers that were time-dependent. Similarly, we show that three-dimensional pellets of MSCs-chondrocytes also resulted in the production of extracellular matrix. These data were confirmed by transmission electron microscopy and inmunoflorescence, where we observed the production and secretion of collagen II in the cells that acquired the differentiated phenotype.

Our results support and suggest a safe and useful method for obtaining a large quantity of cells differentiated to chondrocytes, which may be useful in patients affect from degenerative chondral processes.

AC-O-20 IN VITRO REPAIR OF CHONDRAL DEFECTS WITH MESENCHYMAL STEM CELLS CD271+

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Introduction: Population of mesenchymal stem cells (MSC) is formed by subsets which have different capacity of cellular differentiation. This *in vitro* study compares the capacity to repair chondral defects between the subsets of MSC CD271+ *versus* MSC CD271-.

Material and Methods: MSC were isolated from bone marrow of osteoarthritic patients (mean age: 64 years). These cells were characterized by flow cytometry for hematopoietic (CD34 and CD45) and mesenchymal (CD44, CD73, CD90, CD105 and CD166) markers, and its differentiation potential (adipogenesis, osteogenesis and chondrogenesis) was determined. The MSC were magnetically separated (MACS® *Separation Columns* LS, Miltenyi-Biotec) in two groups: CD271+ and CD271-. Implants of MSC CD271+ or MSC CD271- in focal defects of articular cartilage (2 mm of diameter) were cultured in

chondrogenic differentiation medium supplemented with recombinant human transforming growth factor-beta3 (rHuTGF-beta3) during 8 weeks. The repaired tissue were analyzed by histochemical (hematoxilin-eosin and safranin O) and immunohistochemical (types I and II collagens) methods. A semiquantitative histologic scoring system was used to evaluated the repaired tissue.

Results: MSC resulted negative for CD34 and CD45, and positive for CD44, CD73, CD90, CD105 and CD166. Furthermore, they showed capacity for chondrogenic, osteogenic and adipogenic differentiation. In both groups of implants, the repair tissue showed morphology like articular cartilage (extracellular matrix with a hyaline aspect and numerous lacunae containing cells of rounded shape) and the expression of types I and II collagens. However, both groups showed differences: the chondral defect filling with repaired tissue was lower in the MSC CD271- implants (32.2 ± 9.7%) than in those with MSC CD271+ (59.9 ± 16.6%); safranin O stain of the repaired tissue was negative in the CMM CD271- implants and moderate positive in MSC CD271+ implants; and the integration between the repaired tissue and the adjacent normal cartilage resulted of higher quality in the MSC CD271+ implants. Total score was 9.5 ± 0.89 and 12.19 ± 1.01 for implants CD271- and implants CD271+ respectively.

Conclusion: Our results indicate that the MSC CD271+ subset brings a chondral repair of higher quality than the subset CD271-.

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AC-O-21 BILATERAL VARIATIONS OF THE STYLOHYOID FORMATIONS

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The demominated stylohyoid apparatus (Arnould et al., 1969), also called stylohyoid chain (Stafne and Hollinshead, 1968) consists of the styloid process, stylohyoid ligament, and the hyoid lower horn (Stafne and Hollinshead, 1968). According to Ommell et al., 1998, the stylohyoid chain in humans includes the styloid process, stylohyoid ligament and the hyoid bone, which derive from three pairs of embryological cartilage: the epihial, the ceratohial, the hipohial and a middle cartilage, the basihial. The last one when joining with the cartilages of the third arch will form the body of the hyoid bone.

The stylohyoid formations, a more precise terminology for us, constitute an embryological unit because the containing embryological structures derived from the second branchial arch. The Reichert's cartilage concept, hitherto regarded as a continuous structure (Hamilton-Mossman, 1975; Sperber, 1989, Sadler, 1996; O'Rahilly and Müller, 1996, Moore and Persaud, 1999), has recently been amended by Rodríguez-Vázquez et al (2006). Based on this development model, an explanation is given about the variation of the stylohyoid apparatus in a cadaver during a programmed dissection practice. We analyzed the relationship of this variation, especially with the glossopharyngeal nerve because some neuropathies and cervical pathologies hitherto considered as idiopathic, may be based in these variations.

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D-O-01 – D-O-015 Teaching in Anatomy

D-O-01 ARE ASSIGNMENTS UNDER ACADEMIC SUPERVISION AN USEFUL METHOD TO LEARN MORE THAN IN A TRADITIONAL CLASS?

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The aim of this work is to present second course of Medicine professors and students' opinions. During the first four-month period of the academic year, all of them participated in an educational experience in order to help the approach to the European Convergence process.

This experience involved the execution of an assignment under academic supervision (AUAS) coordinated between different second course subjects (Anatomy, Physiology, Histology, Genetics, Epidemiology, Cronobiology...). All the students developed the same work based on a clinical case. The students were divided into different work teams, each one supervised by a tutor. The role of the tutor was to advise the students, to solve the doubts about each matter, to supervise the composition of the text, the structure of its presentation and, finally, the oral presentation.

The purpose of this experience was to strengthen team work, to encourage self-learning, to make integration of knowledge easier and to improve students' oral and written expression.

The public oral exhibition was evaluated by a work tutors committee. Later everyone answered different questions raised in a survey. With this questionnaire we expect to know the opinion of both groups related to the project's coordination, the tutor's role, the educational methodology used and the satisfaction level of this experience. Suggestions were also collected to improve it.

Professors' opinions show high satisfaction levels related to the raised objectives. Students don't agree with the experience, they think that it's useless, consider that they have lost the time and that they haven't learnt more than with their traditional lessons. They don't want to repeat it neither with other subjects nor in other courses.

D-O-02 ANATOMY STUDENT'S ATTITUDES, BELIEFS AND REACTIONS TO THE DISSECTION ROOM, THE OVERALL EXPERIENCE OF DISSECTION

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One of the first situations in which a future health professional has to face death is in the Dissection Room (DR), where the experience may be made into much more than a tool for technical learning.

In this study we set ourselves the following aims: 1. to discover the emotional reactions, attitudes and beliefs of new students faced with human cadaver dissection. 2. To evaluate the changes produced in these variables by the exhibition and practice of dissection.

Participants were 425 students, all enrolled for the first time in the subject of Human Anatomy (79.6% women and 20.4% men), with a mean age of 18.61(SD=2.13).

The two new instruments designed for this study were administrated in the first and last session of the compulsory practical dissection sessions of the course. In both sessions two points of measurement are established, immediately before and immediately after the dissection session. The questionnaires designed included aspects related to the DR, Perception of preparedness, emotional control and related thoughts during the dissection and responses related to the cadaver.

The results showed that the attitudes and beliefs shown by the students before dissection change as the students gain more experience. Thus, among other things, there is a drop in the number of students stating that they have to make an effort to control their feelings and emotions so as not to interfere with the task of dissection. Practice gives them control over their emotions and increases their concentration on the task, helping them to face up to the dissection.

Conclusions: the observation that the practice of dissection with human cadavers may play a decisive role in the doctor-patient relationship significantly reactivates the role of dissection in undergraduate studies, where the teacher should combine the teaching of professional anatomy with reflexive anatomy.

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D-O-03 DIFFERENCES IN LEARNING STYLES BETWEEN STUDENTS OF THE FIRST AND LAST YEARS IN NURSING SCHOOL (BY CHAEA)

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Background: Bologna process brings numerous changes in High Education in Europe. Every change should be based on a deep knowledge of the learning method of students. This paper aims to identify the prevalent learning style among Nursing students at our institution (School of Nursing, University of the Basque Country) and the evolution from first to last year students.

Material y methods: A study was performed in the Nursing School during the academic year 2008-2009, in a sample comprising 200 students. belonging to 1st and 3rd years. The instrument used was the Honey-Alonso (CHAEA) learning style questionnaire. Data analysis was based on the contrasting of the differences between the scores in the four learning styles, through the ANOVA test and the comparison between years.

Results: For first year students, although quantification for all styles showed middle values, the leading style is the reflexive, with a mean value of 12.5. Theoretical style showed also values slightly higher than middle ones (mean 12). In contrast, pragmatic style is slightly lower than middle value (9.2). Active style is the only is 10. Same behaviour was obtained from last year students, although all values were significantly lower. Thus, reflexive style has a value of 11, theoretical and active 9 and finally pragmatic 78.

Conclusion: Learning styles in our teaching institution show a preponderance of reflexive style over active, that should be taken into account towards the new Grade title in order to enhance self-learning skills. All values suffer a diminishing from 1st to 3rd school year that nevertheless is close from middle values.

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D-O-04 EVOLUTION ON THE USE OF THE PREFERRED CHANNEL OF INFORMATION UPTAKE IN STUDENS OF THE NURSING SCHOOLS (BY VAK)

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Background: Among the different ways to uptake information the main ones are the visual, auditive and kinesthetic channels. These channels play a pivotal role in learning processes as people use preferentially one of them to acquire new knowledge. The identification of this point may be relevant to facilitate teaching-learning tasks related to the Bologna process. The aim of this study was to identify the evolution of the preferred learning channel among students from the first to advanced course in Nursing School of the Basque Country University (UBC).

Material and Methods: The study was performed during the academic year 2008-2009 in a sample of 218 students, 143 of the first course and 75 of the third one. The instrument used for the study was the "VISUAL, AUDITIVE AND KINESTHETIC" (VAK) inventory adapted by M.E. Romo & D. Lopez. This inventory was composed by 45 items from which 15 items, selected for each channel, were valued from 0 to 2. Data analysis was based on the contrasting of the differences between the scores in the three channels through the ANOVA test.

Results: Both student groups showed a homogeneous distribution of the sample in the three preferences. In both courses, the mean values for visual and auditive learning channel were around 19 and 16 for kinesthetic. The comparative analysis showed a preference for the visual channel respect to the others. This point indicates the high receptive capacity of the students.

Conclusions: According to our data, the students of nursing school do no vary their learning method along the degree. During the degree course they keep a passive attitude using a visual and auditive learning style instead of a kinesthetic. Therefore, for the Bologna process the student passivity should be changed. For this purpose, the teaching-learning tasks have to give priority to active participation of students.

Acknowledgements: This work has been supported by PCI Program 09 (AECI-MAEC) and PIE 09 (UBC).

D-O-05 AN ASSESSMENT MODEL BASED ON A MULTIPLE CHOICE EXAMINATION

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Assessment has culturally been considered as an administrative process, through which the sufficiency of an academic period can be obtained.

Current legislation has developed extensive regulations on assessment, although these are focused on basic and secondary education.

We understand assessment as being part of the educational process, with different types: initial, ongoing, final, etc. In turn there are different assessment techniques: written, oral, multiple choice, etc.

On many occasions we have been involved in assessment disputes when it has been understood that we are not using a procedure which is sufficiently objective. Apparently the most objective method is a multiple choice examination. For many students this type of examination presents important psychosocial incongruities, given the fact that an incorrect response can lead to points being deducted.

We propose modifying the multiple choice model by offering students the possibility of choosing an option which does not deduct points but which prevents them from achieving the highest score.

This model is currently being tested and is achieving high levels of satisfaction amongst students.

D-O-06 DECISION MAKING IN THE TEACHING OF ANATOMY

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Over the last 30 years Anatomy has passed from being four annual subjects with daily classes to two subjects with classes on alternating days. This has forced us into making important decisions with regards to its content. Current directives aimed at building the European Higher Education Area propose a more radical approach with regards to Anatomy. When drawing up the new study programme we need to consider the "management chain":

1. The State Administration must establish the legislative framework; 2. The legislative framework is developed by the Regional Administrations; 3. The Universities are responsible for defining and applying the educational model; 4. The Faculties must implement the programmes and oversee their teaching; 5. The departments coordinate the teaching programmes; 6. Finally teachers and students are jointly responsible for the teaching/learning process. Teachers must be responsible for drawing up the teaching programme for our teaching activity. In order to fulfil this function, these six variables must be taken into account: 1. Legal regulations; 2. Epistemological factors (Anatomy is a descriptive and instrumental subject); 3. Students with prior training; 4. Our educational capacity as teachers; 5. Resources provided by the institution; 6. Sociological factors (current moment of the programme).

A unique programme or a programme of minimum content could be proposed, although this may affect the variables listed above. Finally we believe that it is necessary to establish an ongoing training programme for Anatomy, in which teaching professionals will be trained with regards to cross skills.

D-O-07 POPULARIZATION OF SCIENCE AS A TOOL FOR TEACHING ANATOMY. JOURNEY THROUGH THE HUMAN BODY AT THE GRANADA SCIENCE PARK

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One of the most requested themes among visitors to science museums refers to the knowledge of the human body. Therefore, at present, there is a need for a very particular anatomical teaching. This is commonly done at universities with health science departments, where students are highly motivated. On the other hand, for the general public of all ages and levels of knowledge, to learn the insides of a heart and how does it work, which bones support their bodies, how do nerve cells transmit the impulses, how do nephrons filter, etc.. is an exciting challenge and a growing need.

For this reason, the Granada Science Park in southern Spain (www.parqueciencias.com) has opened a spectacular exhibition: **Journey Through the Human Body**. With nearly a hundred exhibits (1879m^2) and offering interactive experiences, objects and historical items, simulations, virtual reality, computer tools, models, real items, preserved and plastinated human and animal bodies or body parts, scenography, illustrations, photographs, videos ... takes the visitor to a fascinating journey through the human body. We therefore cover all organs and systems of the body, beginning with the evolution of life and using comparative anatomy continuously to demonstrate common origins.

The exhibition also focuses on the history of anatomical science through anatomical objects and materials, and holds a plastination laboratory visible to all visitors, for the first time available in a Science Museum.

This exhibition has achieved major scientific support by Spanish medical societies, including the Spanish Anatomical Society, other official organizations and related industries. From now on there is an important work of revitalization mainly through agreements with all of them. Finally, the response from visitors, in its few months of life, has been absolutely overwhelming.

D-O-08 APPLIED ANATOMY: FROM DESCRIPTION TO FUNCTION AND TO PALPATION FOLLOWING STUDENT CENTERED ANATOMY TEACHING (SCAT)

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Student centered anatomy teaching (SCAT) aims to improve the study of clinical and functional anatomy. It is based not only on lectures in which the teacher explains the contents, but also includes observation and manipulation of artificial anatomic models and real speciments. As a result of the Bologna Declaration and the introduction of the European Credit-Transfer System, there is a pressing need to redesign the objectives of university-student education as well as the methodology applied in obtaining such objectives. The SCAT methodology includes:

Bone collection: learning guides with bone, muscular and visceral models

Problem Based Learning (PBL)

Dissection laboratory: dissection guides, by objectives

Multimedia tools: projections of interventions relating surgical actions with the surgical anatomy

To complement these activities, new docent materials are being created and published. The manuals start with self assessment of the student's knowledge topic. Following topics are distributed by levels of difficulty (basic, medium

and difficult), which allow students with any level of knowledge to find significant information, from students that are getting the study of bone and muscular systems, to professionals who need a reference manual to find the description of a muscle (with origin, insertion, action, innervation, vascularization) or how to do its palpation. In addition, includes the description of the most important anatomical regions, as well as sectional anatomy. So as to create a useful tool for any student and professional of health science, we have worked on specific and general objectives from various degree programs (medicine, dentistry, physical therapy, podiatry and nursing), which are already using these books. The last contribution to the SCAT methodology is the "Myology manual. Description, function and palpation of head and trunk".

D-O-09 TEACHING ANATOMY: POSITIVE SYNERGY BETWEEN REFLECTIVE AND B-LEARNING

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According to the new paradigm of the European Higher Education Area, where teachers guide the student to acquire knowledge, we propose a reflective and blended (b)-learning (RBL) for a human anatomy subject.

The aim of this study was to compare the academic results of students of the second year of medicine degree, when the classic method (lectures and practice. 226 students of the 2005-06 course) or the RBL (virtual support and practice; 371 students of the 2007-08 and 2008-09 courses) was applied to the pilot theme (peritoneal cavity) teaching. At the practice exam of the "Human Anatomy: cardiovascular and splancnology", students had to identify structures that were previously marked, except to pilot practice where the location of abdominal organs and spaces was asked. Before the practice session the RBL group had access to a dossier published "ad hoc" (virtual campus) and had to answer a questionnaire to evaluate their preparation. In contrast, the classical group received lectures of the topic. Both groups performed the same practice with the assistance of the same professor: two corpses for five students; a guide (more complete in the RBL group) with reflective exercises to be performed on the bodies and the same tutor.

The results were better in the RBL group than in the classical group: 20.83% more of students obtained the first class grade and the grade average was 10.65% higher. This fact cannot be attributed to differences between the academic levels or to the degree of previous preparation to the practice: very similar global academic achievement in both groups; no correlation between the grades of the pilot practice and that of a previous questionnaire. Therefore we can assume that the demonstrated improvement it was due of a higher formative performance of the practical session, probably linked to an improvement of the practice guide.

In addition, the results in the pilot practice were better than in the rest of the practices, especially in RBL group. These differences seem to be due to the reflective character of the exercises in the practice pilot.

Conclusion: The combination of reflective and b-learning can improve academic outcomes.

D-O-10 TEACHING INNOVATIONS IN THE STUDY OF HUMAN ANATOMY

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In our Department of Human Anatomy and Embryology http://www.ugr.es/~cmorfolo/, besides using classical dissection, e.g., http://www.ugr.es/~mlsoler/ DISEMANO.pdf, to teach Human Anatomy, we are also applying new approaches such as plastination http://www.ugr.es/~mlsoler/Practicas%20Corazon%202008_2009.pdf. This technique, in combination with plastic models, facilitates the acquisition of knowledge by students. We are also using three-dimensional (hypermedia) models, showing students *via* a computer the volumes that are not available in a conventional atlas: http://www.ugr.es/~mlsoler/ Programa%20bbaa.htm and http://www.ugr.es/~mlsoler/ memoria_proyecto_organo_visual_ 2008_2009.pdf.

D-O-11 3-D MODEL FOR STUDY OF THE VISUAL ORGAN

López-Soler, M.*[1], Campos-López, R.[2], Roda-Murillo, O.[1], Guirao Piñeyro M.[1], Pascual-Morenilla, M.T.[1], Sánchez-Montesinos, I.[1]

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We present a three-dimensional model of the eyeball and its annexes that allows students to examine, *via* a computer, the morphology and structure and dynamics of the visual organ in three dimensions as a basis for their subsequent study of ocular diseases:

http://www.ugr.es/~mlsoler/memoria_proyecto_organo_visual_2008_2009.pdf.

This three-dimensional (hypermedia) model shows the student: the elements that form the eye-socket; the layers that make up the eyeball, in colours and textures that match reality as closely as possible; the transparent media of the eye; and the muscles and their functionality. This represents a highly useful learning tool that the student can use at any time.

D-O-12 INNOVATION IN ANATOMY TEACHING: DEVELOPMENT OF 3D INTERACTIVE ANATOMIC ATLAS

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The incorporation of new technologies of communication and information in all areas of society is a fact. Of course, in professional and education has also been incorporated.

Every time we see that there is more Internet accessible contents and does not depend on the technology platform being used. Technologies are those that would have an innovation character.

In the context of medicine and the university, the anatomy is a subject of study and continuous updating on the part of students and professionals. Historically, there are major anatomical atlas very important (not without some errors and confusing graphics on the use of anatomical a payroll), but the advance of new technology means a constant challenge for many to be integrated into the processes of acquiring knowledge.

This challenge is also present and we have assumed a first stage where we can see that technology allows us to establish a project of this magnitude by providing two important features over other existing: –Interactivity, allowing to isolate an element of the anatomical rest to observe their morpholog. –Identification by the International Anatomical Code, all the anatomical representation and viewable through Visor3D are identified by the IAC (International Anatomical Code), with a bi-univocal relationship between each element and its code.

It is irrefutable that the best way of acquiring knowledge in anatomy is to work directly with corpses. Unfortunately there are not enough for everybody, some universities fail to perform a dissection during the first two years of the race. Anatomical study is based on the actual use of material (mass graves) of the simulated material (plastic models) and other material normally provided by teachers (photos, videos, slides, etc.).

Using multimedia and 3D design, modeling is about 1000 different anatomy units, including bones, tendons, ligaments, muscles, fascias, arterial and venous vessels, nerves, and so on. until the Locomotive (osteo-muscular, neurovascular and peripheral osteoarticular) incorporating some new features as: –inclusion of the international anatomical code –including the payroll of each anatomical unit –anatomical description of each element (bones, muscles and joints)

This can be viewed through a Visor3D that allows us to access from a server via the Internet, where the bank of images that correspond to the atlas.

The Visor3D incorporates several features that allow us to navigate through the represented anatomical structures and are:

Zoom, turns, translatory movements, reset, level of transparency of the elements and multiselectión of anatomical elements.

The Anatomy Atlas contains a wealth of anatomical information and we are trying to adapt to the agenda for the university teaching the first course of medicine. Divided into anatomical regions and addressing the Neurovascular and Musculoskeletal Systems.

D-O-13 ATLAS OF ANATOMY: SECTIONAL IMAGING

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This paper is a reflection of the experience acquired both as a university lecturer and a specialist doctor in the training of undergraduates, postgraduates and specialists in diagnostic medical imaging.

The paper attempts to provide an overall picture of anatomical imaging and looks at how such techniques can be applied to the study of the: thorax, abdomen, head and neck, locomotor apparatus, circulation and the nervous system. Each section includes images obtained using different techniques (conventional radiology, computer tomography, magnetic resonance and echography) and the paper finishes with a look at 3-dimensional imaging in the forms of virtual endoscopy and Volume Rendering; this latter part forms the central axis of the paper.

The Atlas of Anatomy in Cross–Sectional Images is an atlas in electronic format on DVD-ROM. Its images have been obtained from 530 individuals who visited our service during the period 2000-2007. Each section includes images obtained using some of the previously mentioned techniques, taking into account each technique's suitability for exploring the organ, apparatus and/or system in question, as well as its clinical relevance to the process of diagnosis.

This study includes 8783 images in which the most important elements have labelled in accordance with international terminology. Given the simultaneously 3-dimensional and cross-sectional nature of Volume Rendering, only the cross-sectional data has been labelled.

D-O-14 RADIOLOGIC AND TOPOGRAPHIC ANATOMY

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Human Anatomy has always been one of the core sciences in medical studies. If anatomical information is fundamental to Medical studies, then it becomes indispensable to the training of specialists in diagnostic medical imaging. This tenet has been reflected in developments at the URV

in recent decades. Throughout the degree course offered here, all Human Anatomy course contents include a corresponding section on Radiological Anatomy.

Diagnostic medical imaging techniques are conventional radiology, ultrasonography, helical and multiplane computer tomography and magnetic resonance. Each of these methods is described in terms of its relevance and usefulness to human anatomical study. Also described are the different ways in which CT and MR present information, namely: MPR, MIP, 3D, Volume Rendering and virtual endoscopy. The latter, that is virtual endoscopy, is a very important method for understanding cross-sectional anatomy because it allows us a perfect perspective of all bodily structures on different spatial planes. The fact that the cross-section can viewed 3-dimensionally makes it a particularly attractive and illuminating technique. Virtual endoscopy provides us with images which were completely unavailable to us until very recently.

This paper aims to describe the work that has been carried out in recent years since the current study plans have been in place. Training is generally given in all the subjects that deal with human anatomy and some of the techniques are given specific and in-depth attention in an optional second year subject in the current study plan.

D-O-15 LEARNING BASED ON AIMS: A MODEL FOR THE ADJUSTMENT OF THE HUMAN ANATOMY AND HISTOLOGY LEARNING IN THE CONTEXT OF THE EUROPEAN HIGHER EDUCATION SPACE

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Abstract: There appears the model designed for the integrated learning of the human anatomy and histology at the Universitat of Girona, in the first year of implementation of the studies of Degree of Medicine.

Aims and design: The model includes:

– The definition of Units of Learning based on Aims (UAOs) corresponding to the different systems.

– The definition of the Aims of Learning in every UAO, and the classification in descriptive, reasoning and integrators of knowledge aims.

– The design of the different Activities of Learning to facilitate the fulfillment of the aims. The activities include a few magistral lessons, seminars of discussion in small groups, laboratory practical activities (self-guided and tutorized), classroom practical lessons, tutorships, and learning integrated seminars to discuss and to resolve of a problem from a point of view of the anatomical and histological knowledge.

– The design of the Activities of Evaluation, which incorporate a written exam and an exam of practical skills in which the student must demonstrate competence in the recognition and comprehension of anatomical and histological structures, on cadáver and on histological preparations, as well as on images corresponding to the main explorations of diagnosis of the different systems.

The evaluation of transverse competences has been carried out by means of the presentation of works in group and discussion on before distributed topics, as well as by the accomplishment of a semistructured portfolio.

Results: The results of this experience in the first year of introduction of medicine studies, refered to the modules given during the course 2008/09, show a high percentage of students who present theyself in the first examination (94,5 %), of which 84 % (module 2: cardiovascular and esplacnology) and 95 % (module 3: locomotor system) they overcome the subject.

Other aspects considered positive of the used methodology have been the progressive implication of the student in his own learning process reflected in a decrease of the necessary time to reach certain aims if we compare it with a more traditional model. Likewise, the integration of the knowledge has been a constant in the different activities, fact that brings us over to the aim to ascend in the levels of the pyramid of the knowledge.

E-O-01 – E-O-04 Embryology

E-O-01 DEVELOPMENT OF THE POSTERIOR CAPSULE SEGMENT OF THE HUMAN TEMPOROMANDIBULAR JOINT (TMJ)

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The TMJ is a complex synovial joint, both in filogeny and development. This complexity explains the lack of descriptions of the posterior joint capsule. Nevertheless it is necessary its study to clarify the articular pathology. Our group has studied human specimens of the Complutense University Embryology Institute Collection ranging from 7 to 17 weeks of development. The posterior joint capsule has an upper sheet with an internal part that reach the tegmen tympani and through the tympanosquamous fissure will related with the lateral side of the Meckel´s cartilage. This last part will give arise to the discomalleolar ligament. The inferior capsule sheet fixed to mandibular condyle. At 13 weeks of development synovial plica appears in the superior and inferior of tmj spaces.

Our results show that the bilaminar zone of the disc (Rees, 1954) correspond to posterior segment of the tmj (Mérida et al. 2007).

E-O-02 DEVELOPMENT OF THE HUMAN LARYNGEAL VENTRICLES OF THE LARYNX

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Introduction: Two different theories have been elaborated to justify the origin of the laryngeal ventricles. One considers the laryngeal ventricles as vestigial structures derivated from the fifth laryngeal pouches and, the other theory considers them as structures completely independent from them. The aim of this study was to dilucidate both theories using a complete series of human embryos and feetuses.

Material and Methods: We used 32 embryos (from 3 to 25 mm) and 35 foetuses (from 32 to 110 mm) belonging to the collection of the Department of Human Anatomy & Embryology I, School of Medicine of the Complutense University of Madrid. Specimens were serially sectioned at a thickness of 10 microns in the standard planes and stained with routine techniques. A 3D digital reconstruction of the most significant specimens was made using the software Kheops Builder 2.3 ®.

Results: The 3rd and 4th pharyngeal pouches appeared connected with the lateral angles of the pharynx during stages 15th to 16th (33-37 days). In stage 17th (41 days) the connection between the derivates of the pharyngeal pouches and the pharynx were lost. In stage 18th (44 days) the primordia of the laryngeal ventricles appeared in the caudal end of the vestibular recess of the laryngeal cavity as lateral swellings.

Development of ventricles occurs during the later embryonic and early fetal periods by an outgrowth of the lateral wall of the vestibular recess.

Conclusion: The origin and development of the laryngeal ventricles is not related with the 5th pharyngeal pouches.

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E-O-03 THE ABSENCE OF SONIC HEDGEHOG PRODUCES SEVERE ABNORMALITIES IN THE DEVELOPING CEREBELLUM

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In the development of the central nervous system, there are key molecules known as morphogens that direct the specification and differentiation programs of the different neuronal populations. One of these morphogens is Sonic Hedgehog (Shh). The genetic cascade induced by this molecule controls the generation of all the ventral structures of the nervous system. Besides that main function it plays other crucial roles in specific regions of the brain. In the cerebellum, Shh is strongly expressed by the Purkinje cells during the formation of this structure. The central role of Shh in the development of the cerebellum is the control of the proliferation of the granule cells precursors. The Purkinje cells liberate this factor inducing a high proliferation rate of the precursors of the granule cells. To identify other possible functions of this molecule we generated a conditional mutant mouse. We deleted the expression of Shh in the cerebellum before the generation of the Purkinje cells. In a drastic reduced number we were able to obtain some conditional mutant at postnatal stages. They displayed a clear cerebellar ataxia with tremors and locomotor impairments. The resulting macroscopic morphology of this mutated structure was a high reduction in the relative size of the cerebellum. The histological analysis displayed a strong disorganization of the cerebellar layers. The molecular and granular layers were absent and the Purkinje cells were not displayed in a monolayer. However the deep nuclei of the cerebellum appeared unaffected, this result was expected due to the fact that these nuclei are generated early on. Their specification program is clearly independent of

the Shh cascade. Strikingly this phenotype was not homogeneous, the caudal portion of the cerebellum displayed a less severe aspect and the three layers could be identified. Other surprisingly result was the presence of a big mass of ectopic Purkinje cells in the surface of the inferior colliculus. These results point out to new roles of Shh in the development of the cerebellum.

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E-O-04 UNILATERAL ABSENCE OF THE TENDON OF THE STAPEDIUS MUSCLE. EMBRYOLOGIC CONSIDERATIONS

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In the right side of a human fetus of 14 weeks PCd, a unilateral absence of the tendon of the stapedius muscle was found. In the head of the stapes, no tendon was attached. Only the existence of the muscle belly was noted, being less developed than in specimens of the same stage. The location and arrangement were anomalous, since it was situated in front of the facial nerve instead of medially, as was normal. The belly of the stapedius muscle was prolonged caudally and laterally, crossing in front of the facial nerve and continued with a more condensed connective tissue as a pseudotendon. This pseudotendon ended in the zone where the prolongation of the otic capsule continued with the cranial end of Reichert's cartilage, at the level of what will be the caudal portion of the vertical part of the facial canal . This anomalous arrangement of the muscle was associated with the absence of the condensed mesenchymal tissue, found forming the anlage of the pyramidal eminence in specimens of the same stage. Our observations in relation to the development of the stapedius muscle (Rodríguez–Vazquez, 2005; Rodríguez–Vázquez et al. 2006) were found to be reinforced, explaining in a logical way the case presented in the present work. This unilateral right agenesia of the tendon of the stapedius muscle, with the presence of the belly of the muscle in an anomalous arrangement is described for the first time in a human fetus.

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N-O-01 – N-O-017 Neuroanatomy

N-O-01 DEVELOPMENT OF A 3D DTI-BASED ATLAS OF HUMAN BRAIN WHITE MATTER

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Diffusion Tensor Imaging (DTI) is a Magnetic Resonance Imaging (MRI) modality that can be used to study the spatial organization of the human white matter (WM) tracts *in vivo* when combined with the so-called fiber-tracking techniques. DTI has become progressively more relevant to everyday clinical practice in different neurological pathologies such as vascular, tumoral or degenerative diseases in which an accurate anatomic knowledge of the WM is needed.

The aim of the present project was to create an electronic atlas to analyze, in a virtual 3D environment, the spatial relationships of the main human brain WM tracts.

Ten consecutively recruited healthy adult volunteers without history of neurological disease were included in a preliminary study. The MR acquisitions were performed on a 1.5T MRI Philip system. A conventional DTI protocol was performed using an eco planar single-shot sequence. Fifteen directions (b= 1000) were used with a matrix = 112x112, FOV = 230 x 230 mm, slice thickness of 3 mm without interslice gaps for a total of 45 slices including the whole head. The atlas was prepared using an additional DTI high definition study (65 slices, thickness=2mm and 32 directions) coregistered with a 1 mm isometric T1-weigthed sequence. DTI and T1 image data volumes were transferred to an off-line workstation and processed with specific software (DTIStudio and DTIWeb, http://trueta.udg.edu/DTI) in order to reconstruct different fiber tracts and to generate a RGB directional colour map.

Our electronic atlas allowed the simultaneous visualization of: 1) a volume rendered model of the skull-striped brain from the T1-weighted MRI; 2) slices of the RGB colour-map in 3 orthogonal planes; and 3) the principal association, projection, and commissural WM tracts, including: superior longitudinal/arcuate, inferior longitudinal, inferior occipito-frontal, uncinate and corticospinal tracts as well as the "U" fibers, cingulum, optic radiations and corpus callosum.

The use of a multimodal and fiber tract-based atlas has proven to be a powerful tool to understand from a 3D point of view the brain WM architecture and is undoubtedly of great value in dealing with RGB colour maps interpretation.

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N-O-02 HOW BRAIN GLIOMA GROWTH INDUCES MICROVASCULAR ABERRATION?

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Glioma growth depends on microvascular adaptation and angiogenesis. Our study is focused on structural and functional changes that occur in the microvasculature during the glioma development.

Vascular morphology, morphometry and permeability studies are performed in endogenous gliomas induced in rats. Vascular permeability is showed in vivo by MR images and ex vivo by extravasation of intravital dyes. Tumour samples are histologically analyzed by H&E, LEA histochemistry and immunohistochemistry for GluT-1(glucose transporter-1), EBA (endothelial barrier specific antigen), occludin (tight junction protein), $VEGF_{165}$ (vascular endothelial growth factor) and eNOS (endothelial oxide nitric synthase) are also performed.

Microvascular network becomes aberrant as we move from low to high-grade glioma. Vessel density decreases, whereas the relative area occupied by the vascular network increases. The homogeneous angioarchitecture of low grade gliomas changes to anarchic one after the "angiogenic switch". Malignant gliomas show different vascular patterns, following a gradient from the neoangiogenic border to the hypoxic core. The *tumour core* contains scarce, huge, dilated vessels with profiles expressing GluT-1, $VEGF_{165}$ and eNOS. The *peripheral tumour tissue* shows light dilated vessels co-expressing EBA-GluT-1 and occludin, and on the *border with normal areas* displays glomeruloid vessels overexpressing VEGF and eNOS but lack of EBA and occludin positivity. Glucose uptake is maintained for some vascular endothelial sections in areas where BBB function is lost.

In conclusion, microvascular network becomes aberrant depending on the glioma malignancy process, undergoing a sequence of adaptive changes which involve the distribution and permeability of vessels. The sequential expression of VEGF and eNOS induced by intratumour hypoxia produces vasodilatation, increase of permeability and finally BBB breakdown in aberrant vessels. This explains the disturbances of blood flow and the oedema production.

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N-O-03 MIGHT PINEAL CLUSTERS BE THE SOURCE OF GLIAL PROLIFERATION IN THE COW PINEAL GLAND?

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In previous work we have observed the presence in the cow pineal gland of cavitary structures formed by cells with an epithelial aspect located in the deep portion of the gland; i.e., at subependymal level. When we used antisera raised against proteins considered to be markers of glial cells –such as GFAP, vimentin, and S-100– we observed that the walls of most of the clusters displayed immunoreactivity against those markers. As well as what we have proposed to date, apart from those used previously to perform the present study we used other markers able to demonstrate the existence of melatonin, β-3-tubulin and somatostatin S14 among others, using cows with ages between 1 and 7 years as experimental animals. The immunoreaction was visualized by the use of 3, 3'-diaminobenzidine or Fast Red. The results can be summarized thus:(a) The clusters were not localized only in the deep subependymal layer of the gland but were also seen at the periphery of the mid-portion of the gland and even at its apex; (b) intense reactivity for the three glial markers was seen in most of the cells delimiting the clusters. The glial cells of these clusters showed long cytoplasmic prolongations that continued with the rest of the pineal glia, forming a mosaic of cells and processes surrounding the pinealocytes and the pineal vessels. Additionally, the walls of the clusters seemed to give off numerous glial cells that were confounded with the rest of the pineal cell population; (c) the cells forming some of the clusters displayed immunoreaction for melatonin, β-3-tubulin and somatostatin. The somatostatin positive clusters were located in the medial portions of the gland. The concurrence of gliosis and of numerous clusters composed of glial cells suggests that such structures could be responsible for the glial proliferation observed in the cow pineal gland. Moreover, the existence of clusters of pinealocytes or of somatostatin suggests that they would essentially be pluripotent structures whose cells are able to express different proteins.

N-O-04 CYTOCHEMICAL, IMMUNOCYTOCHEMICAL AND ULTRASTRUCTURAL STUDY OF THE INTERGLIAL SPACES. ROUTE FOLLOWED BY MELATONIN FROM THE PINEALOCYTES TO THE THIRD VENTRICLE

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The application of histochemical (Alcian blue-PAS, phosphotungstic acid and Mallory-Azan stain) and immunocytochemical (for differentiation between cells of the glial lineage and melatonin-secreting pinealocytes) techniques and others using transmission electron microscopy allow different regions of the cow pineal gland to be distinguished on the basis of the cells and/or fibres that integrate each of them. Briefly, our observations included the following: a) accumulations of pinealocytes separated by the somata of glial cells and their prolongations, which formed a true network with a trabecular aspect (interglial spaces), which from the apex ended by connecting themselves with the reticular space. b)This latter was seen to be formed by some myelin fibres coming from the habenular commissures or the posterior white commissure next to glial cells and some isolated pinealocytes and some prolongations of cells with a tanycyte-like aspect. These latter projected backwards from the ependymal epithelium, ending around the vessels or crossing the reticular space, which was separated from the third ventricle by the epithelium of the pineal recess. c) Although this epithelium was organized in highly different ways in each of its portions, a striking observation was the fact that in the zones between its lateral portions the epithelium was extremely thin and its cells were very flat. d) Whereas in the perivascular spaces there were abundant connective tissue and collagen fibres, together with somata and glial prolongations, in the interglial spaces the vessels were very sparse and thin and we failed to observe the presence of connective tissue or collagen, although mast cells, plasma cells and cells with a phagocyte-like aspect were observed. The existence of interglial spaces and their connection with the reticular structure suggest that the melatonin secreted by pinealocytes finds a very easy route to reach the epithelium of the pineal recess and from there the third ventricle. This could account for the extraordinary concentration of melatonin present in the cerebrospinal fluid.

N-O-05 METHODOLOGICAL TOOLS FOR RESEARCH IN MULTIPLE SCLEROSIS

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Multiple sclerosis (MS) is the major cause of disability in young adults. The design of protocols which simulate this disorder is essential for the search of a therapy. In the experimental autoimmune encephalomyelitis (EAE), a model of MS, an induced attack against the myelin causes functional and histological alterations.

Glutamate and adenosine tri-phosphate (ATP) are excitatory neurotransmiters and oligodendrocytes are vulnerable to over-stimulation of their receptors.

Objectives: To design a methodological approach to evaluate the level of demyelination in mice with EAE.

To evaluate the ability of ATP receptor blockers, in particular those of P2X7 receptors which are highly expressed in oligodendrocytes, to ameliorate EAE.

To examine if the severity of EAE is attenuated in knockout mice lacking the glutamate receptor subunit GluR6, also abundant in oligodendrocytes.

Materials and Methods: Chronic EAE was induced in C57BL/6 mice by immunization with myelin oligodendrocyte glycoprotein. EAE symptoms were maximal at three weeks post-immunization and then treated with P2X7R antagonists. In addition, mice lacking GluR6 were also subjected to EAE.

Functional status was determined by evaluation of the motor behavior and measurement of the conduction velocity of the corticospinal fibers. After recording, mice were fixed and the spinal cord extracted and processed for histology.

Results: Administration of P2X7R antagonists caused a significant improvement of the neurological score in EAE mice. The latency of the pyramidal fibers in mice with EAE is clearly increased as compared to control mice. In animals treated with P2X7R antagonists the latency returned to values near controls. Histological examination showed demyelination in the spinal cord of EAE mice treated with placebo, which was reduced in EAE mice treated with P2X7 antagonists.

EAE symptoms and the latency of the corticospinal cord were reduced in knockout mice lacking GluR6. In turn, the number of inflammatory foci was also reduced in these mutants.

We have developed a procedure using the EAE model to evaluate the efficacy of antagonists of glutamate and ATP receptors as potential therapeutic drugs to treat MS. The results show that blocking P2X7 and GluR6-containing receptors may prove useful to treat this disease.

N-O-06 A MORPHOLOGICAL APPROACH TO GLUTAMATERGIC AND PURINERGIC SIGNALLING IN HUMAN OPTIC NERVE OLIGODENDROCYTES

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In addition to neurons, glial cells express functional glutamate receptors and transporters. In particular, white matter oligodendrocytes express mainly ionotropic glutamate receptors of the alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) and kainate types, so they are sensitive to glutamate excitotoxicity caused by overstimulation of these receptors. Therefore, glutamate uptake from the extracellular space by glutamate transporters is essential to prevent excitotoxicity. Oligodendrocytes contribute to glutamate homeostasis in white matter because they express the main glutamate transporters: excitatory amino acid transporter 1, 2 and 3 (EAAT1-3). Like glutamate, extracellular ATP is a major excitatory neurotransmitter in the central nervous system (CNS) and in the same way, a sustained activation of ATP-gated ionotropic (P2X) receptors, those containing the $P2X_7$ subunit, also causes oligodendroglial excitoxicity.

Recent studies have shown that glutamate and ATP excitotoxicity may be components in the etiology of multiple sclerosis (MS), in which oligodendrocyte death is a hallmark. Since these studies were carried out in experimental animals, an important question is whether human oligodendrocytes also express the above related receptors and transporters. Here we have addressed this issue by an immunohistochemical study on autoptic samples of human optic nerve provided by the Netherlands Brain Bank.

Human optic nerve oligodendrocytes express all the AMPA and kainate receptor subunits studied, suggesting that human oligodendrocytes, as their animal counterparts, might be vulnerable to a glutamatergic signalling deregulation. These cells also express EAAT1, the main glutamate transporter expressed by experimental animal oligodendrocytes. Interestingly, the expression of EAAT1 appears to be more intense in MS optic nerve samples, suggesting that this enhanced expression could be a regulatory response of oligodendrocytes to toxic levels of glutamate present in MS. Finally, $P2X_7$ receptor subunit was also located in oligodendrocytes both in samples of optic nerves from controls and from MS patients. In the latter, cells of the microglial lineage also showed $P2X_7$ immunolabelling. Therefore, signalling through this receptor might affect direct or indirectly the oligodendroglial survival in MS.

In summary, human oligodendrocytes participate in glutamate and ATP signalling both in physiological and pathological conditions as MS, although their significance remains to be elucidated.

N-O-07 $P2X_4$ PURINERGIC RECEPTOR IMMUNOSTAINING REVEALS SPECIFIC NEURONAL DEGENERATION IN TRANSGENIC RODENT MODELS OF AMYOTROPHIC LATERAL SCLEROSIS

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We have described the distribution of P2X family of ATP receptors in $SOD1^{G93A}$ rat and mouse models of amyotrophic lateral sclerosis (ALS). We observed that strong $P2X_4$ immunoreactivity was selectively associated with degenerating motoneurons in spinal ventral horn. Degenerating $P2X_4$-positive motoneurons did not display apoptotic features such as chromatin condensation, positive TUNEL reaction or active caspase 3 immunostaining. In contrast, these neurons showed other signs of abnormality, such as loss of the neuronal marker NeuN and recruitment of microglial cells with neuronophagic activity. Similar changes were observed in affected motoneurons of the cerebral cortex and brainstem in $mSOD1^{G93A}$ both in rat and mouse. Degenerating $P2X_4$ cranial motoneurons were observed in the facial, ambiguous and trigeminal but not in the oculomotor nuclei. In addition, the exquisite ability of $P2X_4$ antibody to detect neuronal degeneration allows the identification of neuronal damage in brain regions not usually considered as affected in transgenic animal models of ALS. These include: neurons in the locus coeruleus, reticular formation, and Purkinje cells of the cerebellar cortex. After western blot analysis, it was found, that, in addition to $P2X_4$ ATP receptor protein, $P2X_4$ antibodies recognize a hitherto unidentified low MW band which is seen in cytosolic extracts from SOD^{G93A} but not in Wild Type samples.

After MALDI-TOF, we found that the low MW, $P2X_4$-immunoreactive protein band seen in the cytosolic fraction of transgenic animals was SOD1. After several biochemical studies we deduced that P2X4 antibody recognizes a form of misfolded mutant SOD1 which is only expressed in neuronal cells undergoing degeneration.

N-O-08 OTOPROTECTION OF DEXAMETHASONE, MELATONIN AND TACROLIMUS BY ROUND WINDOW LOCAL DELIVERY IN GENTAMICIN-DAMAGED COCHLEAE

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Hair cells of the inner ear undergo apoptosis after treatment with aminoglycoside, thereby inducing hearing loss. This study aims to determine the protective efficacy of three compounds that act at different points in the hair cell death cascade and which are currently being used clinically on gentamicin (GM)-damaged cochlea. We reported in a previous study the otoprotective effect of dexamethasone (DXM), melatonin (MLT) and tacrolimus (TCR) when administered systemically, and in this study compounds are now delivered locally by the help of mini-osmotic pump (MP) or gelfoam. Five groups of male Wistar rats were used, each MB was implanted with a catheter that releases its contents for 7 days in the round window (RW) of the cochlea of the ear, while in the right ear a gealfoam with compounds was applied. The groups were: saline (blank), gentamicin (GM), GM + DXM, GM + MLT, GM + TCR. The time-stability of compounds into the MB was checked by HPLC. Distortion Products of Otoacoustic Emissions and Auditory brainstem response were determined at 10, 15 and 20 days after the surgery. Cochlear morphological studies were carried out as well. The auditory functionality and morphological studies show that all three compounds exhibited otoprotective properties when delivered locally into the RW either MB or gel foam. This means an advantage in reducing DXM and TCR side effects by systemic administration. Taken together, our study confirms that in cochleae exposed to GM there is an increase in the production of ROS that leads to an inflammatory response and that the MAPK pathway activation is related and that the compounds in study were able to preserve the auditory function and hair cells of the Organ of Corti.

N-O-09 IS THE RAT RECURRENT LARYNGEAL NERVE AN EXCLUSIVELY MOTOR NERVE?

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In order to add knowledge to favour the laryngeal orthopic transplants, our group is developing a rat laryngeal model to study the proccesses of section/reinnervation of the laryngeal nerves. The present study is devoted to identify the real origin of the laryngeal nerves.The classical statement of the neurophysiology of the larynx establish that the laryngeal nerves, superior (SLN) and inferior or recurrent laryngeal nerve (RLN) are mixed. However, recent studies based on central projection of them in the brain stem seems to show that the RLN is only motor. Therefore the aim of the present study is to study the nuclei of real origin of the fibres convey by the laryngeal nerves of the rat using biotinylated dextrane amines (BDA), a powerful neural tracer.

The study was developed in 32 adult male Sprague-Dawley rats, applying BDA and TRD into the SLN, RLN or both. The size and the shape of the motoneurons were studied by means of a computerized morphometric analysis called Visilog 5.4.

Our results showed that the rat RLN does not contain afferent fibres, whereas its efferent fibres were originated within the ipsilateral nucleus ambiguus (NA). However, the SLN carries efferent fibres originated within the ipsilateral nucleus ambiguous (NA) and dorsal nucleus of the vagus (DNV), and its afferent fibres reach the tractus solitari (TS) and the nucleus tractus solitari (NTS).

In the rat the RLN convey exclusively efferent fibres from the NA to the intrinsic laryngeal muscles, while the SLN convey efferent fibres from the NA to the cricothyroid muscle, from the DNV to seroumucous glands of the whole larynx and, probably, all modalities of sensory fibres (afferent fibres) of the mucousus membrane that reach the TS and the NTS.

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N-O-10 SPATIAL COMPARTMENTALIZATION OF AMPA GLUTAMATE RECEPTOR SUBUNITS AT THE CALYX OF HELD SYNAPSE

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The mature calyx of Held ending on principal neurons of the medial nucleus of the trapezoid body (MNTB) has very specialized morphological and molecular features that make it possible to transmit auditory signals with high fidelity. In a previous work we described an increased localization of the ionotropic AMPA glutamate receptor (GluR) subunits at postsynaptic sites of the calyx of Held

principal cell body synapses from postnatal development to adult. The aim of the present study was to investigate whether the pattern of the synaptic distribution of GluR2/3/4c and 4 in adult MNTB principal cell bodies correlated with preferential subcellular domains (stalks and swellings) of the calyx. We used a postembedding immunocytochemical method combined with specific antibodies to GluR2/3/4c and GluR4 subunits. We found that the density of GluR2/3/4c in calyceal swellings (19±1.54 particles/?m) was higher than in stalks (10.93±1.37 particles/?m); however the differences for GluR4 were not statistically significant (swellings: 13.84±1.39 particles/?m; stalks: 10.42±1.24 particles/?m). Furthermore, GluR2/3/4c and GluR4 labeling colocalized to some extent in calyceal stalks and swellings. Taken together, the distribution pattern of GluR subunits in postsynaptic specializations are indicative of a spatial compartmentalization of AMPA subunits in mature calyx-principal neuron synapses which may support temporally precise transmission required for sound localization in the auditory brainstem.

Acknowledgments

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N-O-11 CHANGES IN THE CEREBELLAR CORTEX DEVELOPED *IN VITRO* IN ORGANOTYPIC CULTURE

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In order to study, under controlled conditions, the development of cerebellar cells and the reorganization of their intrinsic circuits, organotypic cultures of isolated cerebellum were prepared. Parasagital 300-µm-thick sections of the cerebellum were obtained from young rats from P0 to P12 and kept for 4 -6 weeks *in vitro*, following the method of Gähwiller. The third day in culture, an antimitotic cocktail was added for 24 hours.

Cultures were fixed and the cells of the cerebellar cortex were identified by the detection of specific antigens by immunofluorescence or immunohistochemistry. As a general feature, some delay in the development was observed, affecting mainly the cells of Purkinje and the unipolar brush cells. Most cultures showed the usual cytoarchitecture of the cerebellar cortex, although some differences were observed. On the one hand, granular cells were far less numerous than in normally developed brain, most probably due to the addition of the antimitotic when granular cells are still being generated. On the other hand, the cells of Purkinje were not arranged in a monocellular layer but in groups, and the dendritic tree lacked the normal orientation. However, these differences were less prominent in cultures obtained from animals older than P7. Reorganization of intrinsic fibres was also observed, probably due to the lack of both classes of afferent fibres and the significant loss of granular cells and, consequently, of parallel fibres. In the granular layer, the axons of unipolar brush cells were clearly observed, forming a net of so-called intrinsic mossy fibres, able to spread excitatory input. The observed results suggest that the Purkinje cells need the afferent fibres to orientate their dendritic tree in the right direction. The fact that older cultures, in which granular cells are less affected by the antimitotic, show better organization and orientation of the Purkinje cells, points towards the parallel fibres as responsible for the orientation and development of the Purkinje cells' trees. Besides, axons of unipolar brush cells expand in the granular layer when mossy fibres are lost.

N-O-12 DOPAMINE TRANSPORTER GLYCOSYLATION CORRELATES WITH THE DIFFERENTIAL VULNERABILITY OF MIDBRAIN DOPAMINERGIC CELLS IN PARKINSON'S DISEASE

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Midbrain dopaminergic (DA-) cells show differential susceptibility to degeneration in Parkinson´s disease (PD). The metabolism of dopamine (DA) plays a pivotal role in this process. Given that cytosolic levels of DA, and consequently DA metabolism, depend mostly on DA uptake, differences in dopamine transporter (DAT) expression have been involved in the differential vulnerability of DA-cells. Taking into account that DAT activity is closely related to its glycosylation status, we have investigated possible differences in DAT glycosylation and function between DA-cells showing different degrees of vulnerability. The use of antibodies that preferentially detect glycosylated or non-glycosylated DAT forms revealed internuclear differences in the expression of the glycosylated, but not the non-glycosylated, DAT form. Glycosylated DAT levels were higher in the soma and terminal membranes of nigrostriatal neurons than in those of mesolimbic neurons. This expression pattern correlated with the finding of two-fold DA uptake and 20% higher DAT Vmax in the dorsal striatum than in the ventral striatum, and the loss of these differences after deglycosylation of dorsal striatum synaptosomes. The topographic pattern of glycosylated DAT expression in the human midbrain and striatum showed a close anatomical correlation with DA- degeneration in parkinsonian patients. This correlation was confirmed in rodent and monkey models of PD. In addition, *in vitro* studies showed that HEK cells expressing a partially deglycosylated DAT form are more resistant to MPP+ than those expressing the wild-type form. In summary, our results strongly suggest that DAT glycosylation levels could account for the differential vulnerability of midbrain DA-cells in PD. Further studies focused on the mechanisms underlying DAT glycosylation differences may provide insight into the pathogenesis and new therapeutic targets of PD.

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N-O-13 CHANGES OF THE THALAMOCORTICAL CONNECTIONS FROM THE LD NUCLEUS AFTER VISUAL DEAFFERENTATION

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The connection from the laterodorsal thalamic nucleus (LD) to the ipsilateral retrosplenial cortex changes after visual deafferentiation, and the involment of ephrin A5 in these changes was examined in this study.

Iontophoretical injections of Biotin Dextran Amida (BDA) 10000 into the lateral dorsal nucleus of adult control Sprague-Dawley rats labelled terminal fields into ipsilateral retrosplenial granular and agranular (Rsg/a) cortex (Van Groen and Wyss, 1990; 1992; 2003). After suppression of retinal afferents carried out in neonatal animals, terminal fields originating in LD labelled in adult stages, showed an amplification to the adjacent secondary (V2M/L) visual fields and even primary visual fields in more caudal regions (V1M and V1B) Therefore suppression of retinal connections to the LD causes sprouting of the axonal branches coming from the dorsal neurons of the LD nucleus that invade the ipsilateral secondary visual field.

A family of molecules that can be related to the changes in the topography of the axonal terminar fields are the ephrins and their receptors, the Eph. We have studied the expression of one of these ligands, ephrin A5, in the cortex by in situ hibridization technique at two postnatal ages (P4 and P7) in control and in neonatally visually deprived animals. Results showed that at the postnatal age P4, this ligand was expressed in the retrosplenial and visual cortex of visually deprived animals, whereas in controls this signal was absent. At the postnatal age of P7, the expression of ephrin A5 in those areas disappeared in the deafferented animal.

The ephrin A5 expression pattern can act as a repulsive cue, blocking axonal arrival to the retrosplenial and visual cortices until the postnatal day 4 (while this ephrin is being expressed), and allows axonal reorganization after this age, when its expression decreases.

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N-O-14 ULTRAESTRUCTURAL DIFFERENCES BETWEEN AXONAL INITIAL SEGMENTS OF IPSILATERAL AND CALLOSAL SUPRA- AND INFRAGRANULAR PROJECTING NEURONS OF THE RAT VISUAL CORTEX

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This study examines axon initial segments (AISs) of rat visual supra- and infragranular pyramidal neurons that project within ipsilateral visual cortex or through the callosum. After combined injections of NMDA-BDA in area 17, twelve retrogradely labelled neurons were examined by correlative light/electron microscopy. Their AISs proceeded from cell somata to myelinated axons that run straight to the white matter. AIS lengths were within 20-28 microns for supragranular neurons and 26-32 microns for infragranular ones; no differences were observed concerning corticocortical projections studied herein. A most interesting distinction among either supragranular or infragranular AISs was however related with such projections. Thus, AISs of three supragranular ipsilateral neurons respectively received 28, 29 and 34 synaptic boutons (averaging 1.26 boutons per AIS micron), whereas three supragranular contralateral AISs got 19, 21 and 23 (0.8 bouton per AIS micron). AISs of three infragranular ipsilateral neurons received 20, 22 and 23 boutons each (0.7 bouton per AIS micron), whereas three infragranular contralateral AISs had 15, 15 and 16 (0.5 bouton per AIS micron). Mean AIS diameters of ipsilateral and contralateral supragranular neurons were 0,98±0.05 and 0.84±0.05 microns, respectively. Mean AIS diameters of ipsilateral and contralateral infragranular neurons were 0.97±0.06 μm and 1.07±0.09. Distributions of synaptic boutons along AISs were also analysed. Supragranular AISs received more boutons on their intermediate third but infragranular AIS did so on their first third to decrease progressively thereafter. The results suggest that (a) AISs of neurons with corticocortical ipsilateral backward projection to area 17 are more innervated than those of neurons with callosum projection to the same area, (b) AISs of corticocortical-projection neurons at supragranular layers are more innervated that their cortico-cortical-projection partners at infragranular layers and (c) synaptic distributions along AISs of corticocortical-projection neurons are layer-related rather than projection-related.

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N-O-15 INHIBITION OF NOGO-R EXPRESSION IS FOLLOWED BY VISUAL CORTICO-COLLICULAR SPROUTING AFTER VISUAL DEAFFERENTIATION IN ADULT ANIMALS

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After visual deafferentiation carried out in the first postnatal days the cortico-collicular visual connection sprouts considerably. After visual deafferentiation in adults this plastic reaction is absent and therefore there is not amplification of the cortico-collicular terminal field (García del Caño et al., 2002). It has been shown that a part of the responsibility of the lack of axonal growth in adult CNS relays on myelin-associated neurite growth inhibitors, such as Nogo-A and its receptor Nogo-R (Schwab and Bartholdi, 1996). In our study, we have done the laser capture microdissection of layer V pyramidal cells of the visual cortex which are the origin of the cortico-collicular connection in order to see the changes in the expression of the gene of Nogo-R. In adults, the expression of that gene is increased,

this result prompted us to further explore the post-deafferentiation changes of Nogo-R/Nogo-A system in the visual cortex and the superior colliculus. Western blot analysis in adults showed an increase of both Nogo-R and Nogo-A. These results were confirmed using antibodies against Nogo-R and Nogo-A. Consequently, we planned to inhibit the expression of the Nogo-R and see the effects of this inhibition on the status of the cortico-collicular terminal fields after visual deafferentiation in adults. Intracortical administration in primary visual cortex of specific short interfering RNAs (siRNA) was followed by an amplification of the cortico-collicular connection due to sprouting of the cortico-collicular fibers when adult animals were visually deafferentiated.

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N-O-16 GENETIC CHARACTERIZATION OF FOREBRAIN SUBDIVISIONS AND THEIR CONTRIBUTION TO THE FORMATION OF THE AMYGDALAR MOSAIC

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The amygdala is a telencephalic center essential for control of emotional and social behavior. Recent studies indicated that the amygdala is a mosaic-like structure consisting of multiple subdivisions with different molecular features. However, it was unclear how these subdivisions relate to the basic developmental units of the forebrain. To investigate this, we carried out two sets of studies: 1) We used in situ hybridization to study the expression of a battery of developmental regulatory genes in the mouse and chicken forebrain during embryonic development, in order to analyze the basic histogenetic divisions of the forebrain and their apparent amygdalar derivatives. 2) We used organotypic cultures of forebrain slices and fluorescent cell trackers (CMFDA or CMTMR) to fatemap distinct progenitor domains, in order to study cell migrations from different forebrain subdivisions to the amygdala. Our gene expression data suggest that the different molecular subdivisions of the amygdalar mosaic originate in distinct histogenetic subdivisions of the telencephalon and hypothalamus. The basal amygdalar complex, characterized by expression of Tbr1, Lmo3 and Lhx9, appears to originate in the ventrolateral pallium. The central amygdalar nucleus, characterized by expression of Dlx5, Lmo4 and Pax6, appears to originate in the striatal subdivision (or lateral ganglionic eminence). The medial extended amygdala includes subdivisions and neuronal subpopulations that appear to originate in the ventral pallium (expressing Lhx9 and Tbr1), the caudal pallidal subdivision (also called anterior peduncular area, expressing Lhx6), the preoptic area (expressing Shh), or the supraopto-paraventricular domain of the hypothalamus (expressing Lhx5). Many of the suggestions based on the combinatorial gene expression patterns were corroborated by experimental fatemap of distinct progenitor domains. In conclusion, the mosaic-like molecular organization of the amygdala correlates well with the embryonic origin of amygdalar neurons in different forebrain subdivisions. This may help to understand the wide range of connections and functions of different amygdalar subdivisions.

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N-O-17 AXONAL CONNECTIONS BETWEEN THE CLAUSTRUM, NEOCORTEX AND LIMBIC CORTEX. A COMPARATIVE APPRAISAL

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The claustrum is topographically and reciprocally connected with the neocortex and cingulated cortex. The possibility that the claustrum mediates a "global" function has been recently addressed. The present study is aimed to compare axon connections between the claustrum and cingulated cortex to those between the claustrum and neocortex. New-Zealand rabbits received CTb and WGA-HRP injections in different sensory, motor and cingulated areas to study axon projections from the claustrum. Other New-Zealand rabbits received BDA injections in the insular (dorsal) claustrum to trace anterograde axon projections. Retrograde tracers labelled few neurons in the contralateral claustrum. In the ipsilateral insular claustrum, labelled neurons with axon projection to either neocortical or cingulated areas made up labelled patches in topographically equivalent claustrum domains; patches belonging to projection cells to cingulated areas were nonetheless wider than patches of projection neurons to the neocortex. Moreover, retrograde tracer injections in anterior cingulated areas labelled cell patches that entered the piriform (ventral) claustrum. BDA labelled axons went from the insular claustrum to both ipsilateral neocortical and anterior and posterior cingulated areas; few axons went to contralateral cortices. In the cortex we observed three types of claustrocortical axons. The first one was made up by axons proceeding vertically from the white matter to end tufting in layer II-III; these axons typically gave off short axon collaterals in layers VI and IV. The second and third types were both tangential axons, with short collaterals, but along layers VI or I respectively; of these, layer I tangential axons were particularly abundant in cingulated areas. All axon-types had small en-passant enlargements and spine-like boutons. Labelled axons had bigger diameters and more enlargements and buttons in cingulated areas than in the neocortex. Our results suggest that the limbic cortex is even more strongly innervated by the claustrum than the neocortex.

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POSTERS

AC-P-01 – AC-P-027 Clinical Anatomy

AC-P-01 ANATOMICAL STUDY AFTER TRANS-OBTURATOR SURGERY FOR FEMALE STRESS INCONTINENCE

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The different surgical procedures used in the treatment of female urinary incontinence represent minimally invasive and effective method that provide relief of symptoms in 90% of patients. In these techniques, tension free suburethral tapes stabilize the mid-urethra at the time of an abdominal pressure increase without modifying cervico-urethral mobility. To avoid various peri- and post-operative complications associated with retropubic approaches, transobturator passage in which the tape is inserted through obturator foramen from outside to inside or from inside to outside, has been proposed.

Anatomical studies using six cadavers were performed in order to identify the exact route of the needles and tape after transobturator outside-in or inside-out techniques. Dissections of the perineal and crural areas enabled the identification and description of the muscular structures through which the tape passes and the neighbouring neurovascular structures.

The present anatomical study showed that the needles and the tape in transobturator techniques passed into a virtual space located in the most anterior part of the ischio-rectal fossa. This purely perineal passage was completely outside of the pelvic region, situated above the levator ani muscles, thus avoiding complications such as bladder perforation or intestine damage. The tape passed through the obturator foramen along the upper third of the pubic bone and did not injured neurovascular structures located near the three layers of adductor muscles of the thigh.

In conclusion, knowledge about the exact anatomical position of the tapes contributes to safely conduct the operation and reduces perioperative complications.

AC-P-02 ANATOMICAL BASIS OF TRANSVAGINAL REPAIR OF GENITAL PROLAPSE

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Genital prolapse with or without stress urinary incompetence is a major health care problem. Although, surgical procedures such as total abdominal sacral colpopexia or sacrospinous fixation provide good vaginal support, recurrence of cystocele is very common. Total pelvic floor reconstruction with implants using a minimally invasive vaginal approach has been proposed with the aim to reduce recurrence prolapse rate and hospitalisation cost. The goal of this study was to determinate the anatomical position of non-absorbable Prolene implants used in the treatment of genital prolapse and the relation of these implants with adjacent neurovascular structures.

For this purpose anatomical dissections of the pelvic floor were performed in three cadavers after the placement of synthetic meshes. This procedure enables the visualization of the anatomical structures through which the implants pass and the exact location of regional neural and vascular structures.

The anatomical dissections show that the anterior part of the mesh is inserted between the bladder and the vagina and secured bilaterally through each obturator foramen. The posterior part is placed between the rectum and the vagina and is secured bilaterally by one arm passing through each ischio-rectal fossa and sacrospinous ligament. Intermediate section separates anterior and posterior parts. A safe distance exists between meshes and neurovascular structures like obturator nerve and vessels, pudendal nerve and internal pudendal vessels, although individual differences are present.

In conclusion, dissections of the pelvic floor in corps after placement of implants in the treatment of genital prolapse can help surgeons to a better knowledge of the anatomical structures with risk to be damaged.

AC-P-03 ACCESSORY MENTAL FORAMEN. ANATOMIC STUDY OF A CASE REPORT AND A REVIEW LITERATURE

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The mental foramen is usually located apical to the second mandibular premolar or between apices of the premolars. However, its location can vary from the mandibular canine to the first molar. A significant asymmetric occurrence without any side preference was detected for accessory mental foramen (AMF), which was predominant in males. The highest frequencies for this trait where shown on Central Asian and Sub-Saharan African populations. Several findings of multiple mental foramina have been described in humans. In these cases one foramen is termed the mental foramen and the others are referred to as an AMF. The change in the position of the mental foramen with age, loss of teeth and alveolar bone reabsorption is well known. The aim of this study was to present a case report of accessory mental foramen in an anatomic study of 50 specimens and to present the frequency variations of this mandibular trait. Its importance is because the foramen may not appear on conventional radiographs as panoramic or periapical film, and it is a strategically important landmark in osteotomy procedures on mandible and in local anesthesia in terms of achieving effective mandibular nerve blocks and avoiding surgical injuries to the neurovascular bundles.

AC-P-04 HYOID BONE POSITION ON PANORAMIC RADIOGRAPHY AND TELERADIOGRAPHY

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Given the importance of knowing the normal anatomy of any region of the body, not only for the *a posteriori* diagnosis of a pathology, but also for carrying out clinical explorations or treatments, we set out to study which craniofacial morphological factors might influence the position of the hyoid bone in panoramic radiography (PR) and teleradiography (TR).

The study consists of a retrospective analysis of sixty panoramic radiographies and sixty teleradiographies carried out in the Odontological Clinic of the Medical Faculty of the University of Murcia (Spain) (37 males and 23 females, with an age range of 6.83 to 14.16 years) upon wich a variety of cephalometric studies commonly used in odontology were made. We also used some related with the hyoid bone, as BaHMe angle and height of the hyoid bone respect SN plane.

A factorial analysis was carried out to ascertain the relation between the different variables studied. Groups were compared by ANOVA and paired T-test were applied. Relations between qualitative variables were tested with Pearson's chi-squared test. A $p<0.05$ was taken as significant. All the statistical tests were carried out with the statistical package SPSS 15.0.

A statistically significant relation was found between the height of the hyoid bone with respect to the cervical column and the SN plane, so that if the SN plane, as it passes through the H point in the direction of the cervical column, coincides with the middle third of the fourth cervical vertebra in TR, the bone always appears under the mandibular body on both sides of the PR.

We also found a significant relation in TR between the position of the hyoid bone and the factors that depend on the jaw's morphology and the factors that depend on the morphology of the base of the cranium (direct relation with NSH angle).

The values of the right and left mandibular angles in PR is practically identical to the value of this angle in TR (direct dependence according to Pearson's correlation). This lends weight to the use of PR as the method of choice for measuring the mandibular angle.

AC-P-05 A STUDY OF TWO CASES OF THE ELONGATED STYLOID PROCESS OF THE TEMPORAL BONE

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The styloid process, stylohyoid ligament, and the small horn of the hyoid bone form the stylohyoid apparatus, which originally derives from the Reichert cartilage of the second brachial arch during embryogenesis. The styloid processes vary markedly in length, and the stylohyoid ligament may calcify to make a rigid connection with the hyoid bone.

The styloid process, the thin and long osseous part of the temporal bone, lies caudally, medially, and anteriorly towards the maxillo-vertebro-pharyngeal recess, which involves the carotid arteries, internal jugular vein, facial nerve, glossopharyngeal nerve, and the vagal and hypoglossal nerves. The attached structures include the stylopharyngeus, the stylohyoid, the styloglossus muscles and the stylohyoid ligament that reaches the hyoid bone.

Although the elongated styloid process (ESP) is generally asymptomatic, it can cause atypical facial pain. The facial pain, headache, a sensation of a foreign object lodged in the throat and difficulty in swallowing have been associated with ESP and is known as Eagle's syndrome.

We present two cases of elongated styloid process and/or ossification of the stylohyoid ligament in two male cadavers of 50-60 years of age from the Anatomy Department of the Medical Faculty of de University of La Laguna. Both of these ESPs were unilateral.

The length of the styloid process is variable. Moffat et al. 1977 performed a study on the styloid process and reported that the normal length is between 1.52 cm and 4.77 cm. Monsour and Young 1986 concluded that the diagnosis of an elongated styloid process could be made whenever the styloid process was longer than 40 mm. In our cases, styloid processes were longer than 40 mm, and calcification of the stylohyoid ligament can also be seen.

AC-P-06 A UNILATERAL MULTIPLE VARIATIONS OF THE BRACHIAL PLEXUS

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Brachial Plexus variations were encountered in the right upper limb of an adult male cadaver during the routine dissection course for undergraduate teaching at the Department of Anatomy, College of Medicine, King Saud University. The ventral rami of C4 and T2 were shared in the formation of the variant brachial plexus. The anterior and posterior divisions of the upper trunk fused with the middle trunk to make a single unit which divided into anterior and posterior subdivisions. The posterior subdivision joined the posterior division of the lower trunk, forming the posterior cord and giving the usual branches, while the anterior subdivision joined the anterior division of the lower

trunk to form the anterior cord. No lateral or medial cords were found in this cadaver; only anterior and posterior cords, according to their relationship with the second part of the axillary artery. We also observed a medial pectoral nerve with two routes and the absence of a musculocutaneous nerve.

AC-P-07 STUDY OF THE DORSAL ARTERIAL NET ON THE HAND

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Study of the dorsal arterial net on the hand, in 50 anatomical specimens.

The dorsal radial artery is only absent once. The dorsal ulnar artery appears in each hand, but always is a branch with scarce calibre. The anterior interosseous artery has been observed three times, but only once well developed. The dorsal radial and dorsal ulnar arteries appear at the same time in 46 occasions, 38 single arteries and 8 as anasthomosis**.** These threes arteries are anasthomosed in 4 occasions. The interosseous and dorsal ulnar arteries never appear alone. These observations allow to establish different models according to the number of the intermetacarpal arteries.

AC-P-08 MICROANATOMY OF THE ULNAR NERVE: UTILITY IN THE OBERLIN TECHNIQUE

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Introduction: Fascicular intraneural distribution of the peripheral nerves is always referred in the works of Sunderland, Bonnel and Rabishon. Since Oberlin described the technique of the transference of the fascicles for the extrinsic musculature of the ulnar nerve to revive the biceps muscle, the knowledge of the intraneural anatomy has taken importance.

Objectives: Study the distribution and fascicular course of the ulnar nerve proximal and distal to the elbow to be able to apply more accurately those basis in the technique of nerve transference of the ulnar nerve according to the Oberlin technique.

Material and methods: The dissection of 12 criopreserved upper extremities is performed. Previously the vascular tree has been injected with black latex. After the location of the ulnar nerve in its course proximal and distal to the elbow, intraneural dissection of this nerve is carried out using magnification glasses.

Results: Proximal to the elbow the emission of the extrinsic fascicles is not clearly defined. At the elbow, a more clear morphological differentiation of the fascicles can be seen, however, the anatomical variability in thickness and number makes difficult to predict which is the principal extrinsic component. Just distal to the elbow, a more clear differentiation of the branches for the FCU and FDP is seen. Altogether we have appreciated that the disposition of the fascicles of the ulnar nerve has a spiral run from proximal to distal at the elbow modifying their disposition along their course.

Conclusions: According to our studies, the microanatomical disposition of the fascicles of the ulnar nerve at the elbow is not as simple as it has been described in the literature existing variability in number, thickness and disposition that makes it advisable the use of intraoperative registers to localize the fibres for the extrinsic and intrinsic musculature and sensitive fibres when the Oberlin technique is carried out, or at least the use an electric stimulator for their identification.

AC-P-09 3D RECONSTRUCTION OF THE KNEE AND ELBOW JOINTS

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We describe a computer application meant to facilitate the comprehension of the knee and elbow joint, as well as the x-ray's interpretation of the helical computerised tomography. The 3D reconstruction program used was Amira®, by which a digitalizing tablet assigns a colour to each anatomical structure.

The so developed computer application is an extraordinary tool in the range of anatomic and radiologic lecturers, as well as for medical professionals.

We thank the members of Abadia´s Company for their technical assistance.

AC-P-10 VARIATIONS IN THE PATTERN OF DIVISION OF THE POPLITEAL ARTERY: IMPORTANCE IN KNEE SURGERY

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Introduction: Vascular complications of knee surgery or in traumatisms of this region usually carry important sequelae including amputation of the extremity. Unfortunately, the anatomy of the principal artery at this level is not constant, something that orthopaedic surgeons frequently do not give enough importance.

Objectives: Define the patterns of division of the popliteal artery, taking the popliteal muscle for reference.

Material and methods: The dissection of 20 criopreserved lower extremities injected with black latex has been performed. The course of the popliteal artery and its divisions is described in relation with the popliteal muscle. Photographs of the dissections are taken.

Besides, 10 other lower extremities injected with latex have been sliced every 1.5 centimetres in order to see the disposition of the studied vessels in relation with the popliteal muscle taking photographs of them.

Results: The popliteal artery is firmly tided, proximally by the adductor major hiatus, distally by the tendinous arch of the soleus and at each side by the superior and inferior genicular arteries. That makes the popliteal artery more prone to traumatic lesions, as it can not displace freely.

The most common pattern of division found is that the popliteal artery passes posteriorly to the popliteal muscle and divides into the anterior tibial artery and the tibioperoneal trunk at the level of the inferior border of the popliteal muscle.

We found two cases of high division of the anterior tibial artery, proximal to the popliteal muscle, passing posteriorly to the muscle in one of the cases and anteriorly in the other one.

Conclusions: The disposition of these vessels must be taken into account as they could be injured during orthopaedic surgery of the knee, specially during the implantation of total knee prosthesis, with the central retractor to displace the tibia ventrally. It is also important to emphasize the disposition of the artery in the traumatic lesions, particularly in dislocations of the knee or proximal metaphyseal tibia fractures.

AC-P-11 STUDY OF THE CERVIC DIAPHISARY ANGLE IN HUMANS

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The angle formed by the femoral diaphysis and the femoral neck, is called cervic diaphyseal angle or angle of inclination.

In the bibliography the values of this angle are quite disparate, around 125º-126º, varying between 138º-139º, being inferior in women and in people with an advanced age (120º), and bigger in the newborn (around 150º).

It is called Coxa vara when the angle is less than 115º. Coxa valga when the angle is bigger than 140º.

The inclination angle reduced pathologically (coxa vara), will affect to the knee, because the load will pass to the internal condyle of the femur, producing a genu varum (arc like legs) and affecting also the internal meniscus.

The pathologic increment of the angle of inclination (coxa valga), will also affect to the hip and to the knee, the load of the weight will lie essentially on the external condyle of the knee, affecting to the external meniscus (genu valgum) (Knees in X).

We intend to analyse in this study more than 200 human femurs which proceed from the library of bones of the Department and the excavations in different places of the Valencia region, the value of the human cervic diaphyseal angle, due to its own importance not only in the coxofemoral joint itself but also in neighbouring joints like the knee, important joint in the everyday activity because of the weight it bears and the mobility it presents.

Today the use of the coating prosthesis, which permits a minor invasion of the bone, because they only introduce in the head of the femur the point of the prosthesis, which has the form of a thumbtack, gives even more interest to the angle of inclination of the femoral head, because the prosthesis has to be placed exactly in the centre of the femoral head, a mild deviation would cause the fracture of the femoral neck by the decompensation of the weights that is bearing.

AC-P-12 LOCALIZATION, FRAGMENTATIONS AND BIOMECHANICS IMPLICATIONS OF OSSA SESAMOIDEA PEDIS

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Ossa sesamoidea pedis are bones usually located in the plantar face of the foot. Two of them are constant and located in the plantar face of the first metatarsal-phalangic articulation, in relation with the head of the first metatarsal bone. These two little bones have a special morphology with an oval shape and the medial one is bigger than the lateral one. Other dispositions of supernumerary bones are possible. One of the objectives of this work is to study the different locations of them in the foot.

These bones are structurally very solid. With very low rate, the medial bone is fragmented in two or three pieces. This fate usually is the result of an irregular movement of the bone along an irregular surface. We have analyzed the biomechanics implications of intact and fragmented ossa sesamoidea pedis as well as trying to explain the origin of the fragmentation.

The radiographies and documentation correspond to ten different patients with different pains and alterations of the walk in relation with the location of some bone like ossa sesamoidea pedis or supernumerary bones that came to the Clinic of Podology of Extremadura University at University Centre of Plasencia to solve their biomechanics problem.

AC-P-13 ANATOMY AND IDENTIFICATION OF THE MEDIAL DIVISION OF POSTERIOR BRANCHES OF THE LUMBAR SPINAL NERVES. ANESTHESIC AND ULTRASOUND APLICATIONS

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The knowledge of spine and its nervous structures is very important in several areas of medicine as anaesthesia field. One of its applications is in treatment of chronic low back pain. This ache is a frequent pathology in population and one of its treatments is anaesthesia of medial division of posterior branches of spinal nerve. These branches supply the lumbar zygaphysial joint. In order to block these nerves there are several methods. Fluoroscopy is the standard, but now, ultrasound guided methodology can help to give a body landmarks of the spine to approach them. The aim of this study is to describe and localize the medial division of posterior branches of lumbar spinal nerves in relation with the several bone structures in order to facilitate their localization with ultrasound. The study was performed in two steps in 6 adult criopreserved (3 men and 3 female) cadavers. The first part of the study applied ultrasound to identify and locate the zygapophyseal joints and other bone structures of the lumbar spine. In the second part of the study, cadavers were dissected via a posterior approach. We identified the medial division of posterior branches and measured their distances to the articular, transverse and spinous process were measured. The mean distance from the posterior branches of spinal nerve to the articular process was 1,5442 cm, to spinous process 2,5493 cm and to transverse process 2,1558 cm. We report a practical approach for ultrasounds-guided puncture of the lumbar spine, which describes anatomical landmarks in cadaver specimens and establishes the mean distance from the posterior branches of spinal nerves position to the posterior process.

AC-P-14 JOINT ATLAS OF THE LOCOMOTOR SYSTEM USING 3D CT

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Eresa (Special Radiological Explorations S.A.) through his foundation, Eresa's Foundation and the University of Valencia, through the Human Anatomy and Embryology Department, made a collaboration agreement several years ago, which has achieved its first results.

The Edition of the Joint Atlas of the Locomotor System 3D CT.

Using a CT system. Light speed ultra of G.E. A CT multislice, capable of obtaining 65 slices per second.

Endowed with an ultra performix tube of metalized ceramic, with a thermic storage capacity of 6,3 MUH and optidose function, incorporates a matrix detector, of polycrystalline ceramic. Formed by 16 rows of detectors, with more than 900 cells per row, with a cell dimension of 1mm per 1,245 mm.

The reconstruction of the image is made by algorithms of multiple exclusive slices, with times of reconstruction of 0,5 sec. and with maximum coverage areas which vary form 2 per 0,63 mm/sec. to 8 per 2,5-6,7 mm/sec.

The thickness of the image is modified when the collimation, the algorithm of reconstruction and the configuration of the detector vary.

The beam of X rays is collimated by the two central rows to the 16 rows of the detectors.

We obtain images form each of the joints of the locomotor system, from all the angles, in the spinal column we appreciate the different regions with images of each of them, appreciating the conjunctive hole.

Although the explorations where made to subjects who had come by suspicion of pathology not of the locomotor system and since they where young subjects, we can appreciate various osteophytes in the spinal column.

In the images of the cervical column, the vertebral artery is appreciated coming up through the transversal hole of the cervical discs.

In the lower limb, hip, knee, ankle and foot are perfectly appreciated.

In the upper limb, shoulder, elbow and hand are observed in detail.

These are essential images for imaging the anatomy, which will be of use for our classes, because *in vivo* images we can appreciate each of the joints of the locomotor system, as the apophyses, spaces, prominences, trochanters, etc.

The preparation and assembly has been the work of D. Javier García Ramos, Specialized Technician in Images of the Human Anatomy and Embryology Department of the University of Valencia.

AC-P-15 ANTHROPOMETRY SCREEN

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The study of the anthropometry of the human body has been a constant in the investigations of several Departments

of Anatomy in Spain. The Anthropometric Unit of the Human Anatomy and Embryology Department of the University of Valencia has developed an Anthropometric Screen, Patented by the University of Valencia and made by the Enterprise Technology Applied to the Machinery, with headquarters in the Valencia Region.

It is constituted fundamentally by a methacrylate plate with parallel silk-screen printed lines vertical and horizontal with a separation of 5mm between them. Also, the screen is silk-screen printed with an angle measurer, centred on the horizontal and vertical middle lines, allowing the study of the degree of movement of the joints of the subject to be explored.

In the anthropometric screen the patient or subject to be explored is placed, backwards, sideways or frontally to the screen, obtaining the analysis of the general structure of the human body.

The anthropometric screen permits a study, or a complete anthropometric screening, of the human body, in an anterior, posterior or sideways collocation, being able of detecting, following and measuring the evolution of the asymmetry and the evolution experienced with the treatment.

It has general medical application, with special application to Sports Medicine Specialities, Family and Community Medicine, Preventive Medicine and Public Health, Labour Health, Legal and Forensic Medicine, Pediatrics, Rehabilitation, Rheumatology, Traumatology and Orthopedics, Physiotherapy, Osteopathy, etc.

AC-P-16 ANATOMICAL COINCIDENCE BETWEEN TENDER AND TRIGGER POINTS WITH ACUPUNCTURE POINTS

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The tender points have been always related with fibromyalgia, a chronic condition that causes pain all over the body and making it difficult to diagnose.

The defining symptoms of fibromyalgia are often associated with other subjective and objective symptoms which occur in combination. For the classification of fibromyalgia is necessary a history of widespread pain involving all four quadrants of the body for a period of at least 3 months, and the presence of pain in at least 11 of 18 specific points named “tender points” when touched or pressed with force amounting to the equivalent of 4 kg, without referred pain

By the other hand, Myofascial pain comes from trigger points in muscles and the fascia that is interwoven throughout and covering them.Trigger points are discrete, focal, hyperirritable spots located in a taut band of skeletal muscle. They produce pain locally and in a referred pattern and often accompany chronic musculoskeletal disorders.

Referred pain is an important characteristic of a trigger point. It differentiates a trigger point from a tender point, which is associated with pain at the site of palpation only.

We can compare the similarities that exist between myofascial trigger and tender points whit those of the points of traditional Chinese acupuncture.

Considering the fact, the anatomical localization of tender points and Acupuncture points suggest that these points are identical.

The named AhShi points of traditional Chinese acupuncture, are the general name given to sites on the body that become spontaneously tender when disease or injury occurs. The location of AhShi points is not fixed.

Comparisons between the definition and features of AhShi points and trigger points suggest that the two systems represent the same phenomena and can be explained in terms of the same underlying neural mechanism. However, not all acupuncture points are associated with skeletal muscle, therefore not all acupuncture points are trigger points but all tender points are acupuncture ones.

Studying the neurophysiological response to these therapies could help to contribute to the understanding of pain mechanisms and in devising methods of treatment for chronic pain.

AC-P-17 INTRADERMAL FAT IN HUMAN FRESH CADAVERS TO CHARACTERIZE SEX SKIN DIFFERENCES: A MORPHOMETRIC STUDY

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Differences between male and female skin characteristics in certain body areas are of importance for diagnostics and treatment of a female skin condition that is known as cellulite. Although a very high percentage of women are diagnosed of cellulite, very little has been published to characterize this condition at the histological level.

The aim of this work was to investigate whether the distribution of adipose tissue in the dermis is a characteristic sex-dependent. With this purpose we performed a morphometric analysis of the inclusions of adipose tissue at the dermis from 13 human fresh cadavers of both sexes, which were voluntary donors of the dissection room of the Faculty of Medicine (UAB). The samples of skin were obtained from the scapular and gluteal regions and the greater trochanter level (femoral region). Previously, a 22 MHz ultrasound system to image the sample skin was used. The morphometric results of the histology were compared to those obtained by means of the ultrasound system, to assess whether this diagnostic device is suitable to measure *in-vivo* the skin condition for cellulite diagnostic. Digital photographs were obtained from the histological and ultrasound samples. After the dermis and their adipose inclusions were drawn separately (Photoshop CS®), the area of both

were obtained with the Visilog 5® software. The index between the areas of fat inclusions and the total dermis showed to be higher for woman skin than for man one. Although further investigation is required, these results can help to understand the anatomical bases of cellulite.

AC-P-18 SOMATOSTATIN PRETREATMENT DOES NOT IMPROVE SOMATOTROPH CELLS RESPONSE OF PREPUBERAL FEMALE RATS

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Introduction: In a previous study, we showed that the greatest stimulus for GH release, in prepuberal female rats, is achieved with the combination of GHRH and ghrelin, while leading to greater GH storage in somatotroph cells (SC). The purpose of this paper is to study, in female prepuberal rats through ultrastructural and morphometric studies of CS, the SRIH effect on GH storage in SC, in basal conditions and after stimulation combined GHRH and GH secretagogues.

Materials and methods: Twenty-six to twenty-eight-day-old female Wistar rats were used. Half the animals (n=60) received SRIH (*SRIH group*) and the other half (n=60) received saline (*saline group*) at -90 minutes. Stimuli were GHRH, GHRP-6, GHRELIN, GHRH+GHRP-6 and GHRH+GHRELIN at 1 µg/kg body weight in all cases. Trunk blood samples were taken on decapitation at -90, -15, 0, 15, 30 and 90 minutes for GH determination; pituitaries were removed and posterior pituitaries were discarded for ultrastructural and morphometric studies. The amount of secretion granules was determined by the integrated optical density parameter (IOD).

Results: Depending on the amount of IOD were designated four types of SC (I, II, III and IV). In saline group, none of the stimuli was capable of storing greater amounts of GH than in controls. In SRIH group, GHRH with GHRP6 or ghrelin produced greater amount of intracellular GH than in controls, at 30 and 90 minutes after the stimulus. Type IV SC only occurs with stimuli combined of GHRH and GH secretagogues. Area under curve (AUC) of the total quantity of secretion granules is higher in the saline group than in the group SRIH, particularly with the stimulus of GHRH and GHRP-6.

Conclusions: In female prepuberal rats, the largest GH storage and GH release occurs with stimuli combined of GHRH and GH secretagogues, in the absence or presence of SRIH. Throughout the study period, the total GH stored in somatotrop cells in the saline group is the same regardless of the stimulus used, although higher than in animals of SRIH group.

AC-P-19 SOMATOCHART AND SYSTOLIC BLOOD PRESSURE

Sánchez Ferrer, M.*[1], Sánchez del Campo, F.[1], Gil Guillén, V[2], Hernando, J.[3], Garramone, D.[3], Roqués, V.[4], Tejada, S.[3], Almenar, V.[1], Terol, F.[1]

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In our case, we try to analyze the relationship between somatochart, which was built as the model described by Heath and Carter, and the systolic blood pressure, which was measured in the population of a Health Center in Alicante (Spain). It was a random selection, men and women aged between thirthy and sixty-five. We obtained weight, height, limb girths (waist, hip, arm flexed and tensed, calf), bone breaths (biepicondylar of the humerus, bistylod and biepicondylar of the femur). We took the arterial pressure during the same visit.

We built the somatochart of these patients with this data and compared it with the level of systolic blood pressure.

The results obtained showed that those patients whose level of systolic blood pressure was over one hundred and twenty milimeters of mercury, were in the area of the somathochart between X minor or equal -2 (X<= -2) and Y major or equal 2 (Y>= 2). They are endomorphic-mesomorph and mesomorph-endomorph. However, those patients whose systolic blood pressure was under one hundred milimeters of mercury, were in the area between X minor or equal 0 (X<=0) and Y minor or equal 2 (Y <=2). They are mesomorphic-endomorph and balanced endomorph.

AC-P-20 ROLE OF α-ACTIN IN MUSCLE DAMAGE IN COMPARISON WITH TRADITIONAL MARKERS

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In order to identify a reliable marker for the early detection of muscle injuries in sports, α-actin protein and other markers of muscle damage were studied in sera of uninjured sportspeople and those with skeletal muscle injury. Blood samples were obtained from 20 sportspeople with skeletal muscle injury and 48 uninjured sportspeople. Immunoassays were performed to determine cardiac troponin

I (TnI), troponin T, lactate dehydrogenase and myoglobin concentrations. Western blot and densitometry were used to measure α-actin concentrations. Skeletal muscle damage was diagnosed according to physical examination, MRI findings and the biochemical criterion of a creatine kinase value .500 IU/l (Rosalki method, Beckman Instruments SL, Fullerton, California, USA). Results were also compared with previously obtained data on injured and uninjured non sportspeople. The mean serum concentration of α-actin was significantly higher in sportspeople with muscle damage (10.49 mg/ml) than in uninjured sportspeople (3.99 mg/ml). Sera from injured sportspeople showed higher levels of α-actin than of troponin or myoglobin. No significant difference in TnI levels was observed between the groups. According to these results, α-actin is a new and reliable marker of skeletal muscle damage in sportspeople which can be used for the detection of muscle injury. Possible cross interference between skeletal and cardiac muscle damage can be discriminated by the combined use of α-actin and TnI. These data suggest that early measurement of α-actin in sportspeople with suspected muscle damage will allow them to receive earlier and more effective treatment and to return sooner to the practice of their sport.

AC-P-21 MODIFICATION OF α-ACTIN AFTER HIGH-LEVEL COMPETITION MATCH

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To study the effects of high-level matches on serum α actin and other muscle damage markers in teams of rugby and handball players. Blood samples were drawn from 23 sportsmen: 13 rugby players and 10 handball players. One sample was drawn with the player at rest before the match and one immediately after the match. Immunoassays were used to determine troponin I, troponin T, LDH, and myoglobin concentrations. Western blot and densitometry were used to measure α-actin concentrations. Muscle injury was defined by a total CK value of > 500 IU/L (Rosalki method). Mean pre- and post-match serum α-actin values were, respectively, 7.16 and 26.47 mg/ml in the handball group and 1.24 and 20.04 mg/ml in the rugby team. CPK, LDH and myoglobin but not troponin 1 levels also significantly differed between these time points. According to these results, large amounts of ?α-actin are released into peripheral blood immediately after intense physical effort. Possible cross-interference between skeletal and cardiac muscle damage can be discriminated by the combined use of α-actin and troponin I. The significant increase in α-actin after a high-level match may be a reliable marker for the early diagnosis and hence more effective treatment of muscle injury.

AC-P-22 MITOCHONDRIAL APOPTOSIS PATHWAY ACTIVATION: A NEW STRATEGY TO INDUCE APOPTOSIS IN MELANOMA CELLS

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Melanoma represents only 4% of all skin cancers but nearly 80% of total skin cancer deaths, predominantly because of metastatic spread. As melanoma is a highly therapy-refractory tumor, it demands effective therapeutic combinations. In this context, the *E* gene is another potentially interesting bacteriophage lysis gene for cancer therapy. In contrast to most double-stranded DNA phages, which generally encode two genes that elicit host cell lysis (endolysin and holing protein), the small single-stranded DNA phage ϕX174 has only one lysis gene.

Methods: To evaluate whether this *E* gene has a cytotoxic impact on melanoma cells *in vitro* and *in vivo* we selected the B16-F10 cell line. We used a nonviral gene delivery approach (plasmid) to study the inhibition of melanoma cells' proliferation *in vitro* and direct intratumoral injection to deliver *E* cDNA to rapidly growing murine melanomas. The effect and mechanism action of the E protein *in vitro* and *in vivo* was studied by applying several viability, apoptosis and imagen diagnostics assays.

Results: We found that the *E* gene has both a strong antiproliferative effect in B16-F10 cells *in vitro* and induces an efficient decrease in melanoma tumor volume *in vivo*. *In vitro* and *in vivo* analysis demonstrated significant functional and morphological mitochondrial alterations accompanied by a significant increase of cytochrome c and active caspase-3 and -9 in transfected cells, which suggests that tumoral cell death is mediated by the mitochondrial apoptotic pathway.

Conclusion: In summary, we have reported, for the first time, the ability of the *E gene* to induce the death of melanoma cells *in vitro* and *in vivo*. The successful use of this gene as a new anticancer gene therapy system may establish a role for it in cancer treatment.

AC-P-23 A SYNTHETIC URACIL DERIVATIVE WITH ANTITUMOR ACTIVITY THROUGH DECREASING CYCLIN D1 IN MCF-7 CELLS

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In recent years, many studies have shown an association between cell cycle regulation and cancer in as much as the cell cycle inhibitors are being considered as a weapon for the management of cancer. Ultimately a great level of interest has arisen in the G_0/G_1 phase regulatory molecules such as cyclin D1, CdkIs, and p53 as potential therapeutic targets in diseases where the control of inappropriate cellular proliferation would be a therapeutic benefit. As a lead we took (*RS*)-1-(2,3-dihydro-5*H*-1,4-benzodioxepin-3-yl)-5-fluorouracil (DBDFU) and having proved that this compound and other analogs previously reported by us, were not 5-fluorouracil (5-FU) prodrugs, we decided to change the non-natural pyrimidine 5-FU moiety by uracil in DBDFU.

In order to prove our hypothesis, the mechanism of the antitumor and antiproliferative activities of the compounds, the effects on the cell cycle distribution were analysed

The studies have been performed using flow cytometry techniques, western blot, DNA fragmentation and reverse transcriptase-polymerase chain reaction assays.

In the present study, the effects of the uracil benzofused seven-membered *O,N*-acetal DBDU early apoptosis and DNA fragmentation were investigated in the MCF-7 human breast cancer cell line. The expression of cell-cycle-related proteins (cyclin D1, p53 and Cdk1) were also explored to investigate the potential mechanisms of the G_0/G_1 and G_2/M arrest activity of DBDU. In addition, the expression levels of CdkIs, such as p21Waf1/Cip1 and p27Kip1 were examined in MCF-7 cells.

Our result demonstrated that G_0/G_1 and G_2/M cell arrest and apoptosis were easily induced by DBDU treatment in cells with wild-type p53 (MCF-7). Furthermore, the use of a reverse transcription-PCR-based assay at a dose of 5 μM of DBDU, decreased cyclin D1 mRNA, suggesting that DBDU exerts its regulatory action on cyclin D1 at the level of transcription. Our study provides the basis of molecular mechanisms for DBDU in cancer treatment. The structure of DBDU with a naturally-occurring base such as uracil, associated with the inhibition of cancer cell proliferation caused in MCF-7 cells, would make it a very attractive agent, opening a new strategy in cancer chemotherapy using similar compounds endowed with potent antitumor activities with future clinical application in breast cancer.

AC-P-24 CLASSICAL CYTOTOXIC THERAPY IN LUNG CANCER PATIENTS INDUCES MDR 1 GENE EXPRESSION IN PERIPHERAL BLOOD

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Multidrug resistance plays an important role in the lung cancer prognostic because although it can be expressed at a low level in normal and malignant cells, it becomes frequently overexpressed after chemotherapy. This fact implies the presence in the tumor cells of a mechanism able to decrease the intracellular concentration of drugs. However, it is controversial the developed resistance mechanism, the relevance of the modifications in multidrug gene expression levels in peripheral blood and if these modifications can be used as a prognostic factor in lung cancer patients.

Methods: To investigate drug resistance mechanism against taxol (paclitaxel) and carboplatin treatment in lung cancer we used the A-549, A-427 and Nodo (from the Centro Nacional de Investigaciones Oncológicas, CNIO) cell lines. Tumoral cells were treated with clinical doses of drugs and expression of MDR1, MRP3, MRP5, LRP and BCRP was determined by RT-PCR. Expression of P-glycoprotein was studied by confocal microscopy using spheroids tumor models. Proliferation and apoptosis assays and ultraestructural analysis were realized in these cells. On the other hand, peripheral mononuclear cells (PMN) from 38 lung cancer patients were obtained before and after taxol-carboplatin exposure. Resistance genes expression levels were detected by semiquantitative RT-PCR.

Results: Our results demonstrated that mdr1 expression is the main resistance mechanism in lung cancer cells *in vitro*, in relation to the exposure to a combination of taxol-carboplatin similar to that used in clinical treatment. Moreover, mdr1 expression increases in peripheral blood of the patients with drug treatment in comparison to those unexposed. However, MRP, LRP and BCRP expression levels remained the same.

Conclusion: Taxol-carboplatin clinical levels enhance mdr1 expression in lung cancer cells *in vitro* but also in normal peripheral blood mononuclear cells *in vivo*. These results provide evidence that mdr expression detection in PMN circulating cells can be clinical relevance in the prognostic of patients with lung cancer.

AC-P-25 ADDITIVE CYTOTOXIC EFFECT OF *APOPTIN* AND CHEMOTHERAPEUTIC AGENTS, ON MCF-7 BREAST CANCER CELLS

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Although the advancement of diagnostic techniques and treatment in the last decade has greatly contributed to the survival of cancer patients, breast cancer is still one of the leading causes of death in women today and its incidence in the developing world is on the rise. It is estimated that there are approximately 180 510 new occurrences of breast cancer and 40 910 breast cancer deaths (40 460 women, 450 men) in the year of 2007 in the US (American Cancer Society, 2007). New therapeutic strategies are required to overcome the limitations of conventional breast cancer treatment. Thus, the use of novel antitumour drugs that are less toxic to normal tissues and more specific to malignant cells combined to suicide gene therapy offers a potential approach to this type of tumour.

In this context, Apoptin, a virus protein of avian anemia, which induces apoptosis in a large variety of transformed cells but not in primary cells, is regarded as a potential anticancer drug for clinical applications. In this study, we analysed whether chemotherapeutic agents combined with apoptin treatment could result in enhanced cytotoxicity in human tumour cell cultures. Combined treatment with recombinant retrovirus under the control of the tetracycline responsive element (TRE) expressing apoptin and paclitaxel clearly showed an additive cytotoxic effect on human MCF-7 cells. Doxycicline-induction of *apoptin* gene expression in MCF-7 cells combined with low dosis of paclitaxel produced a significant decrease on proliferation rate. In addition, Annexin-V-FITC and propidium iodide assays showed the presence of apoptotic and necrotic cell death, which was confirmed by scanning electron microscopy. Our results indicate that the cytotoxicity-enhancing action by the tumour-specific apoptin in combination with paclitaxel might offer an effective antitumour effect suggesting its potential application as a new combined therapy strategy for this type of tumor.

AC-P-26 EXPRESSION OF GEF GENE IS ASSOCIATED WITH A BETTER PROGNOSIS AND INDUCTION OF APOPTOSIS BY P53-MEDIATED SIGNALLING PATHWAY IN BREAST CANCER CELLS

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The low efficiency of conventional therapies in achieving long-term survival of breast cancer patients with a poor prognosis calls for development of new strategies including gene therapy. One of the most promising recently developments has been the introduction of suicide genes that do not require the use of a prodrug to be effective in tumour cells. We previously reported that gef gene from E. coli, has shown considerable cytotoxic effects in breast cancer cells, however the action mechanism has not been elucidated. Indirect immunofluorescence technique using flow cytometry and immunocytochemical analysis were used to detect breast cancer markers: estrogen (ER) and progesterone (PR) hormonal receptors, human epidermal growth factor receptor-2 protooncogene (c-erbB-2), ki-67 antigen and 53 protein (p53).Gef gene induces an increase in ER and PR expressions and a decrease in Ki-67 and C-ERB-2 gene expressions, indicating a better prognosis and response to treatment and a longer disease-free interval and survival. It also increased p53 expression, suggesting that gef-induced apoptosis is regulated by a p53-mediated signalling pathway. These findings support the hypothesis that the gef gene offers a new approach to gene therapy in breast cancer.

AC-P-27 SOMATOSTATIN AND OCTREOTIDE SHOW AN ENZIMATIC AND MORPHOLOGICAL CYTOPROTECTIVE HEPATIC EFFECT DURING EXPERIMENTAL LIVER PRESERVATION

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Aim: assessment of the enzimatic and morphological hepatic cytoprotective effect of somatostatin (ST) and octreotide (OT) during experimental liver preservation.

Methods: 36 Wistar rats were used. Livers were in situ washed out via portal vein with University of Wisconsin(UW) solution, UW+ST(3,2nmol./ml.) and UW+OT (3,2nmol./ml.) and were cold storaged for 24 and 48 hours (n= 6). Levels of AST, ALT, LDH and CK were assessed in the perfusate during ex situ isolated reperfusion. Morphological study of the livers were performed using optic microscopy after the preservation period.

Results: After 24-hour CIT, ALT, LDH and CK levels were significantly lower($p<.05$) in UW+ST and UW+OT livers than UW livers. After 48-hour CIT, AST, ALT, LDH and CK levels were significantly lower($p<.05$) in UW+ST and UW+OT livers than UW livers. Histopathologic examination showed a distinct ischemic injury after 24- and 48-hour CIT in a higher degree in centrolobular zones than in the periportal ones, with swollen hepatocytes and presence of intracytoplasmatic vacuoles. After 24 hour CIT only a mild protective action of somatostatin and octreoitde was present. However ischemic damage was less evident in UW-ST and UW-OT livers after 48 hour CIT with less vacuolization and hepatocytes swelling and rupture.

Conclusion: Somatostatin and octreotide showed an enzimatic and morphological distinct hepatic cytoprotective effect during experimental liver preservation.

D-P-01 – D-P-015 Teaching in Anatomy

D-P-01 COLLABORATIVE VIRTUAL SCENES FOR ENHANCING ANATOMY LEARNING

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Sufficient ability to visualise in three dimensions is instrumental in identifying structures in the living body as required in clinical examination. 3D representation of anatomic scenes is a valuable tool to view the spatial relationships between structures from numerous viewpoints as well as to aid the learner's understanding of spatial organization of the human body.

Web3D technologies, i.e. graphic technologies supported by the World Wide Web, provide a framework to cost-effectively build anatomic worlds that allow the learner to freely navigate and interact with virtual scenes. To support knowledge construction, desirable features of a digital learning resource include being operating and self-exploratory. In addition, providing the learner with opportunities to collaboratively interact with technology adds a social dimension to the learning process, in keeping with constructivist learning theories.

We propose a solution that makes combined use of various open standard technologies to generate collaborative anatomic scenes:

(i) three-dimensional representation based on surface models stored in X3D file format, i.e. the ISO standard XML-based file format for representing 3D computer graphics in a cross-platform manner,

(ii) JavaScript- and HTML-based user-interface controls to access anatomic model attributes such as position, appearance (colour, transparency), and

(iii) synchronisation between clients by means of Ajax-mediated asynchronous communication with a server-side MySQL database that stores any changes applied to the scene by any user.

A major advantage of this client-server architecture is that it allows users to either view other users alter and rearrange the scene in real-time, or handle the scene themselves concurrently. Possibilities to improve interaction or to enrich such environment may include but are not limited to:

(i) enrichment of anatomic models by adding labels, tags, text or hyperlinks,

(ii) concurrent presentation of clinical diagnostic imaging,

(iii) virtual reality hardware providing an enhanced sense of immersion,

(iv) improved user communication through voice- or IRC channels, or

(v) haptics properties.

Web-based virtual collaborative environments may encourage knowledge construction that requires less cognitive effort than traditional instructional approaches. Further research is required to evaluate the usefulness of these tools for the undergraduate medical curriculum.

D-P-02 ANATOMY IN MEDICAL AND SURGICAL DEGREE: PRELIMINARY EVALUATION FOR AN OPTIMAL EUROPEAN HIGHER EDUCATION AREA SETTING

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The quick implantation of the European higher education area in the Spanish university is going to mean the adoption of new teaching systems. This new model implies new methodologies, new teaching strategies, different evaluation systems, and therefore, new relationship between students and teachers. In Health Sciences, basic science knowledge must be integrated with the clinical skills that students will require in their professional activity. Anatomy has been always considered to play a decisive role in medical education and future professional activity, given a great importance to our subject within Medical and surgical degree.

For the present study we designed a specific questionnaire for Anatomy in Medical and surgical degree, in order to evaluate the current status of the subject and study the necessary changes to adapt it to the European higher education area. Our students where also asked about the competences they must acquired in this subject in the future degree within the European higher education area, in comparison with the present curriculum. The questionnaires estimated, on a scale from one to five, the adequacy of these aspects mentioned.

From all of the results obtained we want to remark those in direct relation with the quick implantation of the European higher education area. So, our questionnaires suggest the great importance and necessity the students of medical and surgical degree give to the subject of Anatomy in both courses. In theory hours, multimedia presentations and the use of blackboards where both well considered. The study with anatomical pieces and dissection were the better evaluated resources for an optimal learn. On the other hand, our students consider few the hours dedicated to practice and the need of more dissection bodies as the most important aspects that must we improved. In conclusion, we can affirm that the students value positively our subject and the future competences proposed for Anatomy in the European higher education area, and so, only a few of aspects must be optimized, in order to acquire all of them proposed for our subject in Medical and surgical degree.

D-P-03 ANATOMY IN PHYSIOTHERAPY GRADUATE FORMATION. PEDAGOGIC VALUATION AND ITS ADAPTATION TO THE EUROPEAN HIGHER EDUCATION AREA

Hita, F.[1], Caba, O.[1], Martínez-Amat, A.[1], Melguizo, C.[2], Prados, J. C.[2], Ortiz, R.[2], Rodríguez-Serrano, F.[2], Vélez, C.[2], Boulaiz, H.[2], Rama, A.R.[2], Marchal, J.A.[2], Perán, M.[1], Carrillo, E.[2], Aránega, A.[2]

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The macroscopic study of human body in Anatomy has been always considered one of the most important and valuated contents when designing the teaching in health sciences area, and especially in physiotherapy degree. From the course 2009/2010 the new degrees are going to be implemented gradually and it adaptation to the European Higher education Area, where the student must acquired several competences, both general and specific.

The purpose of our study was analyze the value, by different groups of students of the degree in physiotherapy of the university of Jaén, of several facts in relation with the subject of Human Anatomy (divided in two blocks, Anatomy of the Locomotor Apparatus and General Human Anatomy), given in the first year, and the evaluation of the competences the student must acquired in this subject in the future degree in the European Higher Education Area in comparison with the present curriculum. The students consulted have received teaching in Human Anatomy from the course 2005/2006 to the present. Our questionnaires estimated, on a scale from one to five, the adequacy of different aspects of the course.

We can highlight, between the obtained results, the high valuation given to the suitability and importance to the subject of Human Anatomy in comparison with the rest of the subjects of the degree, the use of multimedia presentations as the most valuated teaching resource, and the need to increase the number of hours of practice teaching, based on the poor assessment of the number of hours employed in the current curriculum. In relation to the competencies that must be acquired in the future degree, students appreciate them positively, and feel that, after passing the signature in the present curriculum, the acquisition of these skills will be highly interesting.

D-P-04 ANALYSIS OF A PILOT EXPERIENCE TO INSTAURATE THE ECTS IN THE MEDICAL SCHOOL OF CÓRDOBA: THE OPINION OF STUDENTS OF THE FIRST AND SECOND COURSES

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Introduction: The Medical School of Cordoba started a Pilot Experience in 2004-05, supported by the Andalusia government, to adapt its Curriculum to the Bolonia guidelines and the ECTS system. The project is based in the development of Academic Activities designed to acquire generic competences and were supervised by a tutor. The experience was evaluated by the students in order to better understand how these new activities improve the learning-teaching process.

Results: The results presented here analyse the opinions of the 1st (n=112) and 2nd (n=88) year students about the changes introduced in this experience. The students fulfilled a questionnaire of 15 questions in relation with the acquisition of competences related with the subjects of these courses, including Human Anatomy.

Results show that the assistance to classes is higher in students of the 1st year than in students of the 2nd year and the time of study per credit is similar in both courses. The activities were evaluated with higher scores by the 1st year students. On the contrary the students of the 2nd course give low scores to all items in most subjects, including Human Anatomy. Only those items related with the information and contents of the activities "succeeded". The students also considered that whereas the Pilot experience contributes to the Acquisition of Competences it does not favour the auto-learning.

Conclusions:

1) The students are sceptic on the contribution of the Pilot experience to the learning process. This scepticism is higher in students of the 2nd year

2) Auto-learning was not well evaluated by the students

3) The evaluation was well considered by all participants in the study as it will help to correct the deficiencies observed.

D-P-05 THE VAK INVENTORY UNVEILS PREFERRED CHANNELS FOR INFORMATION UPTAKE IN STUDENTS OF HEALTH SCIENCE

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Background: All people use preferently one of the main channels for uptaking information in the learning process. The identification of this point may be relevant to facilitate teaching-learning tasks. The aim of this study was to identify the preferred learning style among biomedical students in our institution (UBC).

Material and Methods: An study was performed in the Nursing School and Faculty of Medicine during the aca-

demic year 2008-2009, in a sample comprising 143 students. The instrument used was the "VISUAL, AUDITIVE AND KINESTHETIC" inventory adapted by M.E Romo & D. López, (VAK). Data analysis was based on the contrasting of the differences between the scores in the three preferred channels through the ANOVA test.

Results: An homogeneous distribution of the sample in the three preferences was found. The mean values were arround 18 for visual and auditive and 16 for kinesthetic within Nursing students as well as within Medical ones. The only significative difference between degrees was for the visual channel, that was preferred significanly by nursing students.

Conclusions: In the first year of biomedical studies no differences were showed by students regarding their preferred channels for uptaking the information, althoug visual channel is higher within nursing students. An important point is the application of this instrument to facilitate the decision making about future academic strategies oriented to develop and potentiate individual learning abilities.

Acknowledgements: This work has been supported by PCI Program 09 (AECI-MAEC) and PIE 09 (UBC).

D-P-06 IMPLEMENTATION OF A JUNIOR FACULTY MENTORING PROGRAM IN MEDICINE

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A priority objective to increase the quality of University teaching should be teacher training. In the hands of teachers is where lies the capacity to develop and improve not only the training process, but the University itself. University that "teaches teachers to teach" will ensure that their students '"learning to learn", making future professionals. In the last year University of Granada developed a mentoring program selecting senior teachers with a comprehensive teaching curriculum from each one university centre. The junior faculty mentoring program at Granada School of Medicine has been designed to help the younger faculty members (clinical or academic) plan their teaching careers with the advice of more experienced colleagues. The main objectives are managing a productive academic career, understanding the formal (and informal/implicit) values, rules and operating procedures in academic medicine, providing a forum for sharing wisdom and experience among faculty members and developing and sustaining a network of professional colleagues. Firstly, junior faculty members did a course of introduction to university teaching and, then, mentors were assigned. Our teaching team is comprised of three experienced members and six younger teachers from preclinical (Human Anatomy and Embryology, Histology) and clinical (Medicine, Paediatrics) knowledge areas. We have developed several training activities: 1). Group or individual seminars to provide novice teachers with the necessary information to ensure understanding of the institutional project of the School of Medicine; to approve the timetable for completion of actions and to reflect, share and discuss the needs expressed by junior faculty. 2). The assistance of beginner teacher to classroom of mentor. 3). The establishment of improvement cycles consisting of an initial interview planning, an observation by video-recording of the teaching activity in the classroom, an analysis of the video-recording and an interview of analysis with the junior teacher. 4). The specific practical training workshops; and 5). The evaluation of diaries, reports, comments and questionnaires. Our experience indicates that mentoring program has enabled us to obtain a valuable material for the implementation by the faculty in subsequent courses. Analysis and monitoring of junior teachers throughout the course improves its teacher performance and helps them acquire skills and abilities in the teaching-learning process, which ultimately benefit the students of the Faculty of Medicine.

D-P-07 TEACHING INNOVATION PROJECT: VOLUMETRIC REPRESENTATIONS AS A METHODOLOGY FOR IMPROVING THE SELF-TEACHING IN THE ANATOMY AREA

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The European Higher Education Area implies the use of active methodologies in which the student is the main objective in the teaching-learning process. In our Area, Health Sciences, it is essential the development of systems for applying principles of self-teaching. In these systems the integration of basic and clinical knowledge improves the academic formation and the professional skills of our students. These professionals have a need to increase the compression of medical imaging (diagnosis, treatment, surgical planning, education, etc.). The visualization of volumes from medical imaging is the process of transforming a precise description of an area or volume obtained from a captured medical image in an image composed of pixels. Currently, volumetric representations are being applied in the educational process to allow the student to have a more realistic visual understanding. Specifically, to study anatomical structures, students can benefit from the manipulation of three-dimensional representations obtained from medical images. The objective is to un-

derstand its shape, constituents, relative location and relations. In this context, a Professor Group from the Anatomy Area in the Andalusian Universities, has developed an experience with Health Science students introducing an innovative educational process in which they work with multimedia material of the most complex human anatomy regions allowing an active teaching method.

D-P-08 NEW STRATEGIES ON AUTONOMOUS WORK: DEVELOPMENT OF A MULTIMEDIA SUPPORT IN RADIOLOGICAL ANATOMY

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The convergence process and the instauration of the European Area of Higher Education are leading on inevitable form to the adoption of renovation process that affect the educational methodology. Within this instauration the development of strategies in which the student is protagonist of its own learning is receiving everyday more and more importance, approaching the work of the academy staff to the development of an activity of guide and regulation of this process. The application of new didactic strategies and development of materials whom adapting will be fundamental for the success of the process.

In this European Area of Higher Education context, a new learning material with useful application as a new interactive methodology strategy has been developed by differents Academic Staff of Anatomy and Human Embryology Area involved in education at Schools and Faculties of Health Sciences. This project tries to create an application that allows to the user the learning of the different bony pieces, its components, its positions and directions in the skeleton, as well as its identification in X-ray images. We made a geometric data base with each one of the bony pieces to study, with a textual identification of each one of its constituent elements, as well as a data base of X-ray images to identify the elements study object. This new method allows student individual work supervised by the Academic Staff.

The above mentioned material is involving a set of knowledges of significant complexity that are common to the different studies of Health Sciences which has enforced collaboration among different professionals. At the same time this material allows its application to an ample rank of students. The result has been the development of an application which allows the individual study in fundamental elements of osteology and their application on understanding the radiological standars of normality. This material will be able to be used in the processes of education learning through alternative didactic systems.

Grant support: This study was support by the University of Jaén trought the "Proyectos de Innovación Docente": La Anatomía Radiológica: desarrollo de una herramienta para su autoaprendizaje (proyect. nº PID20B).

D-P-09 USING NEW TECHNOLOGIES IN MEDICAL ILLUSTRATION FOR TEACHING OF ANATOMY

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Medical illustration allows the meeting between health sciences and art. It begins when the medical teacher needs to express his/her knowledge to other people using visual language, and a closed collaboration between different professionals of different areas of science and art converts Illustration in a multidisciplinary work.

There is no doubt that illustrations are necessary for teaching, and Anatomic Atlas and the illustrations in teaching books, have brought a great benefit for learning. If illustrations are objective, rigourous and efficient we can give better information to people interested in our subject or presentation. Illustrator must be formed in this area acquiring the necessary tools to warrant an optimal quality of teaching.

We show here some examples of the use of new technologies , the new digital programs for image treatment, to improve medical illustrations obtained from different resources: drawing and photography, and show the previous and final results of these works, after applying the Adobe Photoshop program (CS 3) with its different tools of treatment of the image.

Our results confirm that combination of digital methods with traditional art improves a presentation and allows reduce time and effort in our work. Other advantages of digital methods is the possibility of modifying the image, adapting to the new scientific knowledge, the edition of the image and the translation to different formats. It is necessary to signalize that experience of the medical illustrator gives to the image its own character, that makes it personal.

We claim here the importance of Medical Illustration as a Scientific-Artistic Method for teaching Anatomy, as well as other Medical Sciences, to students and any other person who is interested in this subject, and the importance that the development of this kind of work could be a result of cooperation between the artists and the medical professionals, or that this kind of specific formation could be included as an optional subject in the curriculum of medical and art students in the universities.

D-P-10 THE WEBCT SYSTEM FOR TEACHING ANATOMY BY USING RADIOLOGICAL IMAGES

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We have designed a virtual course of Anatomy based on the study of radiological images with the purpose of rein-

forcing masterly lessons and practical teaching. We have chosen the subject of "General Anatomy and Embryology" of the first course of the Degree in Medicine. Previous seminars were developed on Anatomical Radiology to prepare each section. The impact of this novelty in our teaching was evaluated by screening the opinion of the students and by comparing the results of the course respect previous courses. Conclusions of this process are presented in this communication.

D-P-11 SEXED OR GENDERED BODIES: A CRITICAL ANALYSIS OF HUMAN BODIES IMAGES

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The biomedical sciences described and visually presented a "universal model" of human being who presents the characters of *men sex, white race and heterosexual behaviour.* The last years I achieved a critical analysis of the discourses about sexual differentiation of the brain and differential behaviours of women and men. I analysed the different levels of scientific knowledge: knowledge generation in the original articles, knowledge transmission in review journals and finally the university text books, a kind of scientific text which synthesize the standing theories. My hypothesis is that the gender bias progress with the discourse is generalized. The images are included in the scientific discourses and present the same gender bias. I analysed 16.329 images of 12 university anatomical text books recommended, at present, in 20 of the more prestigious Universities in Europe and North America. The images of women bodies in the neutral regions (regions with the same content in women and men bodies) are four times less than men bodies. The white race is the only present in 9 texts and mainly in the rest. I observed social and cultural gender bias in the anatomical images: more or less thinness in women bodies, bodies playing differential sports or the representations of different systems in women and men bodies: central nervous system and arm (including the hands), mainly, in men bodies, circulatory system in women bodies, the eyes are mainly in women faces and the mouth in men faces. It seems that the messages of the anatomical images are: men mind and manipulation versus women nutrition, or, women can see but cannot speak? The results confirm the initial hypothesis and demonstrate the gender bias of the university anatomical texts.

D-P-12 PERCEPTIONS OF CADAVER NECESSITY IN LEARNING GROSS ANATOMY BY STUDENTS

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Generalization in use of electronic media in medical education and, particularly in learning and teaching gross anatomy, besides the cost- requirement of running dissecting rooms, have developed the concept, in many people (including some medical teachers), that cadavers and dissecting rooms are not useful in these days. The aim of this study has been to know the perceptions of the use of cadaver in learning anatomy by students in the age of computers and imaging techniques.

We develop a questionnaire, which was filled for second year students from University of Las Palmas de Gran Canaria in the course 2008/2009. The students have had the experience of learning anatomy with and without cadavers as a consequence of closing in dissecting rooms, due to high levels of formaldehyde during some months. The items including two aspects: 1 assessment of cadaver dissection versus other teaching methodologies, and 2 advantages and disadvantages of cadaver in learning anatomy.

The survey was answered for 66 students. Although most of the students considered that cadaver could not be replaced for other teaching methods, 26% of students though that learning with cadavers must be voluntary. 25% of students considered that cadaver could be replaced by multimedia resources, image projections and films. The main disadvantages considered by students were: 1 bad odor, 2 difficulty in recognizing structures and 3 too much work. The main advantages considered were: 1 visualization of structures in 3D and 2 setting of learned knowledge.

D-P-13 EXPERIMENTAL PROCEDURE OF A NEW METHOD OF EMBALMING AND CONSERVATION FOR HUMAN ENTIRE CORPSES

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The use of human corpses in the teaching and research of our discipline, the Human Anatomy, has generated a specific problematic that already worried and occupied the Anatomical Spanish Society in the First Symposium about Facilities and Environment of a Room of Dissection, celebrated in Barcelona in 1996. One of the analyzed aspects was the relative one to the conservation and storage of the corpses. Following the recommendations contributed in this field, we consider to design a global methodology capable of being applied to the set of procedures used for the embalming, intravascular repletion and conservation of human entire corpses. From the obtained results it is possible to conclude that with our global method of embalming with intravascular injection and conservation of complete corpses: 1.-It is not possible to diminish the concentration of formalin in the liquid of embalming without loss of its conservative capacity; 2.-The organoleptic characteristics of the embalming solution are improved; 3.-There is facilitated the identification of the vascular tree of entire corpses fixed with formaldehyde, introducing a mass of repletion based on natural latex, by means of an own procedure that we have named CALIVIS (CAbanes Latex IntraVascular Injection System); 4.-The hardening of the tissues is corrected and the formalin concentration is neutralized, using the osmotic

flow between the embalming liquid of the raft and the fluids of the corpse; 5.-The formalin concentration is diminished in the embalming liquid without loss of its conservative capacity, diminishing the environmental pollution in the rooms of dissection; 6.-There is extended the spectrum of antimicrobial activity of the embalming solution.

D-P-14 DEVELOPMENT AND PERSPECTIVES IN THE FUTURE OF THE HUMAN ANATOMY IN THE NEW SPANISH MEDICAL CURRICULUM

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A comparative analysis of the differences observed in the design of the new curricula for medical students among the spanish universities is made.

Special attention is pointed on: the number of ECTS, specific competences and integration with clinical materies.

The experience in the design for the university of Salamanca is resalted.

D-P-15 THE CHAEA QUESTIONNAIRE, A USEFUL TOOL TO KNOW THE LEARNING STYLES IN STUDENTS OF FIRST COURSE IN BIOMEDICAL STUDIES (MEDICINE AND NURSING)

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Background: Bologna process brings numerous changes in High Education in Europe. Every change should be based on a deep knowledge of the learning method of students. This paper aims to identify the prevalent learning style among biomedical students (medicine and nursing) at our institution (UBC).

Material and Methods: The study was performed in the Nursing School and Faculty of Medicine during the academic year 2008-2009, in a sample comprising 293 students. The instrument used was the Honey-Alonso (CHAEA) learning style questionnaire. Data analysis was based on the contrasting of the differences between the scores in the four learning styles, through the ANOVA test.

Results: Although quantification for all styles showed middle values, the leading style is the reflexive, with a mean value of 12,7 being higher within Medical students (12,9 vs. 12,5). Theoretical style showed also values slightly higher than middle ones (mean 11,5) being higher in Nursing students (12 vs. 11). In contrast, pragmatic style is slightly lower than middle value (9,2) with similar values for both degrees. Active style is the only that shows significant differences between qualifications, being the mean 9,5. Values are 10 in nursing and 9 in medicine.

Conclusions: Our results indicate that the methods of learning do not change among the students of medicine and nursing, showing in both cases a preponderance of reflexive style over active, being this significantly higher in Nursing students against Medical ones. The rest of styles do not show significant differences.

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E-P-01 – E-P-014 Embryology

E-P-01 DEVELOPMENT OF THE GLENO-HUMERAL JUNCTION: MORPHOMETRIC ANALYSIS

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The early development of the synovial joints within the blastemal mesenchymal cells is distinguishable between the osseous primordial of the prechondral mesenchyme. This cellular accumulation is generally referred as the "interzone" (IZ) and has been proposed as the joint cavitation site and an origin of cells to the articular cartilage.

The objective of this study was to analyze by means of morfometry the transformations in the tissues during the formation of the cartilage of the glenohumeral joint (GHJ). This work concerns to the first phase of the most wide study of the embryology of the synovial joints, which will include also immunohistochemical and molecular analyses (BIO2005-08190).

Embryos of Swiss CD-1 mice of ages (E12-E16), that were including the stages of formation of the GHJ, were submitted to histological conventional techniques. Digital microphotographs (DMPs) at x50 of the histological sagital sections was obtained (Delta Pix® camera), with the extend depth of focus tool activated. From the DMPs a reconstruction of the GHJ image of each section was made. Segments on each articular image were obtained by means of the cells of a lattice superimposed. Histological sections and lattice cells were randomly chosen. Lattice cells were analyzed by Visilog 5: cellular density and parameters of the cellular nucleuses (area; shape indices and orientation). Before the beginning of the joint cavitation (E12) the cellular density was homogeneous so much in the bony primordia (head of humerus (H) and scapula-glenoid cavity and surroundings - (S)) as in the IZ. During the joint cavitation, the cellular density descended globally and the cellular nucleuses in the H and S grew and became more round. The cells near the joint cavity (JC), presumably derived from the IZ, had the nucleuses bigger that in the IZ, but were more lengthened in the concave articular surface (S) and more round in the convex one (H); with regard to the most distant cells of the JC, the nucleuses were globally more similar in the H than in the S.

Conclusion: The morphometric changes observed suggest a radial dynamics of development from the IZ in the formation of the joint cartilage.

E-P-02 INTERACTIONS BETWEEN TGF-B1 AND EGF AND ROLE OF EGF IN THE CLEFT PALATE PRESENTED BY TGF-B3 NULL MUTANT MICE

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We have previously demonstrated interactions between TGF-β1 and TGF-β3 during palate development, and that the presence of both EGF and TGF-β1 is altered in the *Tgf-β3* null mutant mouse (*Tgf-β3* -/-) palate, which shows cleft palate with reduced palatal shelf adhesion and fusion and medial edge epithelium (MEE) cell death. The aim of this work has been to determine whether there are interactions between TGF-β1 and EGF in the palate of *Tgf-β3* -/- mouse palates and whether the changes in the presence of EGF play any role in the reduced palatal shelf adhesion and fusion of these mice. We have performed cultures from embryonic day 13.5 *Tgf-β3* -/- isolated palatal shelves, adding either PBS (control) or TGF-β1 and analyzed the presence of EGF by mean of immunohistochemistry. We also added either PBS (control) or Tyrphostin (which neutralices the EGF receptor) to 18 or 36 hour paired palatal shelf cultures and analyzed palatal shelf adhesion and fusion and the cell death occurring in the adhered opposing MEE. Our results show changes in the presence of EGF in the palate of mice treated with TGF-β1, thus indicating the existence of interactions between them, as well as a great increase in palatal shelf adhesion and fusion and MEE cell death in those cultures in which the EGF action was blocked. We thus conclude that in the *Tgf-β3* -/- mouse palate there are interactions between EGF and TGF-β1, and that the changes observed in the presence of EGF in them have some responsibility in the reduction of palatal shelf adhesion and fusion and MEE cell death presented by these mice.

This work has been supported by a grant from Fondo de Investigación Sanitaria (PI06/0184) and Universidad Complutense de Madrid /CM in 2008.

E-P-03 A FOLIC ACID DEFICIENCY ALTERS TGF-β3 GENE EXPRESSION AND PALATAL CELL PROLIFERATION

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We have previously demonstrated that the progeny of mouse females under a folic acid deficient (FAD) diet for more than two weeks develop cleft palate. Both palatal shelf adhesion and fusion in these mice are reduced, whilst mesenchymal ossification seems to be compromised as well. These alterations occur in the *Tgf-β3* null mutant mice, which also bear cleft palate. As occurs for TGF-β, a folic acid (FA) deficiency could compromise the expression of *Tgf-β3* during palate development. The aim of this work has been to investigate whether mouse embryos with a FA deficiency have a reduced expression of *Tgf-β3* in the developing palate and palatal cell proliferation. We have performed *in situ* hybridization with a *Tgf-β3* rivoprobe and

BrdU essay on embryonic day 14.5 mouse embryos progeny of C57 mouse females fed with either a 0% folic acid + 1% succinil sulfathiazol or a control diet for 2 or 8 weeks. Proliferating cells were measured by mean of the Metamorph image analyser program. Our results demonstrate a reduction in the expression of the *Tgf-β3* gene and a significant decrease of palatal cell proliferation in the mice under a FA deficiency. Both may contribute to the appearance of the cleft palate presented by these mice. We thus conclude that a FA deficiency alters the expression of the *Tgf-β3* gene and compromises palatal cell proliferation.

This work has been supported by a grant from Fondo de Investigación Sanitaria (PI06/0184) and Universidad Complutense de Madrid /CM in 2008.

E-P-04 IMMUNOHISTOCHEMICAL STUDY OF THE DEVELOPMENT OF THE SYNOVIAL MEMBRANE OF THE TEMPOROMANDIBULAR JOINT OF HUMAN FETUSES – PARTIAL RESULTS

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The temporomandibular joint (TMJ) has a crucial function in the movement of the mandibular complex. It is defined as a double joint of the synovial type because of its anatomical characteristics. Just like all synovial joints, the TMJ is involved by a synovial membrane that lines the internal surface of the articular capsule. Finger-shaped projections of the synovial membrane into the TMJ are villi that occur in the anterior and posterior ends of the joint. The synovial membrane consists of two layers: the cellular intima and the other supporting layer, the vascular subintima. The intima layer is made up of cells embedded in an amorphous and fiber-free matrix roughly 1 to 4 cells thick. Previous studies performed in our laboratory found two types cells lining the synovial membrane of the TMJ of human fetuses: macrophage-like type A cells and fibroblast-like type B cells. The macrophage-like type A cells are characterized by the presence of a Golgi apparatus, vesicles and vacuoles dispersed in the cytoplasm, a scarce rough endoplasmic reticulum, dense nuclear chromatin patterns and rare nucleoli, and their function is to synthesize proteins, glycoproteins and proteoglycans. Meanwhile, the fibroblast-like type B cells have a greater amount of rough endoplasmic reticulum. Therefore, these cells are likely to have a phagocytic capacity and translate more than the macrophage-like type A cells. The objective of our study at this point is to determine in which weeks the development of the synovial membrane of human fetuses begins and ends and verify what happens weekly to this structure during the entire gestation period. The immunohistochemical PECAM-1 antibody staining protocol will be used to determine when synovial membrane development begins and ends since it reacts with both macrophage-like type A cells and fibroblast-like type B cells. Fetal ages in weeks were determined by measuring the crown-rump length. This study was approved by the Research Ethics Committee of the Federal University of São Paulo, Brazil, and is being done in partnership with the Department of Anatomy and Embryology II of the Complutense University of Madrid.

E-P-05 TYMPANIC OSSICLES AND PHARYNGEAL ARCHS

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We have performed a study on 11 human embryos regarding the development of the tympanic ossicles and their relationship with the first pharyngeal arches.

After performing measurements to chronologically date the embryos and foetuses, we performed a meticulous dissection of the temporal bones. Afterwards, they were fixed in 10% formol, decalcified with 2% nitric acid, embedded in Paraplast, sectioned in 7-mm sequences and stained with Martin's trichrome technique.

In the 21- and 24-mm CR human embryos, we have observed the head of the malleus and the body of the incus close to Meckel's cartilage, in addition to the handle of the malleus, the long limb of the incus and the stapes. Between them there was a mesenchymal band inside the primordium of the tympanic cavity.

In the 27-mm CR embryo, the various components of the malleus and incus are fusing, and in the 30-mm CR embryo the union is complete.

From our observations, we can conclude that the malleus and the incus are derived from the first and second pharyngeal arches.

E-P-06 THE SUPRACOCHLEAR CARTILAGE IN HUMAN

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The supracochlear cartilage is an accessory cartilage of the chondrocranium that appears during development. Our group has studied this cartilage in 5 human specimens of the Embryology Institute Collection of the Complutense University of Madrid. The specimens range from 10 to 12 weeks of development.

During the period of development studied the supracochlear cartilage is related to the trigeminal nerve ganglion, cavum of Meckel´s duramater and otic capsule.

Its ossification gives rise to the petrosal bone (Meckel,1903) or the supracochlear bone (Orts Llorca, 1986).

E-P-07 ALGORITHMS-BASED MODELING OF MYOCARDIAL DEVELOPMENT

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One aim of stem cell research is to clarify the mechanism underlying organogenesis and histogenesis. In fact, there have been proposed a lot of protocols directed to differentiate human embryonic and other stem cells toward myocardium. Our objective is to develop an algorithms-based bioinformatic model able to predict myocardial differentiation from stem cells. For that purpose we constructed a database including results of papers related to cardiomyocyte differentiation, and control assays performed in our laboratory. Data comprised input and output variables. Input variables concerned the conditions used in the experiments, such as stem cell type and pro-differentiative factor. Output variables characterized the quality of stem cell differentiation, based, e.g., on morphology and expression of cardiomyocyte markers, and was summarized in an empirical quality index ranging from 0 (no differentiation) to 10 (total differentiation). Then, data were typified and used to generate theoric models implemented in Matlab platform, with the algorithms "Support Vector Machine (SVM)" and "CART Decision Tree (CDT)". We configured the predicted results of models in two formats: multi-class classification, i.e., the predicted quality of each experimental design was expressed as a number between 0 and 10, and binary-class classification, which only determines whether the process of directed differentiation has been successful or not. Once the models were compiled, 100 new sets of input values, all them with known empirical quality index, were used to validate the correspondence of that empirical quality indexes and the theoretic quality predicted by the models. We found that for binary-class classification of predicted results, CDT generates a model with more success rate (95.83%) in the correct classification of all samples, than that generated with SVM (81.25%). In addition, sensibility was quite similar for both algorithms, 0.983 for SVM, and 0.975 for CDT, however specificity was significantly higher in CDT [1] than in SVM (0.5). Regarding to multi-class classification of predictions, mean quadratic error of the model programmed with CDT was lower (3.08) than with SVM (4.42). In summary, our results propose that bioinformatic models based on predictive learning algorithms SVM and specially CDT are suitable for modeling myocardial development from stem cells.

E-P-08 ALGORITHMS FOR DESCRIBING CARDIAC MORPHOMETRY IN CARNEGIE STAGES 15 TO 23

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We performed a morphometric study of cardiac development on human embryos to complement the scarce data on human embryonic cardiac morphometry and to attempt to establish, from these, algorithms describing cardiac growth during the second month of gestation.

Methods: 30 human embryos from Carnegie Stages 15 to 23. Shrinkage and compression effects from fixation and inclusion in paraffin were considered in our calculations.

Results: Growth of the cardiac volume and volume of ventricular myocardium through the Carnegie stages were analysed by Anova. Linear correlation was used to describe the relationship between the ventricular myocardium and cardiac volumes. Comparisons of models were carried through the R^2 statistic.

The relationship volume of ventricular myocardium (VVM) versus Cardiac (C_v) is expressed by the equation: $C_v = 0.6266 + 2.4778$ VVM.

The relationship C_v versus Crown-Rump (CR) length is expressed by the equation:

$C_v = 1.3\ e^{0.126\ CR\ length}$.

Also, the relative percentage increase of the cardiac volume (C_v) in relation to embryonic CR length was analysed., where denotes the cardiac volume. This exponential formula is: $(C_v /2.81)\ x100= 46.67\ e^{0.126\ CR\ length}$.

Conclusion: At a clinical level, these results can contribute towards the establishment of a normogram for cardiac development, useful for the design of strategies for early diagnosis of congenital heart disease. They can also help in the study of embryogenesis e.g. in the discussion of ventricular trabeculation.

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E-P-09 BMP-2 IS NEGATIVELY REGULATED BY FGF-8 IN CARDIAC SPECIFICATION DURING EARLY GASTRULA STAGES

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During early gastrulation, groups of cells from the epiblast, known as precardiac cells, ingress through the rostral primitive streak. These cells migrate in an anterior-lateral direction to form a crescent of mesoderm, between the ectoderm and the endoderm, in the most anterior and lateral part of the embryo. This crescent constitutes the mesodermal precardiogenic area or primary heart field, and it forms the endocardial and myocardial progenitors of the bilateral heart rudiments.

Furthermore, the specification of cardiovascular fate requires the close association between the mesoderm of the primary heart field and the adjacent endoderm, with the participation of several molecular factors. Evidence suggests that *Nkx2.5* expression is induced by Bone morphogenetic protein (Bmp) 2, emanating from the adjacent endoderm, also regulated by FGF. We have analyzed the effect in early cardiogenesis of *Fgf8* overexpression in cardiac precursor cells. For this purpose, we have electroporated Fgf8 in precardiogenic cells as they pass the primitive streak. Our study has been complemented by experiments of ectopic administration of FGF8 protein, and also by quail-chick grafts of the rostral end of the primitive streak at the particular stage in which this tissue expresses *Fgf8* but no *Bmp2*. We observe that *Bmp2* expression in the endoderm adjacent to the primary heart field is negatively regulated by Fgf8. Moreover, the domains of expression of *Bmp2* and *Fgf8* in the endoderm do not overlap, being *Fgf8* expression medial to that of *Bmp2*.

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E-P-10 MYOGENIC POTENTIAL OF MULTIPOTENTIAL CELLS FROM HUMAN ADIPOSE TISSUE

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The human body has a strong ability to rebuild bone, cartilage and tendon damage, thanks to the regenerative capacity of progenitor cells present in the structures injured. More attractive still is the possibility of using stem cells capable of differentiating into mesenchymal tissues. Mesenchymal stem cells (MSC) can be obtained from various sources, among which the lipoaspirates provide a large population of MSCs.

Methods: We isolated hMCS from human lipoaspirates to research differentiation capacity into myogenic tissue. The adipocytes were separated from stromal vascular cells in a process of sedimentation and aspiration. The cells obtained were cultured and after 72 h non-adherent were withdrawn. After 3-5 weeks showed a homogeneous population. hMSC were cultured with myogenic induction medium supplemented with hydrocortisone for 5-6 weeks.

Results: hMSCs adherented, grew confluent and formed a kind of morphology fibroblastoide. The phenotype of these cells was identified as CD34 and CD45 negative (hematopoietic markers) and showed mesenchymal markers surface expression (CD90, CD73 and CD105). Having acquired myogenic induction, morphological changes were observed in the culture. The cells were started in parallel and showed a more elongated morphology. Immunocytochemical analysis showed for actin, actinin, and troponin T positive staining. These proteins form part of the contractile apparatus of muscle cells (thin filaments), and desmin, a protein of the intermediate filaments.

Conclusion: The hMSCs in culture medium supplemented with hydrocortisone showed ability to express proteins of the cytoskeleton of muscle cells. A better understanding of genetics and molecular control of this differentiation process, can provide information about how to tackle diseases of muscle type suggesting new therapeutic strategies in the future.

E-P-11 OSTEOGENIC DIFFERENTIATION OF ADIPOSE TISSUE-DERIVED MESENCHYMAL STEM CELLS FOR THE TREATMENT OF BONE DISEASES

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The most important potential application of human stem cells is the generation of cells and tissues that can be used for cell-based therapies. Currently, donated organs and tissues are used to replace damaged tissues, but the need for tissues and organs for transplantation is not substituted in this way. The lipoaspirate fat obtained from liposuction procedures, is being considered in recent years as a unique

source of a large population of stem cells with the ability to differentiate into tissues derived from mesoderm.

Methods: To investigate the ability of osteogenic differentiation of hMCS, we have isolated from this cell population lipoaspirate fat, which was digested 2 h at 37 ° C with collagenase type I. The adipocytes were separated from stromal vascular cells in a process of sedimentation and aspiration. The cells obtained were cultured and after 72 h non-adherent ones were withdrawn. After 3-5 weeks a homogeneous population was revealed. Cultures of hMSC were cultured for 4 weeks with osteogenic differentiation medium consisting basically of 3-glycerophosphate and ascorbic acid.

Results: under the conditions outlined, hMSCs grew in a confluent and adherent way, turning into fibroblast-like cells. The obtained cells were negative for CD34 and CD45 (hemotopoietic markers) and showed high expression of CD90, CD73 and CD105. After osteogenic induction a significant change in cell morphology was observed and staining with Alizarin showed in three weeks time the induction of an intense red staining demonstrating the presence of calcium hydroxyapatite crystals typical of this type of tissue. Successive staining performed in the 4th and 5th week of the steogenic induction showed a gradual increase in staining confirming the differentiation process.

Conclusions: The fat lipoaspirate is now an optimal source for obtaining mesenchymal stem cells capable of osteogenic differentiation, which offers the possibility of a new resource to replace tissue and bone cells in a wide variety of diseases.

E-P-12 HUMAN ADIPOSE-DERIVED MESENCHYMAL STEM CELLS: NEURONAL DIFFERENTIATION POTENTIAL

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During the last few years, a number of studies have addressed the phenotypic plasticity of MSCs. Human mesenchymal stem cells (hMSC) can be isolated from adipose tissue and exhibits stable growth and proliferation kinetics in culture. This cell population is known to differentiate *in vitro* into osteocytes, chondrocytes, fibroblasts and adipocytes. Recently, it was reported by several groups that MSC can also differentiate into neural cells under appropriate stimuli.

Methods: To investigate differentiation capacity of hMCS into neural-like cells we isolated from human lipoaspirates. The tissue was digested for 2 h at 37°C with collagenase, adipocytes were separated from stromal vascular cells after sedimentation and removed by aspiration. Cells obtained were then cultured, after 72 h, non-adherent cells were removed and thereafter medium was changed twice a week. A homogenous cell population was normally obtained after 3 to 5 weeks of culture. The hMSC were cultured with neural differentiation medium contained epidermal and fibroblastic growth factor. Cells were either fixed for immunocytochemistry or flow cytometry to analyze their phenotype and differentiation potential.

Results: Our results demonstrated that MSCs were obtained after 3 days culture and an adherent monolayer was achieved 15 days later. The cells generated a confluent layer of elongated, fibroblastic-shaped cells. hMSCs were negative for CD34 and CD45 hematopoietic markers and there was a high expression of CD90, CD73 and CD105. When hMSCs were cultured with EGF and bFGF, they moved slowly from a fibroblastic to a neural morphology and, exhibited highly refractive cell bodies and prominent process-like extensions after 2 and 3 weeks. Immunocytochemical and flow cytometry analysis showed that 5% ± 2.4% of untreated MSCs were weakly positive for GFAP and 30% ± 4.5% for nestin. After 2 weeks of induction neuronal, differentiated hMSCs were positive for GFAP (22% ± 4%) and nestin (90%± 5.2%).

Conclusion: hMSCs under specific conditions (with growth factors such as bFGF and EGF), normally used for growth and maintenance of neural stem cells, can show neural markers normally expressed at various stages of neuronal development, support neuronal differentiation for a long time.

E-P-13 EXOGENOUS NUCLEOSIDES PROMOTE FAT-DERIVED MESENCHYMAL STEM CELL PROLIFERATION

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Adult stem cells are becoming the best option for regenerative medicine due to they lack pluripotency and they permit autologous transplantation even without *in vitro* culture. However, adult stem cells can undergo less replicative cycles than embryonic stem cells, and this fact could restrict the obtaining of enough stem cell biomass required for an adequate therapy. For its part, numerous papers published in the last years stated that the chemotherapeutic agent 5-azacytidine (5-aza) induces cardiomyocyte differentiation of stem cells cultured *in vitro*. The objective of our research was focused in evaluating the action of nucleoside mixtures NT (thymidine, guanosine, cytidine and inosine; 100µM each) and NU (uridine, guanosine, cytidine and inosine;

100µM each) on adult stem cell proliferation with or without co-treatment with 5-aza. For that purpose, mesenchymal stem cells (MSC) were isolated from human adipose tissue. On one hand, after characterization, multipotency of MSC was studied with the treatment of cells with 10µM 5-aza 24h, and posterior maintenance for 30d with normal culture medium. Then, cardiomyocyte marker expression was quantified. On another hand, we determined the inhibitory concentration 50 (IC50) of 5-aza, and the proliferation rate of MSC cultured for 72h with or without exogenous nucleoside supplementation, and with or without co-treatment of cells with 10µM 5-aza. We found that isolated MSC expressed mesenchymal stem cell markers CD73 (85.8%), CD90 (96.4%) and CD105 (97.5%) but low hematopoietic-related markers CD133 (5.9%), CD34 (1.5%) and CD45 (5.6%). After 30d of culture in cardiomyocyte differentiation conditions, multipotency of fat-derived cells was confirmed as they expressed cardiac troponin I (13.4%), troponin T (61.5%) and myosin light chain 2 (56.9%). Proliferation assays showed antiproliferative effect of 5-aza (IC50: 5.37µM), and that nucleosides significantly stimulated proliferation of MSC maintained with NT (112.6% respecting to 100% of nucleoside free control group) and especially NU (129.3%) nucleoside mixtures. Moreover, the antiproliferative effect of 5-aza was partially reverted by the supplementation with NT (119.1%) or NU (141.5%) mixtures. In summary, exogenous nucleosides promote proliferation of mesenchymal stem cells, and thus, our results support a nucleoside supplementation of medium for expansion and maintenance of those cells in culture.

E-P-14 RETINOIC ACID SIGNALING MODULATES DIGIT OUTGROWTH

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In an attempt to characterize interdigital signals responsible for digit morphogenesis we performed a systematic quantitative analysis of gene expression by real-time quantitative PCR in the three interdigital spaces of the chick leg bud. We have observed that raldh2, which is responsible for the local production of Retinoic Acid (RA), exhibits a dramatic antero-posterior increasing gradient of expression, which is in part coupled with an opposite gradient of cyp26 responsible for the degradation of RA. In addition we found that local application of RA into the interdigit causes a significant elongation of the skeletal elements of the digits located anteriorly to the RA source. We further demonstrated that RA is a cell proliferation promoter and that absence of RA synthesis caused a dramatic drop in cell proliferation. These findings are suggestive for a role of RA signaling in the differential outgrowth of the digits in the latest stages of limb morphogenesis.

N-P-01 – N-P-021 Neuroanatomy

N-P-01 THE HUMAN PACINIAN CORPUSCLES: ONLY MECHANORECEPTORS?

Perez-Moltó, F.J. *[1], Calavia, M.G.[2,3], Cobo, T.[2], Montaño, J.A.[4], García-Suárez, O.[2,5], López-Muñiz, A.[2], Soriano-Lafarga, M.A.[6], Vega, J.A.[2,7]

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Pacinian corpuscles are complex mechanoreceptors widely distributed in the body, whose immunohistochemical profile and protein composition are now rather well known. Typically they are supplied by a single axon with mechanosensory characteristics although poly-innervated Pacinian corpuscles have been also described in the normal human skin, and additional innervation by unmyelinated axons has been also found in Pacinian corpuscles from the cat mesentery. The true nature of these axons, sensory or sympathetic, remains to be established. This study analyzes the immunohistochemical profile of the axons supplying human cutaneous and pancreatic Pacinian corpuscles. Specific antigens of the mechanosensory axons (RT97), the A?- and C-sensory fibres (CGRP, SP, and VRT1), the corpuscular Schwann-related cells forming the inner core (S100P, vimentin), and the endoneurial-perineurial cells forming the outer core and the capsule (EMA, GFAP, vimentin), were investigated using immunohistochemistry; several growth factor receptors ($p75^{NTR}$, TrkA, TrkB, ErbB4) were investigated as well. Around 60% of pancreatic Pacinian corpuscles were poly-innervated (two to ten axons) and each axon had its own inner core. Conversely, the cutaneous Pacinian corpuscles only occasionally were supplied by two axons and showed invariably a single inner core. All axons expressed the pan-neuronal markers PGP 9.5, NSE, TrkA and TrkB. One or two axons in pancreatic Pacinian corpuscles, and all axons in the cutaneous ones, were RT97. The remaining axons in the poly-innervated corpuscles irregularly displayed CGRP, SP and VRT1. The inner core displayed S100P, $p75^{NTR}$, TrkA and TrkB; the outer core-capsule of the cutaneous corpuscles were immunolabelled for EMA and vimentin. In both kinds of corpuscles ErbB-4 was observed in a zone that encircled the inner core thus presumably corresponding to a growth zone or intermediate layer. Differences between pancreatic and cutaneous Pacinian corpuscles were observed for the expression of GFAP, EMA and TrkB. The immunohistochemical profile of nerve fibers in human Pacinian corpuscles suggests that in addition to be mechanoreceptive they can also serve to nociception. Furthermore, taken together, present results demonstrate that, although cutaneous and peritoneal Pacinian corpuscles are similar in structure and function, they differ in their protein composition.

N-P-02 MECHANOPROTEINS IN MECHANORECEPTORS ARE REGULATED BY NEUROTROPHINS

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In mammals the mechanosensitive structures that are responsible for touch sensation are known collectively as mechanoreceptors, among which are the Pacinian corpuscles. To act as mechanotransducers, the constituents of mechanoreceptors are thought to express ion channels activated by force or displacement. Recently, different members of the degenerin/epithelial Na^+ channel (DEG/ENaC) superfamily of proteins have been proposed as putative mechanosensory molecules. DEG/ENaCs are expressed in subpopulations of dorsal root ganglion neurons, and are localized in nerve terminals at the site of mechanotransduction like touch receptors. Consistently, mice deficient in some DEG/ENaC have defects in specific populations of tactile mechanoreceptors. On the other hand, it has been observed that neurotrophins (NTs) influence the expression of some DEG/ENaCs: BDNF and NT-4, acting through their receptor TrkB, regulate the expression of ASIC proteins; NFG-TrkA can induce expression of β- and γ-subunits of ENaC; NT-3 induces a rise in the expression of ASIC1 and ASIC3. Thus, we reasoned that BDNF or NT-4 signaling via TrkB can specifically control the expression of molecules necessary for the mechanosensory function of Pacinian corpuscles. In this study we used immunohistochemistry to analyze the impact of a targeted mutation in the genes coding for TrkB, BDNF and NT-4 in the expression of ENaC subunits (α, β and γ) and ASIC2 by Pacinian corpuscles from *trkB*, *BDNF* and *NT-4* knockout 15 days old mice. We choose this mechanoreceptor because it is grossly normal in number, structure and immunohistochemical properties in mice carrying those mutations. Wild type Pacinian corpucles express axonic β-ENaC and γ-ENaC, whereas ASIC2 was detected in both the axon and inner core. β-ENaC was absent in animals deficient in both TrkB and BDNF, but no varied in NT-4 deficient mice. No changes in the expression or localization of γ-ENaC were noted in none of the experimental groups. Regarding to ASIC2, it was undetectable in TrkB and BDNF deficient mice, but was independent of NT-4. Thus, present results demonstrate that the expression of Deg/ENaCs

in murine pacinian differently depends on TrkB and BDNF, but not NT-4, and that the expression of mechanosensory proteins in Pacianian corpuscles is regulated by NTs.

N-P-03 THE INTERSTITIAL CELL OF CAJAL (ICCS) IN THE HUMAN INTESTINE

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Introduction: The first studies describing the existence of a different cell type in the intestinal muscular coat of amphibians and several mammals were those of Ramon y Cajal. ICCs are distributed in close relationship with the enteric plexuses. On the basis of the staining characteristics (methylene blue and silver chromate), Cajal consider these cells as a type of primitive neuron. Several possible functions were ascribed to these cells on the basis of their morphology and close anatomic relationships with smooth muscle and neurons. Inmunohistochemical techniques labelling kit (a receptor tyrosine kinase) have provided new ways to unequivocally identifier these cells. However, many questions remain unanswered regarding the plasticity of ICCs.

Objectives: Our aim was to study in ICC of the small intestine of human those ultrastructural and immunohistochemical characteristics that support a possible role of ICCs as multipotent progenitors.

Materials and Methods: Intestinal fragments were obtained from elective surgeries with the patients'consent, as approved by CEICA. Immunostaining and confocal microscopy were used to determine ICCs distribution and interrelationship. The ultrastructure of cells was studied by transmission electron microscopy (TEM).

We have applied specific antibodies against alfa-tubulin, Tuj 1 and neuron specific enolase to identify the different neuronal types of enteric ganglia; c-Kit to characterise ICCs, and CD34 as marker of progenitors cells.

Results: There is a degree of maturation in some neurons of enteric ganglia. Distributed around these ganglia there are interstitial cells that are positive for the c-Kit. In this location we have found that these interstitial cells are also immunoreactives against CD34.

Thick axonal trunks constitute part of enteric plexuses, the nuclei of the neurons present with uniformly distributed chromatin and a prominent nucleolus. Around these ganglia they can be observed ICCs. The nucleus of these interstitial cells is very voluminous and it is surrounded by a small perinuclear cytoplasm that expands with long prolongations. These prolongations extend around the nervous trunks and establish close contacts with some of the axons. Furthermore, these prolongations couple with the prolongations of other ICC located in the connective tissue of the muscular circular layer.

Conclusions: The ICCs in the human intestine present some characteristics of progenitors cells.

N-P-04 ALTERATIONS OF THE CHOROID PLEXUS IN THE SPONTANEOUSLY AND INDUCED HYDROCEPHALUS

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Alterations of the brain structures occur in the hydrocephalus such as cortical displacement, cerebrospinal fluid composition alterations and circumventricular structure variations are described and many of these variations could be the cause or consequence of hydrocephalus. The protein p73 is a member of a family of transcription factors and equilibrium between p73 isoforms is necessary for the normal development of the brain and the maturation of the neuroepithelium, on the other hand, p73 deficiency produces many alterations in the brain including hydrocephalus. The purpose of this work is to analyze the alterations of the choroid plexus (CP) in different kinds of hydrocephalus.

Ten control male rats and ten male rats with spontaneous and induced hydrocephalus were used. The control group was composed of rats sacrificed at 26 and 50 weeks of age and the hydrocephalic rats (kaolin induced hydrocephalus, spontaneously hydrocephalus) sacrificed at the same ages as the control. Brains were cut into four serial coronal sections. The sections were immunohistochemically processed using anti-p73 and anti-transthyretin (TTR) as primary antibodies.

We found structural alterations and a decrease in the p73 immunoreactive material, in the induced hydrocephalus when compared to the control group. In spontaneously hydrocephalus we did not find great structural variations of the CP; however, changes in the intensity of anti-p73 reaction were found with respect to the control. The TTR expression varied with the hydrocephalus, in control rats this was clearly observed in the CP and was increased in the spontaneous hydrocephalus. Obstructive hydrocephalus causes CP morphology alterations and a decrease in the p73 expression, as opposed to the CP, where significant structural variations in the spontaneously hydrocephalus were not found.

N-P-05 RECOVERY OF ASTROCYTIC POPULATION AFTER VISUAL DEPRIVATION: EFFICACY OF ENVIRONMENTAL ENRICHMENT AND PHYSICAL EXERCISE

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During development, exposure to visual inputs modulates cortical maturation, inducing numerous changes in all of the components of the visual cortex. Most of the cortical changes thus induced occur during what is called the critical period. Astrocytes play an important role in the development, maintenance and plasticity of the cortex as well as in the structure and function of the vascular network. Visual deprivation induces a decrease in the astroglial population, whereas enhanced experience increases it. Exposure to an enriched environment has been shown to prevent the effects of dark-rearing in the visual cortex. Our purpose was to study the effects of an enriched environment on the density of astrocytes per reference surface at the visual cortex of dark-reared rats, in order to determine if enhanced experience is able to compensate the quantitative effects of visual deprivation and the role of physical exercise on the enrichment paradigm. Pregnant Sprague-Dawley rats were raised in one of the following rearing conditions: control rats with standard housing (12-h light/dark cycle); in total darkness for the dark-rearing experiments; and dark-rearing in conditions of enriched environment without and with physical exercise. The astrocytic density was estimated by immunohistochemistry for S-100 protein. Quantifications were performed in layer IV. The somatosensorial cortex barrel field was also studied as control. The volume of layer IV was stereologically calculated for each region, age and experimental condition. From the beginning of the critical period, astrocyte density was higher in control rats than in the enriched environment group without physical exercise, with densities of astrocytes around 20% higher at all of the different ages. In contrast, when the animals had access to voluntary exercise, densities were significantly higher than even the control rats. The comparison of the effectiveness of enriched experience and physical exercise on recovery of astrocytic population after visual deprivation, shows that enriched environment is more effective when considering the incorporation of physical exercise. This is even so for sensorial areas such as the visual area, where complex enriched experience by itself is not enough to compensate the effects of visual deprivation [(1)].

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(1). Argandoña EG, Bengoetxea H, Lafuente JV (2009) J Anat doi:10.1111/j.1469-7580.2009.01103.x.

N-P-06 DEVELOPMENT OF AN INCUBATOR FOR EXPOSURE OF HUMAN ASTROCYTES IN CULTURE TO MICROWAVES INSIDE A GTEM-CELL: VALIDATION STUDIES

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Common concern about biological effects of microwaves (MW) in the range of 10 GHz is increasing with the expansion of X-band electromagnetic fields (EMF) for meteorology, governmental institutions applications or, more widely extended, speed and aerial traffic control radars. Therefore, it is important that biological effects of EMF in this range of frequencies be understood at cellular level to reveal the molecular mechanisms and the possible health implications. The aim of this study was to evaluate whether 9.6 GHz MW amplitude modulated by ELF-pulsed EMF at low power density, to rule out thermal effects, induce alterations in the morphology, viability and proliferation of human astrocytes, normal and from astrocytoma, maintained in culture. Experiments inside a GTEM-cell for application of MW to *in vitro* mammal cells represent a problem for maintaining cells in culture in humidity atmosphere, at 37 ºC and 5% CO_2. In some laboratories GTEM-cell are introduced inside rooms maintained at 37 ºC. To get a homogeneous temperature distribution in these conditions is by no means easy and by analysing the observed results it could be considered a non-suitable procedure being temperature a very restrictive experimental physical condition for mammal cells culture. More frequently, after culturing cells in a standard incubator, exposures to MW are performed at room temperature, dropping cells culture temperature up to values of 34 ºC, 27 ºC or even 26 ºC low enough to consider the results reliable. Commercial standard incubators have a heating system based on a metallic plate calefactory, being prone to eddy current formation under MW excitation, with EMF distortion and Joule-heat production. To avoid all these problems we have developed a *GTEM-incubator* (built by Olympus firm under our specifications) for MW exposures inside a GTEM-cell controlling humidity, temperature and CO_2. Before using the GTEM-incubator for MW treatment, we made the *incubator validation*. We present the results obtained by testing: astrocytes morphology, growing cycle curves, viability and expression of cytoskeleton proteins: glio-fibrilary-acidic protein (GFAP), beta- and tuc-tubulins. Astrocytes were incubated for 6 and 24 h inside the GTEM-incubator being control samples maintained in the standard incubator for same periods of time.

N-P-07 CATECHOLAMINE APPEARANCE IN THE ADRENAL MEDULLA OF SPONTANEOUSLY HYPERTENSIVE MICE

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Several authors have described that the BPH/2 hypertensive inbred mouse strain has high blood pressure early in life, compared to its hypotensive BPL/1 and normotensive BPN/3 controls. At 21 weeks of age, the hypertensive mouse has a systolic blood pressure which is 60 mm Hg higher than that of the hypotensive mouse. The difference in blood pressure between hypertensive and hypotensive mice is associated with strain differences in heart rate, heart weight, relative heart weight, left ventricular mass, and kidney weight.

The present study investigated the expression of catecholamines in the adrenal medulla, by using antibodies against tyrosine hydroxylase (TH), dopamine-beta-hydroxylase (DBH), phenylethanolamine-N-methytransferase (PNMT), and chromogranin A (ChA) in 15 spontaneously hypertensive mice (BPH/2) and 15 normotensive mice (BPN/3) sacrificed at 30, 230 and 365 days of age. Western blot study was also performed.

We have found immunoreactive material (ir) for TH, DBH, PNMT and ChA at 30 and 230 days of age in the adrenal medulla of hypertensive mice. In mice at 230 postnatal days of age, the TH-ir was slightly decreased in the BPH/2 adrenal medulla, but DBH and PNMT-ir were increased. These results are partially agree with other works on Chromogranin A mRNA expression where it was also elevated in the SHR adrenal medulla, as opposed to the rodent model of hypertension of the present work, where the hypertensive mouse (BPH/2), adrenal chromogranin A and catecholamine enzymes were diminished at 30 days of age but augmented at 230 days of age compared to the control at the same ages. We conclude that the different expression of chromogranin A and the alteration found in the different enzymes of catecholamines, in the different ages, is a variable feature of mammalian genetic hypertension.

N-P-08 VASOPRESSIN AND TYROSINE-HYDROXYLASE EXPRESSION IN THE HYPOTHALAMUS IN SYNDROME OF INAPPROPRIATE ANTIDIURETIC HORMONE SECRETION

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Syndrome of Inappropriate Antidiuretic Hormone Secretion (SIADH) is a rare disease characterized by hyponatremia and hyperosmolality of urine. There are well known causes of this syndrome, such as neoplasmatic processes, different disorders of central nervous system, lung diseases and side effects of various drugs. Abnormalities in water balance are manifested in SIADH as sodium disturbances-hyponatremia and hypernatremia. The aim of the present work is to analyze the hypothalamic distribution of vasopressin (VAS) and dopamine-beta-hydroxylase (DBH) in a case of SIADH.

We used two hypothalamuses from human brains from the Anatomy Department of the University of La Laguna. A 63 year old man who had developed hyponatremia and showed all the symptoms of the syndrome of inappropriate antidiuretic hormone secretion (SIADH), and the other case is of a 64 year old man without SIADH syndrome. Lung cancer was the cause of death in both cases. The hypothalamuses were cut in four series of coronal sections of 10 μm thick. The A series was stained with the Klüver-Barrier method. The B and C series were processed immunohistochemically; using anti-vasopressin (VAS) and anti-Tyrosine-Hydroxylase (TH) as primary antibodies.

The vasopressin immureactive material (VAS-ir) was mainly found located in the supraoptic nucleus and magnocellular part of paraventricular nucleus but VAS-ir was also observed in the periventricular nucleus, preoptic medial area, perifornical nucleus, and hypothalamic lateral area. The TH-ir was found in the arcuate nucleus, periventricular nucleus, preoptic medial and the lateral area. VAS-ir was more intensive and had a higher number of cells and fibres in the SIADH, as opposed to the TH-ir which was similar in both cases.

N-P-09 HYPOTHALAMIC ANGIOTENSIN-VASOPRESSIN SYSTEM IN THE ARTERIAL HYPERTENSION AND ITS CAPTOPRIL TREATMENT

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The paraventricular hypothalamic nucleus (PVN) and supraoptic nucleus (SPO) are two brain structures closely related with hypothalamic-hypophyseal axis and play an important role in cardiovascular regulation and in the salt-water balance mechanism. The hormone vasopressin, well known for its pressor and antidiuretic effects, is primarily synthesized in the magnocellular neurons of the PVN and other hypothalamic structures. The secretion of this hormone is controlled, at least in part, by angiotensinergic inputs that come from the subfornical organ (SFO) and reach the paraventricular (PVN) and supraoptic (SPO) nucleus of the hypothalamus.

We have used a total of 20 ten week old male rats divided into four groups: a control group of five WKY rats,

a control treated group of five WKY rats treated with captopril, a hypertensive group of five SHR rats and a hypertensive treated group of five SHR rats treated with captopril. The brains were cut in four series of coronal sections 10 μm thick. The sections containing paraventricular and supraoptic nuclei were incubated for 24 hours in the primary antibodies and then processed using the method of the Streptavidin-Biotin Complex (DAKO).

We have found inmunoreactive material for anti-VAS (VAS-ir) in neurons and fibers of the nuclei supraoptic and paraventricular hypothalamic nuclei. The reaction was strongly positive for the vasopressin in the control group, and in the hypertensive group the immunohistochemical reaction was weaker than the control. Whereas the captopril treatment produced an increase in the vasopressin IRM in the hypertensive group and a decrease in the WKY group, with the anti-angiotensin II, the immunohistochemical reaction was stronger in the hypertensive than in the control group.

N-P-10 ROLE OF THE LATERAL PARABRACHIAL NUCLEUS OF THE RAT INTO THE NOCICEPTIVE AND ANTINOCICEPTIVE SYSTEMS

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One of the most important advances for the comprehension and treatment of the pain has been the discovery of an endogenous circuit of modulation of the nociceptive information. Recently, it has been involved the lateral parabrachial nucleus (LPB) in the above mentioned circuit, though its role in the transmission and/or modulation of the pain is not sufficiently clear. LPB is strategically placed, from the anatomical point of view, to influence both the transmission and the control of the pain. In the present study, we decide to investigate the role that the LPB might play in the mechanism of transmission of painful different stimuli, as the somatic superficial one (test of the formalin) and the visceral one (test of the abdominal contractions), as well as, in the mechanism of action of different analgesic drugs, as the metamizol (500 mg/kg) and the morphine (10 mg/kg), using the immunocytochemical detection of the protein Fos as a marker of neuronal activity. We used male Sprague-Dawley rats as experimental animal. We can conclude from our results that:

1) LPB plays a role in the somatic nociceptive circuit, principally by the superior (slPB) and dorsal (dlPB) subnucli;

2) LPB plays a role in the visceral nociceptive circuit, principally by the external subnucleus (elPB);

3) LPB plays a role in the mechanism of action of the metamizol, principally by the external (elPB) and central (clPB) subnucli;

4) LPB does not play a role in the mechanism of action of the morphine, at least by the expression of the c-fos proto-oncogen.

N-P-11 FOS-LIKE IMMUNOREACTIVITY IN RAT DORSAL RAPHE NUCLEUS INDUCED BY PREGABALIN

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The dorsal raphe nucleus extends in the medium axis from the mesencephalon to the rostral beginning of the ventromedial medulla oblongata, always ventral to the midbrain aqueduct. The serotonergic dorsal raphe nucleus has been implicated in antinociception, since raphe-spinal cord outputs modulate the gelatinous substance of the spinal cord activity during painful stimuli. Moreover, neural links between the periaqueductal grey matter to the nucleus raphe magnus, also recruiting the dorsal raphe nucleus activity, may crucially involve serotonin-mediated pathways to exert its inhibitory functions on the first synapse of the ascending algesic spinothalamic inputs. Pregabalin is the pharmacologically active S-enantiomer of racemic 3-isobutyl gamma-aminobutyric acid. Pregabalin has demonstrated efficacy in the management of neuropathic pain. However, its exact mechanism of action is unknown. The aim of this study was to assess the involvement of the dorsal raphe nucleus in the pregabalin-induced antinociception. The effect of neuron activation induced by the pregabalin was determined by detecting the expression of the immediate early gene, c-fos, in the dorsal raphe nucleus of male Sprague-Dawley rats. The immunohistochemistry was used to detect Fos protein, the protein product of c-fos proto-oncogene. A significant increase in Fos expression was observed into the dorsal raphe nucleus following i.p. pregabablin (30 mg/kg) injection, compared with control rats. Our results demonstrate that antinociceptive effects of pregabalin could be mediated, partly, through the descending serotonergic system, arising from the dorsal raphe nucleus. The present data also suggest that the dorsal raphe nucleus exerts a key-role in the modulation of the non-opiod antinociception.

N-P-12 NEUROCHEMICAL CHARACTERIZATION OF A PONTO-SEPTAL PATHWAY VIA THE NUCLEUS INCERTUS UNDERLYING THETA RHYTHM IN THE RAT

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Hippocampal theta activity (HPC) is related to memory encoding and retrieval. This oscillation appears in the awake animal during information-seeking behaviours and voluntary movements and is influenced by the level of arousal. HPC also represents the characteristic oscillatory pattern during REM sleep, which is modulated by the orexinergic system. In the generation of HPC, cholinergic neurons in the medial septum-diagonal band (MS/DB) activate pyramidal neurons in the hippocampus, the activity of which is controlled by GABAergic inhibitory septal neurons. Septal glutamatergic neurons are also involved in the generation of HPC but its contribution remains unclear. In the supramammillary nucleus (SUM), populations of glutamatergic and GABAergic neurons modulate the activity of the septohippocampal system. The tegmental nucleus incertus (NI), project to several key structures controlling theta rhythm, including MS/DB and SUM, and receives aferences from the main pontine site involved in HPC activation, the reticularis pontis oralis nucleus (RPO). We have recently verified the existence of a pathway RPO-NI-MS/DB by double tract tracing experiments. In addition, our group has demonstrated that NI participates in the generation of theta activity in the hippocampus. Nevertheless, the neurochemistry of this pathway remains unknown. In the present work, we have tried to determine the nature of the influence of NI on MS/DB. Inmunochemical detection of glutamate vesicular transporter 2 (VGLUT2), parvalbumin (PV), calbindin (CB), calretinin (CR) and orexin (OX) was performed in combination with injection of retrograde tracer (flurogold; FG) in MS/DB. In addition, we have performed the inmunodetection of choline acetyl esterase (AChE). The results of this study suggest that (1) NI is cholinoceptive, (2) there are subpopulations of VGLUT2, PV and CB positive NI neurons which project to MS/DB and (3) some orexinergic fibers in NI terminate on MS/DB projecting neurons. These preliminary results suggest that the contribution of the NI to the hippocampal theta circuit is at least mediated by a glutamatergic projection which could be modulated by the orexinergic system.

N-P-13 ACTIVATION OF C-FOS IN THE LOCUS COERULEUS FOLLOWING BOTH ACUTE NOXIOUS STIMULATION AND NON-OPIOID ANALGESIC STIMULATION

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The locus coeruleus (LC), the noradrenergic A6 group of neurons in the dorsolateral pons, has been implicated as a critical component of the descending pain modulatory system. Electrical or chemical stimulation of the LC inhibits the responses of spinal dorsal horn neurons to noxious stimuli and induces antinociception. However, emerging evidence suggests a contribution of the LC to pain facilitation. Lesions of noradrenergic LC neurons significantly reduced tonic behavioral responses to intraplantar formalin injection. In the present study, the activation of the proto-oncogene c-fos in the LC was examined both following the injection of formalin into the rat hindpaw or following the intraperitoneal injection of an analgesic drug in order to clarify the mechanisms underlying the role of the LC in the nociceptive and antinociceptive systems. In naive rats there were few Fos-immunoreactive neurons (Fos-IR) in the LC. Formalin test and metamizol injection induced an increase in Fos-IR in the LC. However, the number of Fos-IR neurons in the LC in the morphine group was significantly lower than those in all other groups. Our findings suggest that activation of c-fos in the LC is induced by acute noxious stimulation, such as formalin injection, and by non-opiod analgesic stimulation, such as metamizol, but not by opiod analgesic stimulation, such as morphine. Activation of the LC may be involved in both inhibition and facilitation of pain.

N-P-14 CALCITONIN GENE-RELATED PEPTIDE PROMOTES THE EXPRESSION OF GLIAL CELL LINE-DERIVED NEUROTROPHIC FACTOR

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Glial cell line-derived neurotrophic factor (GDNF) is a neurotrophic factor widely expressed in the central nervous system (CNS). It has been shown to be a survival factor for dopaminergic nigral neurons. In the CNS of a strain of calcitonin gene-related peptide (CGRP) transgenic mice, this peptide was broadly expressed with a maximum of localization in hippocampal fascia dentate and in the cerebellar mossy afferences. Examining the nervous tissue of these mice by both immunohistochemistry and western blot an increase of GDNF expression was confirmed in the hippocampal fascia dentate as well as in the cerebellar cortex. These results promoted us to locally administrate CGRP (4mM) and CGRP (2mM) plus dibutyryl cyclic AMP (cAMP) (20mM) in the isocortex. GDNF was localized by immunohistochemestry at the injection sites of the peptide and the dibutyryl cAMP. Twenty four hours after the local injection, an increase of the GDNF expression was observed at the injection site of fixed slices. When dibutyryl cAMP and CGRP were jointly administered the GDNF expression was still more evident. These results could guide the administration of CGRP and dibutyryl cAMP in the substantia nigra of the experimental models of Parkinson disease in order to protect dopaminergic neurons against neurodegeneration.

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N-P-15 DOPAMINE TRANSPORTER AND ALPHA-SYNUCLEIN EXPRESSION IN THE STRIATUM OF THE RAT, MONKEY AND HUMAN

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The dopamine transporter (DAT) is a glycoprotein specific to dopaminergic (DA-) cells and is responsible for the reuptake of dopamine (DA) from the extracellular space. DAT is involved in Parkinson's disease (PD) because it increases the cytosolic levels of DA, and consequently, the oxidative stress in DA-cells. α-synuclein is a presynaptic protein expressed in different neuronal populations including DA-cells. It is known that α-synuclein mutations induce PD, and that α-synuclein is aggregated in PD. In addition, in vitro studies show that α-synuclein regulates DAT by reducing its activity. An interesting fact in PD is that not all midbrain DA-cells show the same susceptibility to degeneration. DA-cells in the caudoventral and lateral region of the sustantia nigra (SNcv), which project to the dorsal striatum (dSt), are more vulnerable than those in the rostromedial and dorsal region of the sustantia nigra (SNrm) and ventral tegmental area (VTA), which project to the ventral striatum (vSt). The aim of this study was to investigate a possible relationship between the expression of α-synuclein and DAT in striatal DA-terminals and the vulnerability of DA-cells. Immunohistochemistry, western-blot, immunoprecipitation, and antibodies which detect the glycosylated (mature) and non-glycosylated (immature) DAT forms were used in rat, monkey and human brains.

Immunoprecipitation confirmed a physical interaction between α-synuclein and DAT. Immunohistochemistry and western-blot revealed a direct correlation between α-synuclein expression and that of the non-glycosylated (non-glyco) DAT form, and an inverse correlation between α-synuclein expression and that of glyco-DAT. α-synuclein and non-glyco-DAT expression levels were higher in the vST than in the dSt, while glyco-DAT expression levels were higher in dSt synaptosomal membranes than in those of the vSt. These findings suggest that α-synuclein down-regulates the trafficking, glycosylation and membrane expression of DAT, and that the α-synuclein/DAT interaction can be involved in the differential vulnerability of midbrain DA-cells.

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N-P-16 INCIDENCE AND DISTRIBUTION OF LESIONS PRODUCED BY 3-NITROPROPIONIC ACID IN DIFFERENT REGIONS OF THE BRAIN OF WISTAR RATS

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The systemic administration of 3-nitropropionic acid (NPA) to rodents and non-human primates causes neurological disturbances that closely mimic Huntington's disease. The neurotoxicity of NPA has been studied mainly in Sprague-Dawley and Lewis rats, in which it triggers the degeneration of medium-sized spiny GABAergic neurons in caudate-putamen (striatum in rat brain). However, it was found that those rat strains have different susceptibilities to the toxin due to unknown reasons. In addition, the occurrence of lesions in other regions of the brain was reported but is still to be clarified. Fewer studies have been carried out with Wistar rats, hence the toxic action of NPA in this animals is much less characterised.

In this work, treatment of Lewis and Wistar rats with different doses of NPA revealed that the later have a quite inferior resistance to the toxic. Treatment of Wistar rats with doses of 40 to 50 mg/kg/day, for 4 days, induced severe motor abnormalities and tissue injury in different brain regions, while doses of 60 mg/kg/day were fatal to these rats. The most noticeable behavioural disturbances observed in the treated animals were hypoactivy, abnormal gait, dystonic movement and paralysis of limbs.

Necrotic lesions in striatum, hippocampus, thalamus and cortex, as well as small haemorrhages in cerebellum, were identified in NPA-administered rats. The analysis of the magnitude of these lesions in animals with different levels of intoxication showed that the various brain regions are not equally affected by the neurotoxin. Striatum is the region most vulnerable to NPA, since striatal lesions were detected even in rats with mild behavioural disturbances and the extension of the lesions increased proportionally to animal motor deficit. The CA1 area of hippocampus is also particularly susceptible to NPA, but widespread damage of hippocampal formation was only observed in most ill rats. Lesions in cortex, thalamus and cerebellum were very localised and present only in animals with extreme motor impairments and extensive brain damage.

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N-P-17 DISTRIBUTION OF 5-HT$_6$ RECEPTOR IN THE HIPPOCAMPUS OF ALZHEIMER´S DISEASE

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Alzheimer's disease (AD) involves dysfunctions of multiple neurotransmitter systems. Among them, serotonergic (5-HT) decrease has been observed in the AD brain making the serotonergic system a potentially important target for new treatments. Although several 5-HT receptors have been shown to alter memory function, the 5-HT_6 receptor is somewhat unusual among the 14 identified 5-HT receptor subtypes in that it appears to influence long-term memory more than anxiety, and it is expressed almost exclusively in the central nervous system. To date, only two studies on changes in 5-HT_6 receptor expression have been published in Alzheimer's patients. Reductions in 5-HT_6 receptor density in the frontal and temporal cortex have been reported and it suggest a role in the pathogenesis of the disease. There is no information on the expression of this receptor in the human hippocampus at the cellular level. Because the hippocampus is of particular importance for the etiopathology of AD and because neuropsychiatric symptoms in AD are associated with reduced metabolism we have chosen this brain region to detail the neuronal distribution of 5-HT_6 receptors.

The present study had two main objectives: 1) To characterize [125I] SB-258585 binding in selected regions of the human hippocampus using autoradiographic studies and immunohistochemistry; 2) To establish if 5-HT_6 receptor patterns are altered in the same regions in AD.

We found 5-HT_6 immunoreactivity confined to the cell bodies of ammonic fields. Also stratum oriens, radiatum and the molecular layer of the dentate gyrus of the hippocampus showed immunoreactivity. Binding studies confirm these findings and revealed higher densities over the hilus, CA2-3 field of hippocampus and granular cell layer of the dentate gyrus when compared with CA1 field.

We measured 5-HT_6 expression in the hippocampus of AD patient's. The same pattern distribution as controls was present but 5-HT_6 receptor densities were significantly reduced in all fields. The quantitative information reported in this study provides complementary information to pharmacological and *in vivo* studies of the 5-HT system in both control and AD patients.

Supported by Grant PAC08-0261-1581 from the Consejería de Educación y Ciencia de la Junta de Castilla-La Mancha (Spain).

N-P-18 VOLUME ESTIMATES OF THE HUMAN ENTORHINAL CORTICAL LAYERS IN CONTROL AND ALZHEIMER DISEASE

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The entorhinal cortex (EC) is a component of the hippocampal formation that is located between the hippocampus and the neocortex. The EC plays an important role in memory processes. When degenerative neurological disorders are present a structural alteration takes place. The aim of this study is to evaluate changes in the volume of the global EC as well as on each cortical layer in Alzheimer' disease (AD) compared to controls. The study was made on thionin-stained 50µm-thick sections obtained from the human temporal lobe that were systematically selected each 1mm along the EC. In each section, the EC was delimited according to the Insausti et al (1995) criteria; two representative subfields (the intermediate EI and the rostral ER) were also individually analyzed in EC. In addition, the volume of cortical layers of the EC was quantified. The CAST-Grid system was used for the estimation of the EC volume; an exhaustive evaluation of the precision of the volume estimates was also performed using stereological tools. The study reveals that the volume of the EC is 754 mm^3 in controls with a decrease greater than 40% in the AD group. ER subfield shows the smallest change while EI subfield has the greatest (48% decrease). This fact is found in all EC layers; however, layer V and, specially, layer II exhibit the most important reduction of the cortical region. These results suggest that quantitative estimates of the EC volume can be helpful to understand disturbances in memory processing through an essential region of the hippocampal formation, and opens the door to the macroscopic evaluation using non-invasive methods as magnetic resonance imaging.

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N-P-19 CORRELATION BETWEEN ANATOMOPATHOLOGICAL SIGNS OF EPILEPSY IN THE LITHIUM-PILOCARPINE EXPERIMENTAL MODEL

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Human temporal lobe epilepsy is characterized by progressive sclerosis of hippocampus together with a reorganization of hippocampal mossy fibres. As in the human disease, in the lihium-pilocarpine experimental model, neuron death affects unevenly the different regions of hippocampus, and both injury level and mossy fibre reorganization display considerable inter-individual differences. The correlation between neuronal damage levels affecting several hippocampal regions, and between them and mossy fibre reorganization was analyzed in lithium-pilocarpine induced epileptic rats.

Ten 12-13 weeks old Sprague-Dawley rats were treated with Lithium chloride (127 mg/kg i.p.) followed by pilocarpine (30 mg/kg i.p.) 18-24 h later. Methyl atropine bromide (1 mg/kg i.p.) was given 30 minutes before pilocarpine to prevent systemic muscarinic side effects. Thirty minutes after the onset of *status epilepticus*, seizures were terminated with diazepam (20 mg/kg i.p.) and pheno-

barbital (8 mg/kg i.p.). Nine animals were selected on the basis of a modified scale of Racine (34 in two observations). Three sham-treated animals were used as controls. After a 4-week survival period, the brains were fixed by transcardial pefusion and serial coronal sections of the hippocampus were processed for Klüver-Barrera and Neo-Timm stains. Neuron death affected mainly lateral CA1-3 regions and, unexpectedly, granular cells of the dorsal blade of the dentate gyrus as well. Hippocampal atrophy, neuron death in $CA1_L$ (CA1 lateral), $CA1_M$ (CA1 medial), CA1-2, CA3 and dentate gyrus, and Neo-Timm staining levels were scored form 0 to 5 for subsequent Pearson and Spearman's correlation analyses. Good positive correlation was found between neuron death scores in $CA1_L$, $CA1_M$ and CA1-2 in all comparisons. Atrophy was correlated only with neuronal damage in $CA1_M$ and DG. Interestingly, dense Neo-Timm staining and severe CA3 injury was observed in several animals displaying mild damage in CA1-CA2 and no significant hippocampal atrophy, and viceversa. Consequently, Neo-Timm staining level and neuronal damage in CA3 were correlated only each other.

The present results indicate that reorganization of mossy fibres and severity of hippocampal sclerosis are independent pathological entities with no causal relationship.

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N-P-20 CHANGES OF EXPRESSION OF VESICULAR GLUTAMATE TRANSPORTER IN RAT VISUAL SYSTEM AFTER DEAFFERENTIATION

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Vesicular glutamate transporters (VGluTs) are responsible for the uploading of the excitatory neurotransmitter glutamate into vesicles for synaptic release. Among the three highly homologous isoforms of VGluTs (VGluT1-3), VgluT2 appears associated with synaptic vesicles in the glutamatergic terminals and is the most abundant in the retinorecipient brain regions including the superior colliculus (SC) (Fujiyama et al. 2003. J Comp Neurol. 465:234-249).

The SC is a mesencephalic layered structure in which the superficial strata (stratum griseum supeficiale –SGS- and stratum opticum –SO-) receive visual glutamatergic input from the retina and the visual cortex, whereas intermediate (stratum griseum intermediale –SGI- and stratum album intermediale –SAI-) and deep strata process multisensorial information (Huerta and Harting. 1984. Comparative Neurology of the Optic Tectum. Plenum Press. pp:687-773)

The present study evaluates changes in the expression of VGluT2 in the SC of adult Sprague-Dawley rats after neonatal unilateral visual deafferentiation, using several techniques such as western blot, inmunohistochemistry and in situ hybridization.

After neonatal enucleation, a decrease of VGluT2 immunoreactivity was found in the upper part of SGS and in the SGI. This result was corroborated by in situ hybridization, while the semiquantitative western blot technique only showed a sligth reduction of VgluT2 expression in these strata.

Neonatal enucleation causes a considerable expansion of the contralateral visual corticocollicular terminal fields, the lack of glutamate coming from the retina could lead on the decrease of VgluT2 expression in the superficial collicular strata.

This work has been supported by Grants: Fondo de Investigaciones Sanitarias (FIS PI052046) and Subvención a Grupos de Investigación UPV/EHU (GIU06/15).

N-P-21 NEURODEGENERATIVE ALTERATIONS IN IRS-2 KO MICE ¿CEREBRAL DIABETES AND NEURODEGENERATION?

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Using ko for IRS-2 (insulin receptor substrate) mice a detailled analysis of the hypothalamus and hypocampus, *in vivo* by means of magnetic resonance and *postmortem* by histochemical and immunocytochemical studies, was carried out.

Neurodegenerative processes were detected by congo red, hyperfosforilation of Tau, deposits of β-amyloid, and morphometric and densitometric analysis.

Correlation among these changes and decreases in the number of the aromatase P450 positive neurons was made.

The results obtained suggest that a tissular specific cerebral diabetes could be related with neurodegenerative diseases and a decrease of neuroprotective role of estrogens could be involved.

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