J. Anat. (1998) **192**, pp. 447–469 Printed in the United Kingdom

Proceedings of the Anatomical Society of Great Britain and Ireland

The Annual General Meeting of the Anatomical Society of Great Britain and Ireland was held at Newcastle University Medical School, from 6th to 8th January 1998. It included a symposium on 'Developmental Neuroscience' on Wednesday 7th January. The following are abstracts of communications and posters presented at the meeting.

TALKS

1 The functional control of the subtalar and transverse tarsal joints of the weight-bearing human foot. By C. E. HARKNESS and G. D. STAINSBY (introduced by S. McHANWELL). *School of Anatomy*, *Newcastle University*

The coordinated movements at the intertarsal joints, and the functional stability of the human foot during the weight-bearing period of the walking cycle, have never been satisfactorily explained. Suggestions have been made that the foot can be regarded as a plate (the *lamina pedis*) which twists and untwists, that the joints have close-packed and loose-packed positions, and that the stability of the transverse tarsal joint is influenced by whether the axes of movement of the talocalcaneonavicular and calcaneocuboid joints are in or out of line. Movements at the talocalcaneal joint have been described as being helicoidal due to the shape of the posterior calcaneal facet. The coordinated movements at the subtalar and transverse tarsal joints have been studied in a cadaveric foot in which the ligaments controlling these joints were dissected and carefully preserved. With the hindfoot everted and the transverse tarsal joints angulated medially, to simulate the posture of fully flat weight-bearing, all ligaments controlling the subtalar and transverse tarsal joints were tight. When the forefoot was held firmly in a plantigrade position, and the calcaneum inverted beneath the talus and the transverse tarsal joints angulated laterally, the talus became laterally rotated but it also moved posteriorly over the calcaneum and the navicular followed. The short plantar ligament and the calcaneal part of the bifurcate ligament remained tight and so stability of the calcaneocuboid joint was maintained. The posterior and lateral talocalcaneal and cervical ligaments remained tight, but the interosseous talocalcaneal and the ligaments around the head of the talus (the navicular part of the bifurcate and the plantar calcaneonavicular ligaments) became slack. A downward force was then applied to the talus to simulate weight-bearing in a heel elevated posture. The talus and the navicular then moved forwards relative to the calcaneum until the interosseous talocalcaneal ligament and the ligaments around the head of the talus tightened. The talocalcaneal joint and both of the transverse tarsal joints were then stable. It is suggested that this sliding movement at the talocalcaneal joint provides the necessary adjustment for the ligaments controlling the subtalar and transtarsal joints to remain tight throughout the load-bearing period, and so help to stabilise the longitudinal structure of the foot when heel elevation occurs.

2 A study of the vascular anatomy of the marrow cavity of the rabbit femur. By M. H. LAWSON, A. N. MATHER, J. POOLEY and P. FLECKNELL (introduced by S. MILLER). *Department of Anatomy*, *University of Newcastle Upon Tyne and Department of Orthopaedics*, *Queen Elizabeth Hospital*, *Gateshead*

Investigators to date have largely relied upon either histological or radiographic methods with which to study the blood vessels in bone marrow. However, controversy persists and recently the nature and even the existence of a central venous sinus seen on radiographic contrast studies have been questioned. The aim of this study was to investigate the anatomy of the intraosseous circulation using a combination of radiographic and histological techniques in order to determine the precise nature of the vessels which appear to be filled by infusion of radiopaque contrast medium in radiographic studies. Experiments were performed on 2 groups of 3 young adult New Zealand White Rabbits anaesthetised with 0.3 ml/kg 'Hypnorm' (Fentanyl/Fluanisone) and 0.5 ml/kg Midazolam. A laparotomy was performed on each animal. In one group the descending aorta was cannulated and 100 ml of a radiopaque contrast medium was infused; in the other group the inferior vena cava was cannulated and 200 ml of the contrast medium was infused. The animals were then killed with an overdose of pentobarbitone and the femora excised. Each femur was fixed in formalin and then decalcified. High resolution anterior posterior and lateral projection radiographs of each bone were made. Measurements were taken from the radiographs and points on the cortex of the bones corresponding to the position of medullary vessels required for histological examination were marked. Appropriate blocks of bone were cut, sectioned with a microtome and stained with Harris's haematoxylin and eosin. The general pattern of vascular anatomy described by others using similar radiographic techniques was observed in this series. A large central vessel with the radiological feature of a venous sinus was seen on all the radiographs made following intravenous infusion of contrast medium and confirmation of the nature of this vessel was obtained by histological examination. The central venous sinus was found to be intimately related to several arterial vessels which we consider may be of functional significance in the control and regulation of the intraosseous circulation.

3 Evidence for arteriovenous anastomoses in the intraosseous circulation of the rabbit. By A. N. MATHER, M. H. LAWSON, J. POOLEY and P. FLECKNELL (introduced by S. MILLER). *Department of Anatomy, University of Newcastle Upon Tyne and Department of Orthopaedics, Queen Elizabeth Hospital, Gateshead*

Studies using contrast radiography have shown that the intraosseous vascular architecture in mammalian long bones conforms to a distinct pattern. A longitudinally disposed central venous sinus branches in the middiaphysis to leave the marrow cavity as the principal nutrient vein and lies in close proximity to centrally placed ascending and descending branches of the nutrient artery. There is evidence to suggest that this anatomical arrangement has functional significance by facilitating intraosseous shunting of blood through arteriovenous anastomoses during exercise conditions. The aim of this study was to investigate the effect of circulating adrenaline, simulating exercise conditions, on the main vessels of the intraosseous circulation as demonstrated by contrast microradiography. Experiments were performed on 18 New Zealand White rabbits. Each animal was anaesthetised with 0.3 ml/kg 'Hypnorm' (Fentanyl/Fluanisone) and 0.5 ml/kg Midazolam and intraosseous pressure was monitored by direct intraosseous cannulation. Arterial pressure was monitored simultaneously through a cannula placed in the central ear artery. Bolus injections of adrenaline solution were administered through a cannula inserted into a marginal ear vein. When the response on intraosseous pressure to injection of adrenaline had been observed the effects of alpha adrenergic blockade using phentolamine and beta adrenergic blockade using propranolol were investigated. A laparotomy was performed on each animal and 200 ml of a radiopaque contrast agent was infused into the inferior vena cava. The animals were then killed with an overdose of pentobarbitone and the femora excised. These were then decalcified and high resolution anterior posterior and lateral projection microradiographs were made of each bone in order to demonstrate the location of the tip of the cannulae used for intraosseous pressure measurements in relation to the intraosseous vessels. Sections of the bones were prepared and stained with haemotoxylin and eosin in order to confirm the nature of the vessels seen on the microradiographs. We observed 2 responses of the intraosseous pressure to circulating adrenaline described by others, a fall in intraosseous pressure – a 'peripheral response' – and a rise in intraosseous pressure – a response measured in the vicinity of the central venous sinus. The peripheral response appears to be mediated by alpha adrenergic receptors and the central venous sinus response by beta adrenergic receptors. These results support the hypothesis that the pattern of the intraosseous vessels has a functional significance and facilitates the shunting of blood through arteriovenous anastomoses during exercise conditions.

4 Diagnosis at a distance – the electronic transmission of anatomical images. By A. VAN DELLEN (introduced by S. MILLER). *University Laboratory of Physiology, Oxford University and Department of Neurosurgery, University of Natal, Durban, South Africa*

With the advent of fax and modem facilities, electronic anatomical images (computer tomography) can now be easily and rapidly transmitted over vast distances. The development of sophisticated radiological imaging services at peripheral hospitals has resulted in many patients having access to computer tomography at a local level. The early access afforded rural victims of neurological insults to this imaging modality has resulted in a dilemma on the part of the attending physician as to whether the patient would benefit from further specialist expertise which is often relatively distant. Patients with neurological deficits often react adversely to transport over distance and the need for this transport would be obviated if no benefit were to be derived from super-specialist attention. With access to images transmitted to a referral centre, in consultation with the referring physician, the decision can jointly be made whether to transfer the patient. The relatively high initial capital outlay involved with electronic transmission of medical data and anatomical images is offset by the cost-savings involved with patient transfer, as well as the avoidance of iatrogenic injury during transportation. The system can also be utilised to support radiological continuing medical education via the reverse transfer of teaching material, aiding in the training of peripheral hospital staff.

AvD is supported by the Rhodes Trustees.

5 An investigation into the mechanism of orbital blowout fractures. By M. URDANG and J. LYNE (supervised by N. WATERHOUSE*, introduced by M. J. HOBBS). *Department of Anatomy and *Department of Craniofacial Surgery, Imperial College School of Medicine*

For over a century, since the first description of an orbital blowout fracture, there has been debate and confusion regarding the aetiological mechanisms of producing these fractures. Past experimental and clinical studies have aimed to support one or other of the 2 principal suggested mechanisms. The buckling mechanism contends that the fracture is produced as a result of transmission of force to the orbital floor from a blow to the orbital rim. The hydraulic theory suggests that the force is transmitted to the floor via a direct blow to the globe. Review of past literature reveals that there are major flaws in the design and execution of previous experimental methods. Most studies have incorporated some or all of the following limitations; low numbers, unquantified forces, nonhuman models, incomplete soft tissues, poor simulation of in vivo conditions and a failure to isolate the position of striking force. No study has ever produced a direct comparison between the 2 mechanisms under identical conditions. We present the results of such a study undertaken on 48 fresh cadaver orbits using the same quantifiable force and under the same experimental conditions. Our results show that the efforts to establish one or the other mechanism as the primary aetiology have been misplaced. Both mechanisms produce orbital blow-out fractures, but with different and specific characteristics.

This study conformed to the guidelines of the Human Tissues Act.

6 The structure of the myotendinous junction of the human and primate heart. By C. MILLINGTON-SANDERS, L. LAWRANCE, J. F. DYE and C. STOLINSKI. *Imperial College School of Medicine*, *Cellular and Integrative Biology*, *Division of Biomedical Sciences*, *University of London*

The myotendinous junction (MTJ) of the heart consists of specialised cardiac myocytes of the papillary muscle linked to collagen fibrils of the chordae tendineae of the mitral valve complex. MTJ's were obtained from 3 postmortem formalin fixed human hearts and one glutaraldehyde perfused primate heart (*Macaca mulata*). The tissue was examined using light and electron microscopy. Glycol metacrylate sections were stained with Gordon-Sweets reticulin method and counterstained with van Gieson or Haematoxylin. Osmium tetroxide stained tissue was embedded in araldite and thin sections were counterstained with lead citrate and uranyl acetate. Silver stained tissue revealed black fibrous connective tissue reticulin fibres in the intercellular spaces and near the MTJ. At the junction the cardiac myocytes were found to be much longer when compared to the cells deep in the papillary muscle. Their junctional system was found to be very extensive and much smoother and distributed over large expanses of the cell membrane when compared to the deeper placed intercalated discs. In tangential sections, the apical myocyte cell membrane showed specialised areas consisting of 60 nm diameter globular densities situated in the middle of a hexagonally arranged network of fibres. Actin filaments were observed to be terminating at the dense globular structures. It is suggested that the cardiac myocytes tend to remain in their primitive form at the junctional region and do not form a standard intercalated disc. The cells apical regions show specialised fibrous networks aiding the transmission of force via the cell membrane.

7 Mouse dermal fibroblasts contribute to new fibre formation in traumatised host mouse muscle. By D. PYE and D. J. WATT. *Neuromuscular Diseases*, *Division of Neuroscience and Psychological Medicine*, *Imperial College School of Medicine*, *University of London*

Dermal fibroblasts implanted into the muscles of the *mdx* mouse, an animal model of Duchenne muscular dystrophy (DMD), result in high numbers of muscle fibres expressing dystrophin, a protein deficient in DMD and *mdx* muscle. The skin cells appear to convert to a myogenic lineage and express myogenic genes within the host muscle. We aim to determine if mouse dermal fibroblasts convert only when introduced into diseased muscle or whether they can also do so when implanted into muscle regenerating after traumatic injury. These cells could then be used to repopulate traumatised muscle. In preliminary experiments donor dermal fibroblasts, engineered to carry a reporter gene coding for β-galactosidase (β-gal) were implanted into muscle regenerating after injury. Dermal fibroblasts contributed to new fibre formation, as evidenced by β-gal-positive fibres within the host muscle. As the β-gal marker was cytoplasmic we could not determine the numbers of implanted fibroblasts contributing to regenerated fibres. Thus, in a 2nd series of experiments implanted cells were prelabelled with the fluorescent nuclear marker DAPI (4,6-diamidino-2-phenylindole). Labelled cells were injected into the tibialis anterior muscle of host mice anaesthetised with a midazolam hydrochloride, fentanyl citrate and fluanisone cocktail. In some experiments pre-implanted host muscle was x-irradiated, knocking out endogenous muscle satellite cells and injury was induced by minced muscle grafting or cold/crush injury. The contribution of skin cells to regenerated fibres was estimated by counting fibres with DAPI-positive central nuclei. The number of DAPI central nuclei in fibres of nonirradiated muscle was significantly greater than in irradiated ($P < 0.05$) but no difference was observed between the 2 types of muscle injury. There are no reports of the use of DAPI to trace cells in multinuclear tissues. We had to ensure that DAPI could not be passed from labelled to nonlabelled nuclei when donor and host cells fuse to form multinucleate myotubes. Thus, using an in vitro model, cells labelled with the cytoplasmic marker β-gal were cocultured with those labelled with DAPI. Following fusion, β-gal-positive myotubes containing both DAPI-positive and DAPI-negative nuclei were seen suggesting that DAPI is not transferred in the multinuclear state. To verify the in vivo contribution of dermal fibroblasts to fibre formation skin fibroblasts harvested from a β-globin transgenic mouse were used as a source of donor cells. Numbers of donor nuclei contributing to fibres can be accurately assessed using in situ hybridisation methods and has been used to determine the contribution of the implanted skin cells to fibre formation in traumatised muscle.

8 Immaturity of skeletal muscle in congenital myotonic dystrophy. By J. P. BARBET, G. S. BUTLER-BROWNE, C. JUNIEN, V. G. GOURDON, V. MOULY and H. RADVIANY. *Hôpital Saint Vincent de Paul*, *URA CNRS 1448* (*Physiologie et Biologie de la Motricité*) *and Unité INSERM 383* (*Génétique*, *Chromosomes et Cancer*), *Paris*, *France*

The severe congenital form of myotonic dystrophy corresponds to a very particular entity, being always maternally transmitted. This exclusive maternal transmission had previously suggested the role of environmental factors, but instead seems now to be linked to limits of transmission of the genetic defect during meiosis. Indeed, the severe form is only found when there has been an extensive amplification resulting in the presence of more than 2000 copies of the trinucleotide repeat in the region q13 on chromosome 19. Clinically, the congenital form of myotonic dystrophy presents at birth with extreme hypotonia and weakness of pharyngeal and respiratory muscles, which lead to respiratory distress and difficulties with sucking and swallowing. The mortality rate is about 50% and of those that survive there is marked mental and motor retardation. Immunocytochemical studies were carried out in a series of 10 affected fetuses, using antibodies specific for markers of cell proliferation, myogenic factors, vimentin, desmin and different isoforms of the myosin heavy chains. This was completed by a biochemical analysis of the contractile proteins. In all these affected fetuses, the skeletal muscles show essentially an arrested or delayed maturation, with abnormalities ranging from the presence of myotubes to

https://doi.org/10.1046/j.1469-7580.1998.19230447.x Published online by Cambridge University Press

incomplete fibre type differentiation, and a general smallness of fibres. The marked increase in numbers of central nuclei, nuclear chains, ring fibres and inflammatory changes associated with the adult form are not found. Immunocytochemical and biochemical studies demonstrated that the delay in differentiation and maturation affected both fast and slow fibres.

Acknowledgements: This work was supported by grants from the Association Française contre les Myopathies, the Centre National de la Recherche Scientifique, the Institut National de la Santé et de la Recherche Médicale and the Fondation pour la Recherche Médicale.

9 Ultrastructural reappraisal of epithelial integrity and cell extrusion in mammalian small intestine (rat, reindeer, seal). By T. M. MAYHEW, R. MYKLEBUST* and A. WHYBROW. *School of Biomedical Sciences, Queen's Medical Centre, University of Nottingham and *Department of Electron Microscopy and Morphology, Faculty of Medicine, University of Tromsø, Norway*

Mammalian small intestine is lined by a continuously-renewing epithelium in which tight junctions (TJs) partition the enterocyte plasmalemma into apical and basolateral domains and help to regulate transepithelial transport. The epithelium is permeable to small molecules and relatively impermeable to large molecules. As if to reinforce these properties, previous studies have emphasised how intact TJs are retained despite cell loss. However, small quantities of macromolecules do transfer and a possible route is via disruption of TJs during cell extrusion. To test this, we conducted ultrastructural studies on segments of small intestine from animals killed humanely and under anaesthesia by nationally approved procedures. Specimens were fixed in buffered glutaraldehyde (pH 7.3–7.4), postosmicated and embedded in resin for TEM. In reindeer and seal (not previously studied), 3 types of extrusion were noted. TJ integrity was preserved in 2 of them and anucleate apical cell fragments (rather than complete cells) passed into the lumen. One type of loss (reindeer only) involved creating large intercellular spaces extending from the preserved apical cap to the lamina propria. Nucleated cell portions were removed from these spaces, probably by lamina propria macrophages. A 2nd type (reindeer and seal) involved cell shrinkage, greater electron density and degeneration of nucleated portions into fragments and membrane whorls which were confined to increasingly narrower intercellular spaces between surrounding healthy enterocytes. It is not clear how these remnants are removed. No evidence of apoptosis was found but apoptosis has been reported in earlier studies. A third type of cell loss, necrosis, was seen in both species and also confirmed in rats. In this case, the epithelial barrier was clearly breached following gradual loss of cell electron density and partial or complete degradation of organelles and membranes. In rat and seal, the cell apex was disrupted and cell contents exposed to the lumen. In reindeer, complete cell remnants exhibiting subtotal membrane degeneration were extruded. These findings imply a greater variety of mechanisms of enterocyte extrusion in which necrosis may be more widespread than admitted previously. At least 3 sorts of extrusion exist: types 1 (whole cell loss) and 2 (apical fragment loss) maintain TJ integrity but type 3 (necrosis) does not. Type 2 loss has 2 variants distinguished by the fate of basal portions of cells in large intercellular spaces (type 2a) or narrow intercellular clefts (type 2b). In all types, the proximity of intraepithelial lymphocytes suggests that they could be involved in cell targeting and killing.

10 Paneth cell numbers and zinc intake in the proximal small intestine in the mouse. By R. R. ETTARH. *Department of Human Anatomy and Physiology, University College, Dublin*

Paneth cells in the small intestine are known to concentrate zinc within their secretory granules. The numbers of these cells can be affected by manipulating zinc intake. In experimental diabetes mellitus in rodents, zinc intake is increased because of hyperphagia. The aim of this investigation was therefore to determine Paneth cell numbers in diabetes and examine the effect of zinc supplementation. Adult male CD-1 mice were given one of the following treatments: (a) an intraperitoneal injection of streptozotocin 250 mg/kg body weight to induce diabetes (b) zinc in drinking water (0.3 mmol/l zinc sulphate) (c) streptozotocin injection plus zinc in drinking water (d) no treatment. Blood glucose estimations were carried out in all groups of mice. Diabetic mice were treated with insulin (2 units/d intraperitoneally). The amount of zinc in food was estimated by atomic absorption spectrophotometry. After 28 d, the small intestine was taken out, divided into 4 equal segments and samples taken at the midpoint of each segment. Samples from the proximal half of the intestine were processed for routine histology, and the number of Paneth cells and crypts calculated from profile counts, cell and crypt dimensions and segmental length values. The data were analysed statistically using the Mann-Whitney U-test. Results for the 2 segments of the proximal small intestine show that there were more Paneth cells in the first segment in diabetic and in zinc-fed diabetic mice than in the corresponding segment in untreated mice ($P < 0.05$ and 0.025 respectively). There were also more crypts in the first segment in diabetic and in zinc-fed diabetic mice than in corresponding segments in untreated mice ($P < 0.025$ and 0.001 respectively). The differences between these groups were absent in the 2nd intestinal segment. Values for Paneth cells, crypts and Paneth cells/crypts in zinc-fed and in zinc-fed-diabetic mice were similar to corresponding values in untreated and in diabetic mice respectively. These findings show that Paneth cell numbers are increased in the proximal small intestine in diabetic mice. The numbers of these cells in the proximal small intestine are unaffected by zinc supplementation in normal and in diabetic mice.

11 Cartilage microfibrillar network development in human embryonic knee. By D. M. LAWTON, W. B. OSWALD, L. MOORE and A. J. FREEMONT. *Department of Pathological Sciences, University of Manchester*

Normal embryonic skeletal cartilage contains microfibrils in a network that immunostains for fibrillin-1 (a force-bearing elastic molecule, characteristic of one class of microfibril)

and chondrocytes that express the fibrillin-1 gene (Lee, *Dev. Biol*. **163**, 1994; Lawton, *J. Microsc*. **178**, 1995; Keene, *J. Histochem. Cytochem*. **45**, 1997). Conventional preparation, and tinctorial contrasting, of the network has been effective in a case of thanatophoric dysplasia [(FGFR3 mutation) A Darby; pers.comm.)] but, apparently, never in normal tissue. We investigated normal human embryo knees at 15 and 21 wk, using a Karnovsky's picric acid + aldehyde fixative/ 70% ethanol/ LR White resin. Network was present extensively in femoral and tibial hyaline cartilage. Absence of network in fibrocartilaginous meniscus was in contrast to adjacent tibial plateau, which was layered: Superficial zone – network rich; adjacent deeper zone – network poor; deeper zone still – network rich. An important exception was the cartilage zone immediately above the flattened cell zone, where network was mostly absent. Some network 'crossed' the fibrous perichondrium and was attached to perichondrial fibroblasts. Cell-cell network interconnections correlate with, areas of cell division, developing congruent convex/concave articular surface zones, and chondrocyte hypertrophy in growth plate. Network interconnections are zonally pleiomorphic, and may have several functions. They may stabilise cell arrays during rapid synthesis of extracellular matrix (ECM), give positional information and polarity to cells for directional secretion of ECM, and localise ECM. Consistent with this is the lack of interconnections in the region that will come to be occupied by the resting zone of the growth plate. These observations have implications for understanding mechanisms that coordinate long bone growth at growth plate level, and may be important in answering the key question 'How is new length first added to long bones in the form of new perichondrial length, co-zonally with new chondrocyte height?'.

Ethics Committee Reference: CM/97/181

12 The frequency of mononuclear cells with subplasmalemmal linear densities in a peripheral nerve autoimmune model. By S. O. A. AHMED (supervised by C. L. CRAWFORD). *Department of Neuromuscular Diseases*, *Division of Neuroscience and Psychological Medicine*, *Imperial College School of Medicine*

Subplasmalemmal linear densities (SPLDs) are discrete ultrastructural features characterised by a linear layer of electron dense material of variable length immediately subjacent to the plasma membrane associated with an extracellular basal lamina-like component; they are found on cells of the mononuclear phagocytic system and occur in a number of human diseases such as sarcoidosis, multiple sclerosis, and AIDS encephalopathy, among others. An autoimmune animal model in Dutch Bantam rabbits has been developed in which injection of homogenates of human sensory nerve produce skin lesions and subsequent skin testing at remote locations with the homogenate gives rise to epithelioid cell granulomas at the test site with mononuclear cells positive for SPLDs. It had been shown previously that the antigen inducing such a state of granulomatous hypersensitivity lay in the nonmyelin component of the sensory nerve and the nuclear fraction – obtained by centrifugation – in particular. The present investigation was carried out to investigate the nature of the antigen that gives rise to these SPLD-containing mononuclear cells in the highest frequencies. The results showed that the granulomas induced with the deoxycholate extracted nuclear subfraction induced a very high fraction of such SPLD carrying cells, producing in one case 2 times and in another 3 times as many as were contained in the biopsy where myelin was used for the skin test. It was of note that by weight the protein content in the myelin test solution (9.6 mg) was 6000 times higher than that in the nuclear fraction (1.5 μg). We suggest that a similar nonmyelin antigen may be involved in human disease processes; investigations with such a model may have implications for our understanding of diseases with an unclear pathogenesis where an autoimmune mechanism is suggested, such as in sarcoidosis and multiple sclerosis.

13 The bilateral effects of neonatal capsaicin treatment on the number and mean volume of neurons in the tenth thoracic dorsal root ganglion of the rat: a stereological study. By M. McCARTHY (supervised by C. KENT). *School of Biomedical Sciences*, *University of Nottingham*

Capsaicin is a pharmacological tool of potential therapeutic value used in the study of pain. In order to obtain quantitative information about the long term effects on the peripheral nervous system of neonatal capsaicin treatment we have investigated its effect on sensory cell bodies in the dorsal root ganglion (DRG). Systematic random sampling techniques and unbiased stereological methods were used to estimate the number and mean volumes of A and B-neurons in the left and right tenth thoracic DRG (T10 DRG) of female Wistar rats. Five rats were treated with a single subcutaneous dose of capsaicin, 50 mg/kgbw, 12 h after birth, and 5 litter mate contols were injected with the vehicle solution. At the age of 6 mo, the animals were killed by an intraperitoneal injection of 0.15 mg/gbw sodium pentobarbitone and tissues fixed by intracardiac perfusion of 3% glutaraldehyde and 1% paraformaldehyde in 1% sodium cacodylate buffer. The left and right T10 DRG were removed from each animal after laminectomy, embedded in JB4 resin and serially sectioned at 4 mm. The Nissl stain Cresyl Fast Violet was used to distinguish between the A and B-cell populations. Using random systematic sampling and physical disectors, total numbers of A and B-cells in each ganglion were estimated by multiplying the Cavalieri estimate of ganglion volume by the numerical density of each neurone type. Unbiased estimates of mean cytoplasmic and nuclear volumes were obtained by dividing volume densities (estimated by point counting) by numerical densities. Results were analysed by 2 and 3 way ANOVA with cell type, side and treatment as the main effects. There was no evidence of asymmetry between the left and right DRG in either the vehicle or capsaicin-treated animals for any of the parameters. The mean (coefficient of variation, CV) number of neurons per DRG was estimated as 3320 (9%). Of these, approximately 23% were A-cells with a mean volume of 79800 μm^3 (25%) and 73% were B-cells with a mean volume of 13100 μm^3 (17%). After neonatal capsaicin treatment 56% of the original population was destroyed. The loss of B-cells, constituting 43.1% of the total neuronal population (59.5% of the B-cell population), was significantly greater than that of A-cells, which

https://doi.org/10.1046/j.1469-7580.1998.19230447.x Published online by Cambridge University Press

constituted 11.2% of the total neuronal population (50.8% of the A-cell population). The mean volume of A-cell cytoplasm increased significantly after treatment. In conclusion, neonatal capsaicin treatment in the rat destroys peripheral sensory neurons and results in a deficit of appproximately 60% of B neurons and 50% of A in the neurons in the T10 DRG at 6 mo of age. The effect on A cells has not been recorded in other published studies.

14 Gene transfer to facial motoneurons after the retrograde axonal transport of a modified herpes simplex type I viral vector. By P. ERABADDA (supervised by I. P. JOHNSON). *Department of Anatomy and Developmental Biology*, *Royal Free Hospital School of Medicine*, *London*

The delivery of some growth- and survival-promoting molecules to the central nervous system is hindered by their short half-lives, undesirable systemic side-effects and failure to cross the blood brain barrier. This study explores the possibility that functional copies of genes may be transferred directly to the cell bodies of motoneurons through the retrograde axonal transport of a herpes viral vector. A replication-defective and nonlytic form of herpes simplex type I virus (gift from Prof. D. S. Latchman and Dr R. S. Coffin, University College London) containing either the bacterial reporter gene lac-Z, or the mammalian gene for heat shock protein 27 (HSP) was applied unilaterally to the proximal stumps of the cut right facial nerve of groups of 4 anaesthetised (2% Halothane) adult rats. Controls received phosphate buffered saline (PBS) to the cut nerve. After 3 d, anaesthetised (sodium pentobarbitone) rats were perfusion-fixed with buffered 4% paraformaldehyde and 70 μm vibratome sections cut through the facial nucleus. Alternate sections were immunostained for HSP, calcitonin gene-related peptide (CGRP), or stained histochemically for the lac-Z gene product, beta galactosidase (b-Gal). After application of the lac-Z-containing virus to the cut nerve, ipsilateral b-Gal staining of the cell bodies of facial motoneurons was found. Similarly, after application of the HSP-containing virus to the cut nerve, ipsilateral HSP-like immunostaining of motoneurons was found. Image analysis revealed increased CGRP-like immunostaining of ipsilateral motoneurons when PBS or the Lac-Z containing virus was applied to the cut nerve, but not after application of the HSP containing virus. These results show that the retrograde transfer of functional copies of the heat shock protein 27 gene to rat facial motoneurons can modify one aspect of their response to peripheral nerve injury.

Supported by the Peter Samuel Royal Free Fund.

15 Organ culture of neuromuscular junctions in a mouse exhibiting unusually slow wallerian degeneration (C57Bl/Wlds) By *N. DAVIE (supervised by S. H. PARSON) *Department of Human Biology*, *University of Leeds*. **Present address: Department of Histochemistry*, *Imperial College of Science*, *Technology and Medicine*, *Hammersmith Hospital*

Following section of a peripheral nerve, distal nerve stumps and nerve terminals are rapidly removed by a process of wallerian degeneration. In a mutant strain of mouse (C57BL/Wld^s) the time course of these events is markedly slowed, resulting in the maintenance of intact, functional but disconnected distal nerve compartments for up to 5 d following nerve section. The process of neonatal poly-neuronal synapse elimination, is however, normal in these mice (Parson et al., *Eur. J. Neurosi.* **9**, 1997). In the present study we have used these unique features to develop a novel nerve, muscle organ culture paradigm in which to investigate synaptic phenomena. The levator auris longus muscle and the posterior auricular nerve were aseptically dissected from 200 neonatal and young adult mice killed by cervical dislocation. These wholemount nerve/muscle preparations were pinned at approximate resting length in Sylgard lined glass Petri dishes. Preparations were cultured in supplemented M199 medium at 37 °C in a humidified 95% air/5% CO_2 atmosphere on a rocking platform: The medium was changed after 1 h and every additional 24 h period. After 24, 48 or 72 h in culture, preparations were fixed in buffered 4% paraformaldehyde and stained with combined silver/ acetylcholinesterase for visualisation under the light microscope. Similar preparations were also dissected from wild-type mice and cultured under identical conditions. After 72 h in culture, muscle from Wld^s mice were healthy and striated in appearance and often demonstrated spontaneous fasciculations. Intramuscular nerves were intact and nerve terminals showed characteristic claw-shaped endings, although some had a 'blebby' appearance. The morphology was comparable to that of muscle and nerve 3 d after nerve section in vivo. Preparations from wild-type mice were quite different: muscle fibres appeared healthy, but intramuscular nerves were fragmented and often absent. In these cultures, nerve terminals were rarely seen after 24 h in culture, with nerve terminal degeneration being visible as early as 6 h. The appearance of degenerating terminals and axonal branches in the control mice was noticeably different from that of 'blebby' terminals. Further experiments suggest that the process of synapse elimination may proceed for up to 3 d in these cultures, which raises some interesting questions for the role of patterned neuromuscular activity in synapse elimination. The combination of Wld^s mouse and the novel in vitro system outlined here may prove a useful tool in the study of the cellular and molecular cascades involved in the orchestration of synaptic remodelling at the neuromuscular junction.

S.H.P. was supported by grants from The Nuffield Foundation, The Royal Society and The Wellcome Trust.

16 Determination of a standard site for the measurement of bone mineral density of the human calcaneus. By B. BURSTON (supervised by D. McNALLY and H. NICHOLSON). *Department of Anatomy*, *School of Medical Sciences*, *University of Bristol*

Ultrasound of the calcaneus may be used as a cheap, radiation-free and easy to use indicator of skeletal status, and hence osteoporotic fracture risk. However, at present ultrasound is not widely used as it suffers from high precision errors. As ultrasound parameters are largely determined by bone mineral density (BMD), an increase in the accuracy and precision of BMD measurements, should reduce the precision error associated with ultrasound

measurements. This study therefore set out to define an anatomical site on the calcaneus at which accurate and precise measurement of BMD can be made. Ten dry calcanei and 10 cadaveric feet were scanned using a DXA scanner. Nine 1 cm^2, anatomically defined regions were selected in the posterior part of the calcaneus for analysis. The centre of region number 1 was positioned halfway along the line joining the anterior border to the calcaneal tubercle and the peak of the posterior superior tubercle and the remaining 8 regions were placed around this central area. The BMD in these 9 regions was compared with the whole bone BMD and the variability of BMD within each of the 9 regions was measured. The reproducibility of the technique was assessed by taking 10 repeated measurements of 2 bone and 2 cadaveric specimens, each specimen being removed and repositioned between measurements. Region 1 was found to be the most representative of total BMD in cadaveric feet. This region also showed the least variability of BMD and consistently gave the lowest coefficients of variation in the reproducibility study in both the bone and cadaveric specimens. This region is hence the most suitable site on the calcaneus for measuring absolute values of BMD and changes in BMD. The surface position of region 1 was found to be consistently five ninths of the way along the line from the posterior border of the lateral malleolus to the most angular part of the heel. The identification of the surface location of region 1 relative to anatomical landmarks of the foot, has facilitated the same anatomical site to be measured in all subjects. This allows meaningful intersubject comparison of BMD values to be made. Preliminary data suggest that precision errors using ultrasound are also reduced when measurements are taken at this region of the calcaneus. The reduction in the precision error of ultrasound's assessment of skeletal status may provide a cheap and safe way to identify those people at risk from osteoporotic fracture.

17 The growth of the cranial base in extant hominoids: a geometric morphometric study. By S. N. COBB and P. O'HIGGINS. *Evolutionary Anatomy Unit*, *Department of Anatomy and Developmental Biology*, *University College London*

The form of the cartilaginous cranial base is under tight genetic regulation and is also influenced by adjacent soft tissue, mechanical and hormonal factors. Thus the principal differences in adult cranial base morphology among extant large-bodied hominoids are expected to be established prenatally with no postnatal differences in growth allometry. This hypothesis, together with the corollary hypothesis that more closely related hominoids share more similar postnatal allometries are to be tested. The 2D cartesian coordinates of 18 basicranial landmarks were obtained from cranial radiographs (in norma basilaris) of known age growth series of *Homo*, *Gorilla*, *Pan* and *Pongo*. The landmarks were registered by a least squares fit, which removed differences in size, translation and rotation. Size was recorded separately as centroid size. In the apes, size of the cranial base shows a linear relationship with age whereas in humans, this relationship is curvilinear; a large rate of increase in size during the first 2 postnatal years gives way to more gentle increase later. The shape differences in each species were examined by a Principal Components Analysis (PCA), shape variations on those Principal Components (PCs) found to have a significant correlation with age were visualised using cartesian transformation grids from thin plate splines. Among the apes only the scores for PC1 significantly correlated with age, the visualisation of the allometric shape differences shown by PC1 for each species showed a difference in allometric trends between the 3 species, with the African apes appearing distinct in comparison to Pongo. The main differences between the apes appeared to be the growth processes responsible for anteroposterior lengthening of the whole cranial base and lateral stretching of the mid to posterior cranial base. The Homo sample showed a significant correlation between age and both PC1 and PC2 that appeared to be concentrated in the first 2 postnatal years. The Homo sample was therefore divided into 2 groups, group 1 with those under 2 y old and group 2 with those over 2 y old. The shape variability of group 1 was described by PC1, this represented a considerable lateral stretching of the cranial base, especially of the petrous temporal bone and occipital condyles. PC1 and PC2 of group 2 described a combined shape change, significantly correlated with age, of a relative anteroposterior stretching of the base (PC1) and a widening of the posterior base (PC2). This study has for the first time clearly highlighted differences in the growth of the cranial base amongst great apes and humans. Preliminary studies have also shown how this comparative method can help to interpret fossil morphologies.

18 Apoptotic blebbing of HT-22 cells is prolonged by an ICE-related protease inhibitor and involves cytoskeletal rearrangement. By J. E. R. DAVIES (supervised by J. P. BENNETT). *Division of Biomedical Sciences*, *Imperial College School of Medicine*, *St Mary's Campus*, *London*

The neuron-derived HT-22 cell line was used in a sequence of experiments to examine the morphology of blebbing during apoptosis caused by excitotoxic concentrations of the neurotransmitter glutamate. Significant loss of substratal adherence preceded cell rounding-up 195 ± 39 min (mean $\pm$ S.E., $n = 10$ cells) after the addition of 10 mM glutamate. Cells were then observed to undergo intense periods of blebbing, with the smooth cellular surface being replaced by the undulating blebbing membrane. Once blebbing began, videomicroscopy analysis revealed that it continued for a period of 46 ± 41 min (mean $\pm$ S.E., $n = 10$). The addition of an ICE-related protease inhibitor (benzyloxycarbonyl-Val-Ala-Asp[O- methyl]-fluoromethylketone) significantly prolonged the duration of blebbing to 302 ± 41 min (mean $\pm$ S.E., $n = 10$, $P < 0.05$). Cytoskeletal changes were suggested as being integral to the blebbing state and were investigated with confocal immunofluorescence labelling of HT-22 cells. This revealed marked cytoskeletal changes between control cells and those treated with glutamate. The location of F-actin remained submembranous, including blebs, with a loss of stress fibres corresponding to substratum detachment. The normal characteristic microtubule staining pattern was reduced in extent, with some indication of unpolymerised tubulin being present within blebs. Vinculin labelling illustrated a loss of focal adhesion sites in glutamate-treated cells, and it was

https://doi.org/10.1046/j.1469-7580.1998.19230447.x Published online by Cambridge University Press

concentrated in regions not associated with the substratum. Similar results were seen with talin, spectrin and filamin, suggesting the occurrence of processes for disassembly of adhesion complexes and segregation of the proteins to new sites in the cell. These results imply a specific series of cytoskeletal changes is associated with the blebbing phase of apoptosis. Inhibitor studies suggest an ICE-like protease is necessary for completion of blebbing and for entry into the next stage of apoptosis.

19 Development and axon guidance of cranial motor neurons in the chick and rat. By A. VARELA-ECHAVARRIA, A. HACKER, A. CATON, A. NAEEM and S. GUTHRIE. *Department of Developmental Neurobiology, UMDS, Guy's Hospital*

Within the developing central nervous system, cranial motor neurons develop in a column on either side of the ventral midline floor plate. They later diversify to form various subpopulations based on their segregation into columns, axon trajectories and synaptic targets. Initially all subpopulations of motor axons extend away from the floor plate. Subsequently, somatic motor (SM) axons exit the neural tube ventrally in small groups, whereas branchiomotor (BM) and visceral motor (VM) axons extend into the periphery via large single dorsal exit points. Different motor neuron subpopulations express different repertoires of expression of genes of the LIM homeobox family, perhaps reflecting a role for these genes in specifying motor neurone phenotype. The distinct axon trajectories of motor axon subpopulations in vivo may be generated via differential responses to axon guidance cues in their environment. We have investigated this idea using a coculture system in 3 dimensional collagen matrices. Tissue explants containing different subpopulations of cranial motor neurons were cultured in proximity to tissue explants or cells secreting candidate guidance molecules. We have found that floor plate tissue is chemorepulsive for motor axons, an effect that can be mimicked by the secreted molecules netrin-1 and semaphorin D. Ventrally-projecting (SM) and dorsally-projecting (BM,VM) motor axon subpopulations may become segregated due to their differential responses to these chemorepellent molecules. Once they have reached the periphery, VM and BM axons grow towards the parasympathetic ganglia and the branchial arches respectively. Branchial arch explants elicit strong chemoattraction of cranial motor axons in vitro, an effect that can be blocked by antibodies against Hepatocyte Growth Factor (HGF). HGF is produced by the developing muscles plates of the branchial arches, implying a role for this molecule in generating patterns of nerve-muscle connectivity in the head.

20 Genetic analysis of neural patterning in zebrafish. By C. B. MOENS, W. JACKMAN, V. PRINCE* and C. B. KIMMEL. *Institute of Neuroscience, University of Oregon and *Department of Biology, Princeton University*

The vertebrate hindbrain develops in a pattern that is overtly segmental. Neural crest migrating from the hindbrain forms the segmented skeleton of the pharynx, positioned just ventral to the hindbrain. The hindbrain segments, termed rhombomeres, correspond to the pharyngeal segments, the branchiomeres, in 2:1 manner: The crest forming cartilage in one branchiomere and motor axons innervating muscles in one branchiomere each come from 2 adjacent rhombomeres. If, as has been supposed, rhombomeres and branchiomeres are patterned as subregions of larger whole 'head segments' that include both CNS and periphery, then why is the correspondence between them 2:1 and not 1:1? Mutational analysis provides insight into this question. Using an RNA in situ screen for disruption of hindbrain marker genes in haploid zebrafish embryos, we identified mutants in '*valentino*' (*val*), a gene that we subsequently showed to be the zebrafish homologue of mouse *kreisler* (*kr*). *kr/val* encodes a bZIP transcription factor that is expressed in the primordia of 2 adjacent rhombomeres, r5 and r6. In the absence of *kr/val* function, no rhombomere boundaries develop posterior to the r3/r4 boundary, and our analysis reveals that a region the size of a *single* rhombomere, with some character of *both* r5 and r6, takes the place of the r5-r6 rhombomere pair in *val* mutants. This region has neither odd nor even identity, accounting for why segment borders are missing. From these findings we propose that normally, under control of kr/val^{+}, r5 and r6 develop from a single primordial segmental unit. This primordial brain segment and a branchiomere correspond 1:1, rescuing the 'head segment' hypothesis. The expansion and subdivision of primitive neural segments may have been a feature important for elaboration of the vertebrate head, derived during early vertebrate evolution. In support, marker expression studies suggest that in *Amphioxus*, the sister group to the vertebrates, the correspondence between neural and peripheral segments is 1:1, not 2:1.

21 The origin of cell diversity in the cerebral cortex of the rat: the roles of genetic and epigenetic factors. By J. G. PARNAVELAS. *Department of Anatomy and Developmental Biology, University College London*

An important challenge in developmental neurobiology is to understand how a seemingly homogeneous population of epithelial cells in the early mammalian embryo gives rise to the diverse array of neurons and glia that make up the mature central nervous system. It is now known that neurons and glia acquire their phenotypes both from their genetic material and their environment. My colleagues and I have been investigating the question of cell diversification in the cerebral cortex by examining the influence of both genetic and epigenetic factors. We have used a lineage marker (BAG retrovirus) in combination with a marker of proliferating cells (bromodeoxyuridine) to study the pattern of generation of pyramidal and nonpyramidal neurons, the principal neuronal types in the cerebral cortex of the rat. We found that pyramidal neurons are generated by 2 different patterns of cell division, symmetric and asymmetric; their distribution in clusters suggests that their migration is strictly radial. In contrast, nonpyramidal neurons, the interneurons of the cortex, lose the spatial relationship with their clonal relatives and disperse through tangential migration. Radial and nonradial migratory routes may expose young neurons to yet unknown but probably different cues that may be important for the acquisition of

a specific phenotype. Our data suggest that local environmental factors contribute to the isolation (and phenotype) of nonpyramidal neurons. Such factors may also act on progenitors prior to their commitment to a particular phenotype or to maintain and support a cell induced to a particular phenotype by intrinsic cellular programmes. We investigated these hypothesis by following individual cortical cell lineages in a defined and manipulable environment. We found that neurotrophic factors, neurotransmitters and extracellular matrix molecules differentially regulate cell proliferation, survival and differentiation of cortical progenitor cells. Knowledge of the roles of genetic and epigenetic influences and their relative importance in cell phenotype determination is essential if we are to understand what determines the choice of differentiation pathway taken by individual epithelial cells, and when these choices are made during development.

22 Early neural activity and developing retinal receptive fields in turtles and chicks. By E. SERNAGOR (introduced by S. MILLER). *Department of Child Health*, *The Medical School*, *Royal Victoria Infirmary*, *Newcastle upon Tyne*

Long before the onset of visual experience, embryonic retinal ganglion cells (GCs) in vertebrates are known to fire spontaneous bursts of activity which are correlated between neighbours, resulting in waves sweeping across the retina. This immature retinal activity is believed to play a fundamental role for wiring the developing visual system, both at retinal and extraretinal levels. The first aim of this study is to investigate the cellular mechanisms underlying the generation and propagation of these spontaneous waves. For that purpose, retinae were isolated from chick and turtle embryos (following egg cooling and decapitation of the embryo). Extracellular recording of spontaneous activity from turtle GCs and calcium imaging of spontaneous waves in chick embryonic GCs under different pharmacological conditions reveal that cholinergic and glutamatergic connections as well as extracellular potassium contribute to the activity generation and propagation through short and long range synaptic connections. The 2nd aim of this study is to investigate the role this early spontaneous activity versus early visual experience in shaping receptive field properties of developing GCs in the turtle. To address this question, 2 opposite approaches were used: chronic blockade (by exposure to curare, a nicotinic cholinergic blocker) and prolongation (by dark-rearing) of spontaneous firing of GCs. Curare (1 mM) was applied intra-ocularly through the slow-release polymer Elvax 40W implanted at different developmental stages. Prolongation of the period of spontaneous bursting by dark-rearing leads to a profound expansion of receptive field areas, an effect that is counteracted by exposure to curare during the period of dark rearing. Receptive field areas measured in posthatching turtles reared in normal light and dark cycles are significantly smaller if the retina is exposed to curare from embryonic stages. These changes in receptive field areas correlate with changes in dendritic arborisation in GCs, suggesting that early spontaneous cholinergic bursting activity, rather than light, controls the development of receptive field areas, presumably through dendritic outgrowth in GCs. As GCs develop more complex receptive field properties, visual experience becomes more involved in their maturation. The development of concentricity of receptive fields depends both on spontaneous activity and on visual experience, while maturation of orientation selectivity depends primarily on visual experience.

23 The role of nerve-muscle interaction in rat motoneuron development. By R. NAVARRETE, G. MENTIS, E. DIAZ and L. MORAN. *Division of Neuroscience and Psychological Medicine*, *Imperial College School of Medicine*, *Charing Cross Campus*, *London*

During early stages of development, motoneurons are critically dependent on their target muscles for survival and differentiation. Previous studies have shown that neonatal axotomy causes massive motoneuron death, most of which occurs in the 1st week postinjury. This cell death is believed to be due to the loss of neurotrophic support from the target but little is known about the functional alterations that precede cell death. In order to gain a better understanding of the factors involved in motoneuron target dependence we have investigated the electrophysiological and morphological properties of motoneurons following neonatal axotomy. In newborn rats (P0), tibialis anterior motoneurons were retrogradely labelled by intramuscular injection of fluorescent tracers (fast blue and diamidino yellow). Two days later, the common peroneal nerve was crushed unilaterally. Intracellular recordings were obtained from visually and antidromically identified motoneurons using an in vitro hemisected spinal cord-hindlimb preparation. In normal motoneurons during the 1st postnatal week, the input resistance and rheobase, both electrophysiological parameters related to cell excitability, decreased markedly as a function of age. Following axotomy, the developmental decrease in motoneuron excitability was prevented and the input resistance, time constant and the duration of the after-hyperpolarisation (AHP) were significantly increased compared to control. The firing pattern of these cells was characterised by tonic, low frequency firing with little or no adaptation. In contrast, most normal motoneurons showed an initial high frequency burst followed by low frequency tonic firing. These changes may be related to the shift towards a more tonic firing pattern in axotomised motoneurons which persists into adulthood. Morphological analysis of intracellularly labelled motoneurons using confocal microscopy revealed that during the first 5–6 d after birth the soma and proximal dendrites of normal flexor motoneurons are covered by growth-associated processes (e.g. spines, filopodia and growth cones), most of which disappear during the second postnatal week. In addition, transient electrotonic and dye coupling between motoneurons is seen at this stage. Neonatal axotomy arrested the disappearance of these growth-associated processes and increased the incidence of gap junctional coupling. Sholl analysis revealed a decrease in dendritic branching and a significant decrease in total dendritic length compared to control. Nevertheless, some of the axotomised cells showed evidence of somatic and dendritic sprouting. A small number of injured motoneurons exhibited extremely abnormal features such as swelling of the soma and proximal dendrites, cytoplasmic vacuoles and dendritic varicosities. It is possible that some of the morphological changes observed

https://doi.org/10.1046/j.1469-7580.1998.19230447.x Published online by Cambridge University Press

(e.g. dendritic sprouting) may represent attempts at regeneration, while others (e.g. dendritic varicosities) may be associated with cell degeneration These results demonstrate that disruption of nerve-muscle interaction keeps the motoneurons in an immature state expressed physiologically by increased excitability and morphologically by the maintenance of growth associated processes. It is possible that the enhanced excitability of axotomised neonatal motoneurons may contribute to their death by excitotoxicity.

We are grateful to the Medical Research Council, Wellcome Trust and European Commission for support.

24 Ontogeny and phylogeny of thalamocortical interactions in vertebrates. By Z. MOLNÁR (introduced by S. MILLER) *University Laboratory of Physiology*, *Oxford*

Thalamic axons, which will later mediate most sensory information from the environment, are the first to reach the cerebral cortex, before the majority of cortical neurons have even been born. Anatomical studies on the earliest thalamocortical projections have demonstrated their distribution in topographic order and without gross exuberance before entering the cortical plate (Molnár & Blakemore, *TINS* **18**, 1995) and optical recording studies have revealed that their cortical synapses are functional from these embryonic ages (Higashi et al., *Soc Neurosci. Abstr*, **22**, 1996). During the early embryonic development of the mammalian pallium, unusual precocious cell populations, some of them transient, exist in the cortical subplate, marginal zone and internal capsule. These fascinating cells might assist in the establishment of external cortical circuitry by providing temporary targets for corticopetal and corticofugal projections, and by forming early scaffolds over which the other axons grow, or by acting as guidepost cells within the internal capsule (McConnell et al., *Science*, **245**, 1989; Mitrofanis & Guillery, *TINS* **16**, 1993). After their role has been fulfilled, many of them disappear some time after birth. Partly because of their supposed role in pathfinding, studies on these cells have blossomed in recent years and we are now beginning to understand the strategic position they might hold during cortical circuit formation in mammals. Their equivalent homologous cell groups in reptiles and their evolutionary origin is, however, unknown. The comparative analysis of brain development is useful in this respect. Comparison of immature stages reveal features of evolution that are otherwise obstructed by the complexity of the mature brain. Reciprocally, to look at development by thinking in evolutionary terms helps to focus on the most biologically relevant mechanisms. We have taken advantage of the fact that comparisons revealing common developmental algorithms are often easier to make in developing brains. We have examined the distribution and origin of transient cell populations in the embryonic mammalian cortical subplate, marginal zone, and perireticular and thalamic reticular nuclei (Cordery et al., *Soc Neurosci. Abstr*, **23**, 1997). These cells have numerous similarities in all mammals examined: they are among the earliest generated cells in the pallium, contain distinctive neurotransmitters and form the first connections within and outside the cortex. In my presentation, I shall review the location of cell groups with similar connectivity and molecular markers in the developing mammalian and reptilian brain and systematically analyse the hodological and neurochemical patterns in the rat (between embryonic (E) day 14–17), marsupial, (*Monodelphis domestica* between postnatal d 2–30) and in turtle (*Pseudemus scripta elegans*, between stages 17 and 25). For all our studies we used carbocyanine tracing (from dorsal and ventral thalamus, internal capsule, ventral and dorsal cortex) and calretinin immunohistochemistry on postmortem fixed brains. Our studies suggest that, in both mammals and turtles, axons of similar cells reach both the dorsal and ventral compartments of the thalamus at very early stages and thalamocortical development follows common developmental algorithms. I shall review recent comparative developmental studies using cell-specific and genetic markers which will bring a deeper understanding of the general features of thalamocortical development and of the fundamental principles of construction of the different subdivisions of the reptilian and mammalian pallia and the possible evolutionary origin of the mammalian isocortex.

25 Refinement of cortical connections during development in the mouse. By D. J. PRICE, R. BEAU LOTTO, P. ASAVARITIKRAI and L. VALI. *Department of Physiology*, *University Medical School*, *Edinburgh*

The cerebral cortex receives its major afferent innervation from the thalamus. These afferents terminate mainly in cortical layer 4. In mice, thalamocortical axons begin to grow at about embryonic d 14–15 (E14–15; gestation is 20–21 d) and enter the cortex over the following few days. Thalamocortical axons find their targets in cortical layer 4 at around the time of birth. After birth, there is considerable refinement of these connections, involving both subtle alterations in the axonal arbors and terminals of thalamic neurons and the death of entire thalamic neurons. We are interested in the mechanisms that regulate the survival of thalamic neurons. To study these mechanisms, we use an in vitro system in which we dissociate embryonic dorsal thalamic cells and culture them in defined serum-free medium. We have shown that E15 dorsal thalamic cells survive for up to 3 d when cultured at high density; to survive at low density, they require diffusible factors from the thalamus or added neurotrophic factors such as the neurotrophins or fibroblast growth factors (FGFs). Since dorsal thalamic cells produce these factors, we suggest that neurotrophic factors endogenous to the early embryonic thalamus may promote the survival of its cells. Dorsal thalamic cells taken from older embryos, around E19, or cultured from E15 for 4 d or more do not survive even when plated at high density, suggesting that these cells alter their trophic requirements at the time when they innervate the cortex. E19 thalamic cells are not rescued by neurotrophins or FGFs added to the medium, but do survive when cultured in medium conditioned by cortical slices. These results suggest that as thalamic cells innervate the cortex they switch their trophic requirements from a dependence on factors intrinsic to the thalamus to factors derived from the cortex. The neurotrophic hypothesis, based on a knowledge of the mechanisms that regulate neuronal numbers in the peripheral nervous system, proposes that neuronal numbers are controlled by competition for target-

derived trophic substances. Our results, indicating that thalamic cells switch their trophic requirements to target-derived factors prior to the major period of thalamic cell death, suggest that this concept may be applicable to the modulation of cell numbers in the thalamocortical system.

26 The development of the corticospinal tract in the rat. By E. A. J. JOOSTEN. *Department of Neurology*, *Rudolf Magnus Institute for Neurosciences*, *University of Utrecht*

The corticospinal tract (CST) is a long descending central pathway, restricted to mammals, and involved in both motor and sensory control. The rat CST is a very useful model in experimental research on the development of fibre systems in mammals because of its postnatal outgrowth throughout the spinal cord as well as its experimental accessibility. Hence, mechanisms underlying axon outgrowth and subsequent target finding of developing CST fibres can be studied relatively easily. In order to fully understand the development of the rat CST it is of eminent importance to know which guidance factors are involved in the different phases of CST outgrowth and spinal target finding. At present the most plausible explanation of the mechanisms underlying pathfinding of CST axons during their outgrowth is that a number of guidance cues act together. In this context the role of immature astroglial cells, the adhesion molecules L1 and N-CAM as well as tropic diffusible factor(s) will be discussed.

27 Development of the corticospinal tract in man. By G. J. CLOWRY, E. A. CONWAY, J. A. EYRE, S. MILLER and J. P. TAYLOR. *Department of Child Health*, *University of Newcastle upon Tyne*, *Royal Victoria Infirmary*

Neurophysiological studies of motor evoked potentials following magnetic stimulation of the motor cortex have revealed responses in upper limb muscles in neonates as premature as 28 wk gestation. The latency of the descending volley recorded over C5 vertebral spine, compared with the central motor conduction delay, provides evidence for a monosynaptic excitation of motoneurons, at least at term. Spatial summation of subthreshold group Ia afferents and subthreshold corticospinal excitation of biceps brachii motoneurons confirms the observation. Similarly, spatial summation of group Ia inhibition from triceps to biceps brachii with corticospinal excitation establishes a monosynaptic corticospinal projection to group Ia inhibitory interneurons. Anatomical cytochemical studies were carried out on fixed frozen sections of developing human cervical spinal cord (25–42 wk gestational age) obtained with parental consent at postmortem. Myelination was found to be well advanced in all white matter tracts except for the corticospinal tracts (CST) by 25 wk, however at 42 wk some myelinated axons were also observed in the CST. Expression of growth associated protein 43 kDa (GAP43) was studied between 25–33 wk gestation. At 25 wk, GAP43 immunoreactivity was present throughout the white and grey matter, but by 29 wk was largely confined to the CST in the spinal white matter and to fibres emanating from the CST. These fibres innervated the intermediate grey matter and deep dorsal horn but not the motoneuron pools. This pattern of labelling was similar to that seen 1 mo postnatally in the macaque monkey following anterograde labelling from the motor cortex (Armand et al. *J. Neurosci.* **17**, 1997). By 33 wk, GAP43 immunoreactivity remained in the CST and had spread to all the grey matter, comparable to the macaque 3–5 mo postnatally. Indirect evidence for functional corticospinal innervation of the human spinal cord from as early as 25 wk gestation was provided by the observation of parvalbumin immunoreactivity in neurons in the ventral horn. Experiments in rats have shown that parvalbumin expression follows cortical innervation of spinal neurons and is greatly reduced by a motor cortex lesion performed prior to corticospinal synaptogenesis. In conclusion, these physiological and anatomical studies taken together provide strong evidence that the CST projects monosynaptically to motoneurons and interneurons prenatally in man.

Support from the Wellcome Trust is gratefully acknowledged.

28 The influence of retinoic acid on the morphogenesis of the rat cleft palate and tongue. By E.-N. EMMANOUIL-NIKOLOUSSI, CH. KERAMEOS-FOROGLOU and A. DHEM*. *Laboratory of Histology-Embryology*, *Faculty of Medicine*, *Aristotle University of Thessaloniki*, *Greece and* **Unité de Anatomie Humaine*, *Faculté de Médecine*, *Université Catholique de Louvain*, *Bruxelles*

Retinoic acid (RA) has been viewed in recent years as a potential teratogenic factor for embryonic mammalian species, including man. Exposure on critical gestational days induces a high incidence of cleft palate. This exposure includes doses that are not maternally toxic and do not increase fetal resorption rates. Abbott et al (*Teratology* **41**, 1990) consider that the mechanism by which RA induces cleft palate in embryos depends on their developmental stage. The timetable and dosage level, selected for this study, were based on our previous teratological data concerning RA induction on the craniofacial area (Emmanouil-Niloloussi et al, *Annals 2nd Medit. Cong. Oral & Maxillo-facial Surg*, 1993). For this study, female Wistar/Syrates rats were divided into 4 groups. Rats in group 1 were treated with 150 mg RA/ kg bw on embryonic (E) days 10.5 and 11. Animals in group 2 were treated with the same dose of RA on E11 and E12 and animals in group 3 were given a single dose of 200 mg RA/kg bw on E11. The 4th group of rats were left untreated and served as controls. The palatal shelves of rats in groups 1 and 3 had obvious clefts, while smaller gaps were observed in the anterior and middle portions of the palate of rats in group 2. In all 3 experimental groups, the middle part of the tongue was raised in the cleft region and showed signs of muscular hyperplasia. Our results support the teratogenic influence of RA on diverse cell populations in the palatal shelves and tongue. Cells in the developing tongue seem to be influenced both by the RA induction and by the autonomous intrusion of the body of the tongue into the median cleft. Although we support these 2 theories, our results also suggest that the epithelium on the upper surface of the tongue is especially influenced by RA which affects its epithelial cell lines.

29 Morphological changes in target muscles after nerve repair in the rabbit. By A-J. CARTER, F. KRISTMUNDSDOTTIR and M. A. GLASBY. *Department of Anatomy, University of Edinburgh*

The idea of peripheral nerve repair using nonneural tubes is not new but has met with limited success. Successful repair of peripheral nerves using short coaxial freeze-thawed muscle autografts (FTMG) has been demonstrated experimentally and clinically. The aim of the experiments presented here was to compare a new method of nerve repair by entubulation to repair by means of the FTMG. Assessment was carried out by investigation of the target muscle using histological and histochemical methods. Two groups of 5 adult New Zealand White rabbits were used. In one group, FTMGs were used to bridge gaps of 1 cm in the left common peroneal nerve. In the 2nd group, an equivalent gap was bridged by inserting the cut ends of the nerve into the ends of a 3 cm biodegradable glass tube (BGT) containing at its centre a 1 cm length of autogenous randomly oriented freeze-thawed muscle. After a recovery period of 180 d, the animals were assessed by measuring the wet muscle mass, the volume fraction of connective tissue, the narrow-fibre diameter and the form factors of type I (slow) and type II (fast) muscle fibres. The animals were anaesthetised with Fentamine/Ketamine and killed with a pentobarbitone overdose. Assessment was carried out on the left extensor digitorum longus (EDL) muscle which was removed and immediately frozen in melting isopentane (-160 °C). The samples were then stored at -70 °C until ready for sectioning on a cryostat. Sections were stained for myofibrillar adenosine triphosphatase and with Masson's trichrome for connective tissue They were then examined using a computerised image analysis system calibrated against a 1 mm calibration grid slide. There was no significant difference between the 2 groups in respect of the wet muscle mass or the connective tissue content. The mean narrow-fibre diameter for FTMGs was found to be significantly lower for type I ($P < 0.05$) and significantly higher for type II ($P < 0.001$) fibres than the equivalent measurements in the BGT group. The mean values of form factor in the BGT group were significantly greater for both type I and type II fibres than those in the FTMG group. There was evidence of grouping of the fibre types within both experimental groups. The results indicate that after nerve repair using either of the experimental methods, there was effective reinnervation of the target muscle but it remains inconclusive as to whether the observed morphological changes corresponded to any change in function. It seems likely, from this and other experiments conducted in our laboratory that biodegradable glass tubes may offer an effective and technically simple means of repairing transected nerves.

30 The effects of riluzole on motoneuron survival and muscle recovery in a rat model of motoneuron degeneration. By N. DAVIES, J. BISH and M. B. LOWRIE. *Division of Biomedical Sciences, Imperial College School of Medicine, London*

Amyotrophic lateral sclerosis (ALS) is a neurological disorder characterised by progressive motoneuron degeneration and paralysis. There is evidence that glutamate excitotoxicity contributes to this neuronal death. Riluzole, a drug with antiglutamate properties, has been shown in clinical trials to prolong the life of ALS patients and in vitro to protect cultured motoneurons from glutamate toxicity, but whether it can prevent motoneuron death in vivo is not known. In this study we measured the effect of Riluzole on motoneurons which had been induced to die in rats after peripheral nerve injury. This form of cell death is also thought to involve glutamate toxicity. The sciatic nerve was crushed in one hindlimb of Wistar rats within 1 d of birth under Halothane anaesthesia and sterile conditions. Riluzole was injected subcutaneously twice daily at a dose of either 4 or 8 mg/kg/d, starting at the time of surgery and extending for 10 d. Control groups of rat pups received injections of the vehicle (5% DMSO in saline) with or without nerve crush surgery, or Riluzole without surgery. 2–4 mo later the motoneurons innervating either the soleus muscle or tibialis anterior and extensor digitorum longus (TA/EDL) of both legs were labelled retrogradely by injection of horseradish peroxidase (HRP) into the muscle, under anaesthetic. After 2 d, the animals were killed by perfusion with fixative under general anaesthesia, after which the spinal cord was removed and processed for HRP histochemistry. The soleus, TA, EDL and plantaris muscles were dissected and weighed. The number of labelled motoneurons in each ventral horn was counted using a light microscope. After nerve crush the number of labelled motoneurons on the operated side was reduced to 8.8% (± 2.4 S.E.M., $n = 6$) of the contralateral control count . Neither Riluzole nor the vehicle affected motoneuron counts in control animals and neither doses of Riluzole caused any significant improvement in motoneuron survival after nerve injury (4 mg/Kg: 7.8 ± 3.2, $n = 5$; 8 mg/Kg: 9.0 ± 3.3, $n = 6$). Similar results were found for the motor pool innervating TA/EDL. Thus we conclude that, at doses similar to and higher than those used in patients, Riluzole does not prevent the death of motoneurons induced to die in this animal model. When the muscle weights from the experimental groups were compared it was found that those animals which received nerve crush and Riluzole treatment showed improved recovery of soleus and plantaris muscles (soleus 20% improvement, plantaris 25% improvement, compared to untreated rats, $P < 0.02$ by unpaired t test). These results suggest that the effect of Riluzole on patients may be through a beneficial effect on the existing motoneurons or the muscle rather than through delaying degeneration of motoneurons.

This work was funded by the Wellcome Trust and Motor Neurone Disease Association.

31 CHL1, a cell recognition molecule closely related to L1, is expressed in motoneurons following sciatic nerve crush in the rat. By R. ROSLAN, N. HAQUE, M. SCHACHNER* and P. N. ANDERSON. *Department of Anatomy, University College London and *Zentrum für Molekulare Neurobiologie, Universität Hamburg*

Close Homologue of L1 (CHL1) is a member of the L1 family of neural recognition molecules. As with the other L1

https://doi.org/10.1046/j.1469-7580.1998.19230447.x Published online by Cambridge University Press

family members, CHL1 expression is predominantly observed in the nervous system. In the central nervous system however, unlike L1, CHL1 is not only expressed by certain neurons, but also by glial cells. We have studied the effect of axotomy on the expression of CHL1 mRNA in lumbar spinal cord of adult rats. Adult female Sprague-Dawley rats were deeply anaesthetised with Halothane and the left sciatic nerve crushed with watchmakers forceps. In some animals the left L4 and L5 dorsal roots were also crushed. Between 1–5 wk after sciatic nerve, crush rats were anaesthetised, killed by exsanguination and the lumbar enlargement of the spinal cord was removed and processed for in situ hybridisation using a digoxygenin-labelled riboprobe. CHL1 mRNA was not detected in white matter following sciatic nerve injury, but was strongly upregulated in motor neurons in the spinal cord on the injured side at 1 wk and 2 wk after injury. This upregulation declined again by 5 wk. Motor neurons on the unoperated side were not labelled at any stage and background staining was very low. This is in contrast to L1 mRNA expression which is detectable in neurons throughout the grey matter of unoperated lumbar spinal cord and in which no change can be detected using this method following sciatic nerve crush. Dorsal root injury had no additional effect on CHL1 mRNA expression in the grey matter. Our preliminary studies suggest that the response of motor axons to injury at a distal site is characterised by an increase of CHL1 mRNA in the cell bodies of the injured neurons while regeneration is in progress. Since CHL1 strongly promotes the growth of neurites in culture, its expression by regenerating motor neurons may play an important part in the elongation of axons within the injured nerve.

Supported by the Wellcome Trust and the MRC.

32 A comparison of the developmental expression of nitric oxide synthase (NOS) in dorsal root ganglion (DRG) neurons with that of nerve growth factor (NGF) and its receptor, trkA, in the rat. By T. THIPPESWAMY and R. MORRIS (introduced by S. MILLER) *Department of Veterinary Preclinical Sciences*, *University of Liverpool*

NOS is found in very few DRG neurons in the adult rat but early in fetal development (E12) and following axotomy in the adult it is expressed in many of these neurons. We have recently found that the synthesis of NOS in neonatal (P15) DRG neurons in dissociated tissue culture is negatively regulated by NGF and that NO is neuroprotective. In view of this we have examined the relationship between NOS expression and that of NGF and its high affinity receptor, trkA, in embryonic and postnatal development in situ and in dissociated cultures. Cultures of dissociated DRG from E15, E18, and P0–P21 rats (anaesthetised with ether and decapitated) were grown using standard culture methods. They were grown on poly-D-lysine coated coverslips in a serum rich medium with or without added NGF for 5 d at 36 °C, 5% CO_2. For in situ studies whole embryos (E12-P0, gelatine-embedded), or DRG (P1-P21) were cryostat sectioned. Cultures and sections were stained using immunofluorescence and immunocytochemical methods to reveal the distributions of NOS, NGF and trkA. Apoptosis was also observed using the TUNEL method. At E12, 100% of DRG neurons contained NOS whilst at E18 it could only be observed in 5%, however, between P0 and P8 this rose to 18–20%, returning to $< 5\%$ by P21. NGF could not be observed in the DRGs of the embryos but was found in the epidermis, becoming more intense in the older embryos. NOS and trkA tended not to be colocalised in the DRG supporting the view that NGF acting via the trkA receptor reduces NOS expression. Apoptosis was observed in the DRG in situ by E13 and rose to a maximum by E18. Neither NOS nor trkA positive DRG neurons showed apoptotic features in situ. Interestingly, NOS inhibitors did not cause DRG neurone cell death in cultures derived from embryo (E15 and E16) DRGs whilst it did in those from postnatal animals over P10. In early embryonic development, DRG neurons have not made peripheral tissue connections and are NGF independent, during this period they show high levels of NOS and NO may have a neuroprotective role. As peripheral connections are established the DRG neurons take up NGF and are dependent on this growth factor. During this phase NOS is downregulated, and NO does not influence cell survival. Neurons not making appropriate connections undergo cell death. From approximately P10 through to adulthood, DRG neurons are NGF independent and NO appears to have a neuroprotective function following axotomy.

33 Time-dependent modulation of granule cell dendritic spine density in adult rat hippocampus following passive avoidance learning. By A. O'MALLEY, C. O'CONNELL and C. M. REGAN (introduced by J. BANNIGAN). *Department of Pharmacology*, *University College*, *Dublin*

The entrainment of learning-associated neuroplastic change is likely to involve synaptic rearrangement during the conversion of short-term to long-term memory formation. Previous studies have shown that anti-NCAM, when injected at a defined 6 h posttraining time, induces amnesia at 48 h but not at earlier recall times (Doyle et al., *J. Neurochem*. **59**, 1992). This delayed emergence of amnesia has been attributed to an involvement of NCAM in the transient production of novel synapses which competitively alter connectivity pattern during consolidation. We have identified granule cells in the dorsal crest of the adult rat dentate gyrus which transiently increase their dendritic spine density at 6 h following passive avoidance learning (O'Connell et al., *Neuroscience* **76**, 1997). We have now employed an unbiased stereological counting probe, the disector, to determine if the increased spine expression was transient following passive avoidance learning. Adult male Wistar rats were trained to acquire a one-trial, step-through, light-dark passive avoidance paradigm. Immediately after recall, animals were anaesthetised with pentobaribitol and perfused transcardially with 4% paraformaldehyde/2% glutaraldehyde at pH 7.4. A series of disector-pairs were generated from ultrathin sections taken in the midmolecular layer at a point approximately -3.3 mm with respect to Bregma. Spine density in the midmolecular layer of the

https://doi.org/10.1046/j.1469-7580.1998.19230447.x Published online by Cambridge University Press

dentate crest exhibited a time-dependent, learning-specific increase which was maximal at the 6 h posttraining time. The majority of spines formed axospinous synapses and their density per unit volume was approximately 2.5 fold greater with respect to the control animals. Increased spine density persisted until the 24 h posttraining time and, thereafter, exhibited a time-dependent decrease in their number and finally reached the control value at the 72 h posttraining time. These results confirm transient synapse formation to subserve memory consolidation in a manner consistent with alteration in synapse connectivity pattern.

34 Expression of amyloid precursor protein (APP) in human astrocytes in vitro; isoform specific increases following heat shock. By C. E. SHEPHERD, E. L. CALVERT, M. A. CAMBRAY-DEAKIN and R. C. A. PEARSON. *Department of Biomedical Science, University of Sheffield*

Amyloid precursor protein (APP) is the parent protein from which the β amyloid deposited in the brain in Alzheimer's disease (AD) is derived. Differential splicing generates 3 major isoforms of the protein, full length APP $_{770}$, APP_{751} (minus exon 8, a Kunitz protease inhibitor domain, KPI) and APP_{695} (minus exons 7 and 8). Most APP is cleaved by α secretase in the middle of the β amyloid sequence. Alternative processing (β and γ secretase cleavage) excises the β amyloid peptide from the precursor protein intact, probably intracellularly, within the endosome/lysosome compartment. Our aim was to study the effects of heat-shock on the expression and processing of APP in a human astrocytic cell line. Expression and differential splicing of APP was examined in a human fetal astrocytic cell line (CC2565, Clonetics) using RT-PCR, Northern blotting and in situ hybridisation (ISH). Processing of APP isoforms was studied by Western blotting. Heat-shock comprised incubation at 42 °C for 30 min. Cells and media were harvested after a further 1, 2, 12 and 24 h recovery at 37 °C and the expression of the APP isoforms quantified by ISH, and APP secretion by Western blotting using 22C11 (Boehringer) and a KPI domain specific antibody (kindly donated by Dr D Parkinson). These cells express predominantly KP1 containing isoforms of APP (APP_{770} and APP_{751}), with little APP_{695} mRNA detected. Heat shock led to an increase in the mRNA encoding KPI isoforms of APP, which peaked at 4 h. This increase was confined to the mRNA encoding the APP_{751} isoform, with no change in APP_{695} mRNA and a significant decrease in APP_{770} mRNA. Western blotting showed a significant decrease in secretion of APP into the medium following heat shock. Cross-reactivity of antibodies used with APLP2 was excluded by the demonstration that these cells do not contain significant APLP2 mRNA or protein by ISH using an APLP2 specific oligonucleotide probe and Western blotting using an APLP2 specific antibody (3B11, kindly donated by Dr M.-T. Webster). These data demonstrate increased expression of APP following heat-shock. This is unlikely to be due to a direct action on the promoter of the APP gene, since it is specific for one splice product, APP_{751} mRNA. The increase in expression is accompanied by a decrease in secretion of APP, implying a shift in processing of the protein towards an intracellular route, which is known to be potentially amyloidogenic. Such a mechanism may contribute to amyloidosis in the intact brain in response to cellular stress, such as head injury.

35 Embryonic development of the retinal projections in the turtle (*Pseudemys scripta elegans*). By Z. MOLNÁR and E. SERNAGOR* (introduced by S. MILLER). *University Laboratory of Physiology, Oxford, and *Department of Child Health, The Medical School, University of Newcastle upon Tyne*

Turtle retina has previously been used to examine the role of early spontaneous retinal activity on the development of receptive fields of retinal ganglion cells (Sernagor & Grzywacz, *Curr Biology* **6**, 1996). Ganglion cells fire spontaneous bursts from embryonic stage 22 and throughout the 1st month posthatching. However, little is known about the development of the central visual projections. We have examined their development between embryonic stage 17 and 25 (embryonic ds 20–50, 60 d gestation in total – staging according to Yntema, *J. Morph.* **125**, 1968) and report our results on carbocyanine dye tracing in fixed tissue from the retina and colliculus. Our study was performed using 28 eggs from different embryonic stages on red-eared slider turtle (*Pseudemys scripta elegans*). Embryos were decapitated following cooling. After fixation in 4% paraformaldehyde (in 0.1 mM PBS, 4 °C, pH 7.4), single crystals of fluorescent carbocyanine dye 'DiI': (1,1′-dioctadecyl 3,3,3′3′-tetramethylindocarbocyanine perchlorate, Molecular Probes, Inc.) were inserted into the retina or the tectum (see Godement et al., *Development*, **101**, 1987). After crystal insertion, the brains were stored at room temperature (22 °C) in fixative. Following incubation, the brains were embedded in 5% agar, and 70–100 μm coronal sections cut on a vibratome (Oxford Instruments). All sections were counterstained with bisbenzimide (10 min in 2.5 μg/ml solution in PBS) in order to reveal major boundaries. The sections were coverslipped in PBS and then sealed before examination under epifluorescence illumination and photographed. After stage 17, almost all retinal fibres crossed and ascended along the lateral wall of the diencephalon and some began to invade the lateral geniculate nucleus (LGN) of the perirotundal complex. However, the nucleus rotundus remained free of labelling. Only 3–4 axons/brain could be followed as they formed branches in the ipsilateral thalamus, suggesting almost complete decussation of the optic nerves. No fibres reached the tectum at stage 17. By stage 20, the first fibres began to reach the tectum, and by stage 21, the retinotectal fibres began to form a continuous layer in the upper layers of the tectum. From stage 21–25, these projections became stronger and the labelled layer thicker. Tectal crystal placements at stage 17 did not yet backlabel retinal ganglion cells (examined on retinal wholemounts on both ipsi and contralateral retina). The first backlabelled retinal ganglion cells emerged at stage 20 and their number increased throughout stage 25. Crystal placement in different regions of the tectum labelled ganglion cells in different

https://doi.org/10.1046/j.1469-7580.1998.19230447.x Published online by Cambridge University Press

regions of the retina, suggesting early retinotectal topography. When DiI was applied in the tectum, intensive fibre labelling was observed in the ipsilateral nucleus rotundus. Our study demonstrates that the axons of the retinal ganglion cells reach the thalamus and tectum by stage 20–21, before ganglion cells start bursting spontaneously (stage 22). These findings suggest that spontaneous retinal activity is not necessary for retinal projections to reach their targets. However it remains to be determined to what extent this early activity influences precise mapping of retinal projections.

POSTERS

D. 1 A comparison of anatomy education in Turkey with some Asian countries. By N. S. CANKUR and M. A. KURT. *Department of Anatomy*, *Faculty of Medicine*, *Uludag University*, *Bursa*, *Turkey*

Human Anatomy forms the foundation for clinical medicine and is the most important unit of the basic medical sciences. Thus, its place in the medical school curriculum deserves careful attention. Typically, classes in anatomy include gross anatomy and neuroanatomy and are taught via lectures which are extremely efficient for delivering large amounts of information in a short period of time and via dissections, prosections, models and most recently computer-aided learning packages. Associated subjects such as histology and embryology are taught either by related departments or by the academic staff members in Anatomy departments. With the advances which are taking place in basic sciences (e.g. molecular, cellular, structural and neural biology) and the changes in the mechanism of funding for universities and the introduction of research and teaching assessments, a new paradigm for medical education is being created in most Anatomy departments in developed countries. This has resulted in a necessary decline in the amount of time allotted to lectures and dissections in anatomy courses. Integrated teaching systems and computer-assisted education have therefore been introduced in order to fill this gap. However, this is not yet the case in most Asian countries where Anatomy is still being taught in traditional ways. In this study, we compared anatomy education in Turkey, South Korea, China, India and Japan. The hours allocated for gross anatomy lectures and dissections are documented. Anatomy teaching was in all cases found to be relatively lecture and laboratory intensive, with dissection being the primary laboratory teaching method. Use of anatomical models, audiovisual aids and computer-assisted instruction as auxiliary teaching methods varied between the countries. When student-to-cadaver ratios were compared Japan had the lowest student/cadaver ratio, Turkey the highest. Some of the reasons for the differences in teaching methods between countries are clear and include religion, social factors and prevailing educational orthodoxy. However, in the current study, it was difficult to assess the efficacy of the teaching regimes in the different countries but such an evaluation would seen critical for the rational design of new curricula that is currently taking place in many countries.

D. 2 Creating an anatomical database of the anatomy of the shoulder using an applied anatomy section to demonstrate the relevance of the basic anatomy of this region. By D. A. SHANAHAN, F. C. S. KANU, R. K. JORDAN, M. KHAW*, A. PRIDIE** and P. DRUMMOND***. *Anatomy & Clinical Skills Centre and Department of Orthopaedics**, *The School of Surgical Sciences*, *Cookson Computing Centre****, *University of Newcastle Upon Tyne*. *Department of Anaesthetics*** *and Freeman Hospital*, *Newcastle Upon Tyne*

In Newcastle, gross anatomy teaching is traditionally based on cadaveric dissections and taught in the dissecting room by demonstrators to small groups of students (10–16). This traditional method of teaching must be reviewed because there are more students, fewer staff and the students have less time in the dissecting room. To overcome these difficulties gross anatomy is now taught on the cadaver and the computer. Therefore a number of anatomists and clinicians have constructed databases, ranging from a simple list of muscles and their attachments, actions and innervation to 3-dimensional atlases of the human body. The basic format of current anatomical textbooks and atlases is to present the anatomical data and its clinical relevance. As far as the authors are aware previous authors of shoulder databases have not enhanced this basic format by creating an applied anatomy section. The aims of this work were to create a database of the anatomy of the shoulder and to highlight the relevance of the basic anatomy by creating an applied anatomy section. Text is divided into pages that are linked to other relevant textpages and photographs. The completed database consists of 120 pages, 30 photographs and approximately 5 min of digitised video. Following the title page students are presented with the following menu: osteology, arthrology, myology, neurology and applied anatomy, these are linked to the relevant pages. The applied anatomy section is divided into the examination of the shoulder joint and the electrical stimulation of the brachial plexus. Video clips of the former illustrate movements of the joints of the pectoral girdle, demonstrating the relevance of the osteology, arthrology and myology sections. The infraclavicular part of the brachial plexus was stimulated using a 30 mm Pole needle and electrical stimulator at 2 mA. Each branch of the plexus was stimulated leading to contraction of the muscles that the nerve innervates and as such the stimulated nerve could be identified. Video clips of the contraction of the muscles innervated by these nerves highlighted the actions and attachments of the muscles of the arm and forearm demonstrating the relevance of the myology and neurology sections. The addition of an applied anatomy section to this database highlighted the relevance of knowing the basic anatomy of this region.

D. 3 Learning anatomy in an integrated clinical skill course. By P. DANGERFIELD, P. BRADLEY and T. GIBBS. *Departments of Anatomy and Health Care Education*. *University of Liverpool Medical School*

The topic of anatomy is presently undergoing a period of change within the UK where there are moves away from the traditional standpoint of the old types of course. New approaches have been driven by the General Medical

https://doi.org/10.1046/j.1469-7580.1998.19230447.x Published online by Cambridge University Press

Council's document 'Tomorrows Doctors' which together with other documents called for an undergraduate course which reflects modern medical practice and offers material in context and of relevance to a doctor graduating in the early years of the new century. Anatomy courses have had to change considerably if they are to meet these new criteria. These changes must combine proven educational theory with an integrated and appropriate clinical approach, drawing in the context of competencies and skills rather than the acquisition of pure knowledge. The poster describes the approach to the learning and understanding of clinically relevant anatomy in the new Clinical Skills Resource Centre at Liverpool University Medical School. It describes the aims and objectives of the course, together with the methodologies used. An evaluation from the students and facilitators perspective is provided together with a description of how the course will develop in the future.

D. 4 Teaching relevant anatomy to increased numbers of medical students: the Sheffield solution. By G. H. COPE and M. A. WARREN. *Department of Biomedical Science*, *University of Sheffield*

As well as curriculum revisions in line with GMC guidelines, The University of Sheffield Medical School has had a 60% increase in numbers following the ASEAN initiative, bringing the annual intake to over 320 students. Substantial revision in curriculum delivery has been necessary to provide high quality teaching with a minimal increase in staff numbers. In levels (years) 1 and 2 the course adopts a fully integrated approach to the delivery of core knowledge in basic medical sciences. Activity in the modular, systems-based course centres around the dissection room which has been extended and rebuilt to include 16 study rooms each able to accommodate teams of 6–8 students (one third of the class at a time). Whilst a slimmed-down and clinically oriented dissection of the whole body is going on in the main laboratory, groups of students, in rotation, undertake directed self learning in the adjacent study rooms. These rooms are equipped with microscopes, video players and computers and the students are provided with a range of CAL packages to help to reinforce their learning. During these classes students also undertake related physiological experiments on themselves using the PowerLab® computer and biochemical principles are reinforced with a range of interactive CAL packages. Relevant histology slides and poster presentations present the microscopic anatomy of regions and systems under study. Running in parallel is a 'long-thin' special studies skills module based on a clinical scenario. This incorporates directed self-learning, team work, IT, statistics, epidemiology, evidence-based medicine, written, poster and oral presentations within a series of weekly activities. Each week has a structured set of learning objectives. Support is from a team of general facilitators ('supervisors'), each of whom stays with the same group of students throughout the project. Specialist (subject-specific) tutors provide expert advice at set points in the project. Assessment is based on a log-book and 3 pieces of work (group poster, individual written assignment and oral presentation) and involves peer and staff assessment. Pieces of work which do not meet the learning objectives are resubmitted, following advice from facilitators, until the objectives have been met. The course allows the students to discover for themselves the workings of the human body within a structured environment and at a pace which they find comfortable.

D. 5 Exposing students to computer assisted learning at an early stage of their course. By I. J. STEWART and S. PEEL. *Human Morphology*, *School of Medicine University of Southampton*

At the University of Southampton students of the Bachelor of Medicine course are given a basic lecture and practical course in cell ultrastructure during the first 3 wk of the 1st term in Year 1. History has shown that an estimated half of the students have a very poor understanding of cell ultrastructure: this includes students with, and without, an A level or equivalent qualification in biology. In 1997, to facilitate the students' learning in cell ultrastructure, a computer assisted learning (CAL) program was written to supplement the lecture and practical program and the recommended histology textbooks. The main features of this CAL program include: (1) short paragraphs of text with supporting diagrams on topics in cell ultrastructure, e.g. the nucleolus. Links are provided to electron micrographs illustrating the subject of the text. (2) Quizzes – these include true/false statements and multiple choice spotter questions based on electron micrographs. (3) Wide availability of the program – this CAL program is available on the 'public' workstations (60) in the Biomedical Sciences Building. The program, without the quizzes, is also available throughout the University of Southampton, including the Halls of Residence, via the University network. A questionnaire was given to the students in the 5th week of the term to provide feedback on the extent of usage of the program, and to determine their assessment of its quality. A large proportion (62%) of students stated that they had not used the program. Of these students 35% stated they 'did not know of its existence', although a descriptive handout with information of how to access it was given to each student. Many (49%) stated they were 'too busy' with other things. Interestingly, none stated that they 'knew everything about cell ultrastructure from other sources'. Those students who used the program rated both the text files and the quizzes highly: these had a mean score of 3.94 and 3.97 respectively on a 1 (poor) to 5 (excellent) scoring scheme. As well as providing programs of high quality there is a need also to 'sell' the CAL program to the students.

D. 6 Replacement of dissecting room classes with computer assisted learning. By S. PEEL and I. J. STEWART. *Human Morphology*, *School of Medicine*, *University of Southampton*

The gross anatomy course in Southampton, for each intake of 160 medical students, consists of about 28 dissecting room (DR) classes. Four classes in the 1st term introduce the skeletal, muscular and nervous systems and in the next 5 terms anatomy is integrated into Systems courses. Each DR class is based on planned, prosected specimens and is supported by aids such as radiographs, models and lectures: work books, designed to encourage student based learning, are essential adjuncts. In 1996–7, with the restructuring of

https://doi.org/10.1046/j.1469-7580.1998.19230447.x Published online by Cambridge University Press

the academic year into semesters, time was lost from some courses and the content of DR classes was reviewed. It was decided to redesign 2 DR classes, one on the vertebral column and limb girdles (LM1) and another on the abdominal wall (AW1), as computer assisted learning (CAL) programs. Text files supported by digitised images of prosections, bones and diagrams formed the basis of the programs. Questions were incorporated into the text to promote recall of topics previously studied. Separate quizzes were included for students to assess whether they had learned the content of the programs. A questionnaire was given to the students to assess their response to the CAL programs. Students were also asked for their opinion on the other, commercial, programs available to them. Year 1 students studied the LM1 program at the start of the Locomotor system course having already had the introductory classes and one on thorax in the Cardiopulmonary system course. They were told they were expected to achieve a score of at least 65% on the quizzes. Only 6% of students said they did not use the program. The quizzes were rated the best part of the program and scored 4.3 out of 5 (where 1 was poor and 5 excellent). The text, diagrams and dissections were rated, respectively, 3.9, 4.0 and 3.7. Only one of the 5 commercial programs was rated as highly as the LM1 program (3.9) but only half as many students used this commercial program. Year 2 students studied the AW1 program at the start of the Urogenital and Endocrine systems. They rated the quizzes the best part of the AW1 program (3.6 for the MCQ and 3.9 for the spotter quizzes). The text files, diagrams and dissections were rated, respectively, as 3.0, 3.2 and 3.0. Few commercial programs were used by the Year 2 students. When asked about their preference for studying gross anatomy using CAL or the DR, 19% of Year 1 and only 5% of Year 2 students preferred the CAL . From their free comments on the questionnaires it was clear that students would like more extensive CAL to be available but that it should complement, not substitute for, wet specimens.

D. 7 Early introduction of clinical skills teaching integrated with the teaching of Anatomy in the 1st and 2nd years of the medical curriculum in Newcastle upon Tyne. By F. C. S. KANU, G. G. R. GREEN*, R. K. JORDAN, J. A. SPENCER** and S. MILLER. *Anatomy & Clinical Skills Centre*, *The School of Surgical Sciences*, *Physiological Sciences**, *Department of Primary Health Care***, *University of Newcastle upon Tyne*

The teaching and learning of anatomy is undergoing radical change, moving gradually away from the traditional didactic teaching of very detailed topographical anatomy to a more functional and clinically applied approach. In Newcastle upon Tyne clinical skills teaching is introduced during the 1st and 2nd years in an integrated manner with the teaching of topographical anatomy, neuroanatomy and other core medical subjects. Topics within clinical skills include examination of different anatomical systems of the body, the use of diagnostic instruments, e.g. ophthalmoscopes and otoscopes, and procedures such as basic cardiopulmonary resuscitation and venesection. In addition, emphasis is given to the application of communication skills and the principles of ethics. The teaching of clinical skills is a mixture of didactic and problem-oriented approaches, of which examples will be given on the poster. All components of the clinical skills courses make reference to the core knowledge base, which the students will have already covered in the curriculum. The format of each clinical skills session comprises an initial summary of relevant anatomical and physiological knowledge, followed by a demonstration of the particular procedure, usually supported by a video. After this introduction students practise in small groups, in which an individual student will perform the procedure, supervised by a tutor and encouraged by comments and feedback from his/her peers. A formative assessment of the students' competence in performing a procedure is made at the end of most sessions. Such assessment is always complemented with structured questions to identify relevant core knowledge. Informal comments of the students over the past years concerning the early introduction of clinical skills teaching in the medical curriculum include: 'Teaching of clinical skills facilitates easier recall of core knowledge', 'It makes learning more interesting', 'It appears to accelerate learning during the clinical rotations'. In the long term it will be important to evaluate how the early introduction of clinical skills teaching influences the development of professional skills and knowledge.

D. 8 The intercellular junctions of the human endothelial cell-line, HMEC-1. By R. A. BUDWORTH, M. ANDERSON, R. H. CLOTHIER and L. LEACH. *School of Biomedical Sciences*, *University of Nottingham*

The intercellular junctions of the endothelium are considered to play an important role in the regulation of its selective barrier. Alterations in endothelial permeability associated with many pathological states are usually limited to the microcirculation. We have therefore investigated the expression of pertinent features, important in the formation and control of a selective endothelial barrier, in the immortalised microvascular cell-line HMEC-1. (Ades, *J. Invest. Dermatol.* **99**, 1992). This cell-line is derived from human dermal microvascular endothelial cells. Vascular-Endothelial Cadherin and Platelet-Endothelial Cell Adhesion Molecule – 1, molecules implicated in the formation of cell-cell contacts, were localised using indirect immunofluorescence. Positive immunostaining was observed in the cultures at cell-cell borders with no staining seen at free cell edges. To visualise the presence of junctions between the cells, they were grown to confluence on Transwell Inserts and fixed in Karnovsky's fixative. They were then embedded in epoxy resin and ultrathin sections were cut perpendicular to the endothelial cell surface for examination using transmission electron microscopy. From the electron micrographs the paracellular clefts between the endothelial cells appear very tortuous. These clefts contain both zonulae adherentes, as seen by the dense cytoplasmic plaques, combined with discontinuous tight junction-like areas where the cell membranes converge close to each other, but do not fuse. Finally the localisation of F-actin was ascertained using fluoroisothiocyanate labelled phalloidin binding. Junctional adhesion molecules are tethered to the internal actin via cytoplasmic linking proteins. The cells exhibited

https://doi.org/10.1046/j.1469-7580.1998.19230447.x Published online by Cambridge University Press

both stress fibres throughout the cytoplasm of the cell and a dense ring of F-actin at the cell membrane. This concurs with previous observations seen in other endothelial cell cultures. Our results indicate that this cell-line is indeed forming intercellular junctions. HMEC-1s could be a useful model for studies into regulation of microvascular permeability.

Funded by FRAME.

D. 9 A quantitative ultrastructural study of collagen fibrils within the flexor digitorum profundus (FDP) tendon and paratenon of the rat hind limb. By H. MOHAMMED, R. YOUNG and B. J. MOXHAM. *Anatomy Unit*, *School of Molecular and Medical Biosciences*, *University of Wales*, *Cardiff*

The FDP tendon is subjected to tensional forces throughout most of its length but, within areas in contact with adjacent bone, it is subjected to compressive forces. The aim of this study was to determine the effects of anatomical site and ageing upon collagen fibril diameters and densities in the rat. Samples of freshly excised FDP tendon and paratenon, from both tensional and compressive sites, in 2 groups of Wistar rats aged 8 wk and 24 mo, were prepared for transmission electron microscopy. Collagen fibril diameters in tendon and tendon sheath were measured in cross section and analysed using a Quantimet 500 image analyser. The frequency distribution of collagen fibrils in 8 wk old rat tendon showed a unimodal distribution in both tensional and compressive zones (with a mean diameter of 331 nm $\pm$ 34.3 S.D. and 286 nm $\pm$ 44.4 S.D. respectively), while those of 2 mo old rat tendon showed a bimodal distribution in both tensional and compressive zones (with a mean diameter of 503 nm $\pm$ 153.1 S.D. and 395 nm $\pm$ 122.6 S.D. respectively). These results conflict with previous studies where only the tensional area showed a bimodal distribution (Merrilees & Flint, *Amer. J. Anat.* **157**, 1980). The present study also revealed that the collagen fibrils of tendon sheath in both regions and age groups have a unimodal distribution with bigger fibrils measured in 8 week old rats (mean diameter, 221 nm $\pm$ 23.7 S.D. in tensional area and 229 nm $\pm$ 26.9 S.D. in compressive zone), while those of 24 month old rat paratenon had smaller fibril diameters in both tension and compression regions (mean diameters of 113 nm $\pm$ 13.6 S.D. and 120 nm $\pm$ 12.9 S.D. respectively). This study has demonstrated that there are marked differences in collagen fibril diameters and distributions between tension transmitting regions of the rat FDP tendon and paratenon, and those regions which are normally subject to compressive load.

This study was funded by a Scholarship from the King Abdulaziz University and the Ministry of Higher Education in Saudi Arabia.

D. 10 Histological evidence for multiple, occlusive encoding sites in cat tendon organs. By R. W. BANKS. *Department of Biological Sciences*, *University of Durham*

Mammalian tendon organs normally contain, within each capsule, several separate sensory terminals all derived from a single, large-diameter group I b afferent axon, by a system of myelinated preterminal branches. Commonly there are 3 or 4 orders of myelinated preterminal branches, and branching is typically dichotomous. On the assumption that each terminal produces its own receptor potential and that each heminode is potentially able to act as an encoding site for the conversion of a receptor potential into a train of impulses, any single tendon organ would be expected to possess multiple potential encoding sites. It is known that in general single tendon organs may be readily excited by the active contraction of more than one motor unit, and that the output of the tendon organ in response to simultaneous excitation by 2 or more motor units is less than the linear sum of the outputs elicited by their separate activity, often markedly so. Proske et al. (*J. Neurophysiol.* **54**, 1985) have argued that this is due in part to the presence of at least 2, mutually resetting, encoding sites in each tendon organ, such that impulses originating in the encoder with the momentarily highest intrinsic activity reset any other encoder by antidromic invasion. For this mechanism to work the encoders must be electrotonically distant from each other and their associated sensory terminals must be principally activated by different motor units. In this study the distribution of sensory terminals among compartments of individual tendon organs have been analysed to assess whether the above conditions can be met. Such compartments may be resolved as separate bundles of tendon fibres, each associated with a subgroup, often just one, of the muscle fibres that insert into the whole organ. Thus if sensory terminals lie in parallel compartments then the possibility of their activation by different motor units exists. The analysis was carried out on 46 tendon organs derived from several intrinsic hind foot muscles (9 abductor digiti quinti medius, and one each of adductor digiti quinti medius, quadratus plantae and calcaneometatarsalis) from 7 adult cats killed by pentobarbitone overdose administered intravenously. The tendon organs were silver-impregnated and teased from the whole muscles. Groups of sensory terminals supplied by the (usually 2) first-order preterminal branches are necessarily the most distant electrotonically. These groups of terminals occurred in separate parallel compartments in 29 tendon organs, but were mutually in series in the remainder. There was some evidence of muscle-specific variability. Accordingly, we may conclude that the necessary structural substrate for multiple, occlusive encoding sites does exist in many, perhaps the majority of, cat tendon organs.

D. 11 Regional variation in non-apoptotic and apoptotic nuclei in human placental trophoblast. By T. M. MAYHEW, L. LEACH, R. McGEE, R. MYKLEBUST* and W. H. WAN ISMAIL. *School of Biomedical Sciences*, *Queen's Medical Centre*, *University of Nottingham and* **Department of Electron Microscopy and Morphology*, *University of Tromsø*, *Norway*

Human villous trophoblast has zones of proliferation (cytotrophoblast, CT) and terminal differentiation (syncytiotrophoblast), the latter including syncytial knot (SK) and non-syncytial knot (non-SK) regions. Whilst the prevailing notion has been one of epithelial expansion becoming restricted by a shrinking pool of CT cells, recent studies point to a continuously expanding epithelium in

which recruitment and extrusion result in rather stable numerical relationships between zones. In light of this, the characteristics of trophoblast compartments and their nuclei at term have been reexamined in randomly sampled sections. Using TEM, relative volumes were estimated by point-counting and surfaces by intersection-counting. Between-compartment differences were analysed using Page's L trends test. Group means (coefficients of variation in brackets) for compartment volume fractions were 15% (30%) for CT, 53% (7%) for non-SK and 33% (23%) for SK regions. Within regions, nuclei accounted for 24% (17%), 18% (27%) and 49% (27%) of total volume respectively. CT nuclei tended to be rounded and euchromatic (only 17% (13%) heterochromatin by volume) with prominent nucleoli. In non-SK regions, nuclei were smaller, less rounded and more heterochromatic (39% (10%) by volume). In SK regions, nuclei were very densely packed, heterochromatic (63% (1%)) and pleiomorphic. Nuclei with ultrastructural features of apoptosis (including condensed peripheral heterochromatin and envelope convolution) were found singly and in aggregates and their heterochromatin occupied 77% (5%) of volume. Nuclear surface-to-volume ratios (in $\mu m^2/\mu m^3$) increased from 1.1 (4%) for CT to 1.4 (8%) for non-SK and 1.7 (12%) for SK regions. These findings confirm that nuclear phenotypes alter during differentiation. Nuclei become smaller and more heterochromatic, convoluted and aggregated and nucleoli regress. Comparable features are seen in formalin-fixed, paraffin sections stained with propidium iodide where brightly fluorescent nuclei (condensed chromatin) are commonly aggregated in SK regions. In situ detection of end-labelled DNA fragments in deparaffinised sections shows a peripheral clumped labelling of end-stage apoptotic nuclei in SK regions. To achieve the observed stability between compartments and pattern of differentiation, fusion of uninucleate CT cells must be balanced by extrusion into the maternal circulation of SK regions rich in heterochromatic, and possibly (pre)-apoptotic, nuclei. Similar sequences are seen in other continuously renewing epithelia where apoptotic nuclei may be phagocytosed by neighbouring cells. However, extruded trophoblast fragments may be phagocytosed at remote (extraplacental) sites.

D. 12 Does presensitisation of mouse granulated metrial gland cells alter their cytotoxic activity against Wehi 164 cells? By S. PEEL and I. J. STEWART. *Human Morphology*, *School of Medicine*, *University of Southampton*

In pregnancy, mouse granulated metrial gland (GMG) cells are cytotoxic to some labyrinthine placental trophoblast cells. It is generally believed that mouse GMG cells are a type of natural killer (NK) cell. However, mouse GMG cells in vitro exhibit high cytotoxic activity against the fibrosarcoma cell line Wehi 164 and this type of cytotoxic activity is a characteristic feature of cells defined as natural cytotoxic (NC) cells. The activity of NC cells is normally assessed using an 18–21 h ^{51}chromium release assay: NK cell cytotoxicity is normally assessed using a 4–6 h assay. We have previously shown that metrial gland cells are not cytotoxic to Wehi 164 cells in a 4 h assay but do show high levels of activity in a 21 h assay suggesting they are NC cells rather than NK cells. We have now investigated whether the long assay period involved in showing NC cytotoxicity is required to switch on the effector cells' cytotoxic mechanisms. We have pre-exposed metrial gland cells, and spleen cells as controls, to unlabelled Wehi 164 cells for 24 h prior to their inclusion in a 4 or 21 h cytotoxicity assay against additional ^{51}chromium labelled Wehi 164 cells. Metrial gland cells, and spleen cells, maintained in culture in the presence or absence of Wehi 164 cells were not cytotoxic to ^{51}chromium labelled Wehi 164 cells in the 4 h assays but were cytotoxic in the 21 h assays. Therefore presensitisation of effector metrial gland cells does not appear to switch on the cytotoxic mechanism and shorten the assay period for NC activity. However the maintenance of cytotoxicity by effector metrial gland cells for over 24 h is supportive of GMG cells being a type of NC cell rather than a type of NK cell as NK cytotoxic activity is lost by cells cultured for over 24 h.

D. 13 Identification of cell subpopulations in human tonsillar epithelium. By C. WILSON$^{\Psi}$, A. SAMA*$^{\Psi}$, M. A. CLARK$^{\Psi}$, B. H. HIRST$^{\Psi}$ and J. A. WILSON* (introduced by S. McHANWELL), $^{\Psi}$*Department of Physiological Sciences*, *University of Newcastle upon Tyne and* **Department of Otolaryngology*, *Freeman Hospital*, *Newcastle upon Tyne*

The tonsillar epithelium is believed to play a key role as a barrier to bacterial pathogens and in pathogen sampling. However, the precise functional and structural characteristics of human tonsillar epithelium remain poorly defined. The aim of this study was to characterise human tonsils, in particular the tonsillar surface and crypt epithelium. Frozen sections of human tonsil (obtained with informed consent from patients undergoing tonsillectomy for recurrent tonsillitis) were probed with a panel of antibodies directed against cytokeratin intermediate filaments and a panel of lectins directed against a range of glycoconjugates. Initially 6 cases were studied. Stained tissue sections were examined by epifluorescence and confocal laser scanning microscopy. Antibodies directed against several cytokeratins (7, 8, 18) demonstrated differences in cytokeratin expression between tonsillar surface and crypt epithelium. For example, cytokeratins 8 and 18 were absent from the surface epithelium, although cytokeratin 8 was expressed in the majority of crypt epithelial cells and cytokeratin 18 was expressed in a smaller subpopulation of more superficially located crypt epithelial cells. Cytokeratin 7 exhibited a variable distribution in surface epithelial cells (the basal layer always being negative), however, it was demonstrated in scattered superficial crypt epithelial cells that were typically located adjacent to the crypt lumen. A number of lectins including *Helix aspersa*, *Helix pomatia* and *Ulex europaeus* 1 also demonstrated differences in glycoconjugate expression between the surface and crypt epithelium. For example, *Helix aspersa* failed to bind to surface epithelial cells but bound to a subpopulation of superficial crypt epithelial cells. Our study demonstrates that tonsil surface and crypt epithelium differ in their patterns of cytokeratin and glycoconjugate expression. This observation is consistent with the hypothesis that these 2 epithelia may have different functional

https://doi.org/10.1046/j.1469-7580.1998.19230447.x Published online by Cambridge University Press

roles. In addition, our results suggest that subpopulations of surface and crypt epithelial cells may also be functionally distinct. These hypotheses will be examined in future studies and attempts made to identify factors that might predispose to recurrent episodes of tonsillitis.

Supported by the Freeman Hospital Trust and the Dr William Edmund Harker Foundation.

D. 14 Immunocytochemical and molecular biological evidence for the occurrence of somatic cross-over mutations between mouse vasopressin and oxytocin precursor genes. By M. J. EPTON, T. C. BUDD and J. F. MORRIS. *Department of Human Anatomy*, *University of Oxford*

The precursor genes of the neuropeptides vasopressin (VP) and oxytocin (OT) have evolved by gene duplication and mutation, lie back-to-back on the same chromosome, and have an identical A, B, C exon-intron structure. The B exons are 95% homologous and are separated by an 11 kbp linking sequence in the rat, and by a 3.5 kbp sequence in the mouse. Exon A codes for the signal, hormone, and N-terminal neurophysin; B for the central part of the neurophysin; C for the C-terminal neurophysin (and a VP-associated glycopeptide). In the homozygous Brattleboro rat (which suffers from hypothalamic diabetes insipidus; DI) somatic cross-over mutations occur between the VP and OT genes. Cross-overs occur mainly within the highly homologous B exons resulting in the formation of hybrid genes encoding the N-terminal part of the VP precursor fused to the C-terminal part of the OT precursor (VP/OT transcripts) and vice versa (OT/VP transcripts). The objective of this work is to determine whether similar mutations occur in the mouse, as this would imply whether or not markedly different lengths of intergene sequence allow the occurrence of the mutation. Both immunocytochemical and molecular biological experiments provide evidence for the occurrence of somatic cross-over mutations between the VP and OT genes in the mouse. Using light (Nomarski) and electron microscopy and immunocytochemistry we have identified, in mice with hereditary nephrogenic diabetes insipidus, magnocellular hypothalamic neurons containing large accumulations of hybrid peptide in their rough endoplasmic reticulum. The presence of hybrid peptide was demonstrated by use of antibodies specific for either the peptide (N-terminal region of precursor) or the neurophysin (C-terminal region of the precursor) on serial ultrathin sections of the affected cells. To substantiate the immunocytochemical evidence, total hypothalamic RNA preparations were reverse transcribed and the resulting cDNA used as the template for polymerase chain reaction amplification. Combinations of forward and reverse primers specific for mouse VP and OT cDNA were used to amplify VP, OT, VP/OT hybrid, and OT/VP hybrid cDNA transcripts. The evidence shows that the shorter intergene sequence length in the mouse does not prevent cross-over mutations from occurring; however there appear to be many fewer cells containing hybrid peptide in the DI mouse than in the DI rat.

M. J. Epton is supported by a studentship from the Anatomical Society of Great Britain and Ireland.

D. 15 Mucus gel thickness in the human large intestine. By V. STRUGALA, N. JORDAN, J. P. PEARSON and A. ALLEN. *Department of Physiological Sciences*, *University of Newcastle*

The surface of the large intestine is covered by a layer of mucus gel that protects the epithelium from luminal enzymes, toxins and from shear forces resulting from movement of the stool. The amount of protection that this layer provides depends upon its thickness. In this study, the thickness of the adherent mucus gel layer was measured along the length of the human large intestine. Biopsies were taken from various regions of the large intestine (caecum, transverse colon, descending colon, sigmoid colon and rectum) from patients undergoing routine colonoscopy or flexible sigmoidoscopy and found to have a macroscopically normal bowel. All patients gave written informed consent. The biopsies were wrapped in a liver envelope and snap frozen. Cryostat sections 18 μm thick were mounted onto poly-l-lysine coated slides and stained with the modified periodic acid-Schiff/Alcian blue technique (Jordan et. al. Phys. Soc. 1996) This method prevents dehydration of the gel and gives an accurate measurement of the thickness of the protective mucus barrier. The thickness of the layer was measured 10 times per section at 250 μm intervals and in approximately 6 slides per specimen. Mean mucus thickness values for each region were 23.1 mm in the caecum ($n = 3$), 31.2 μm in the transverse colon ($n = 6$), 45.7 μm in the descending colon ($n = 4$), 62.5 μm in the sigmoid colon ($n = 8$) and 65.7 μm in the rectum ($n = 8$). There was a progressive and significant ($P < 0.05$) increase in mean mucus thickness towards the rectum in man. The layer in the rectum was almost three times greater than the layer in the caecum. The thicker mucus layer in the distal bowel suggests that the mucosa is exposed to more stress than in the proximal region of the bowel. In particular, the effect of shear due to the hardening of the faeces increases towards the rectum and so additional protection is required in the form of a thicker mucus layer. In ulcerative colitis, the mucus layer was discontinuous with a significantly reduced mucus thickness (mean = 38.6 μm; $n = 6$) compared with that of the controls (mean = 61.2 μm; $n = 20$).

D. 16 Three markers for sensory afferents decline during postnatal development in cervical spinal cord ventral horn of the rat. By C. GIBSON, E. LIM, G. ARNOTT and G. J. CLOWRY. *Department of Child Health*, *University of Newcastle upon Tyne*

Most morphological studies appear to show that ingrowth of sensory fibres is highly specific from an early stage. However, there is physiological evidence for innervation of heteronymous motoneurons by muscle afferents in developing human and rat, with the human condition cerebral palsy characterised by a failure to eliminate these inappropriate connections. We have studied 3 markers for sensory afferents at postnatal day (P) 7, P14, P28 and P70. Under Halothane anaesthesia and in aseptic conditions, cholera toxin B subunit (CT-B, 0.5%) was injected into the extensor digitorum communis (EDC) muscle and then the animals were transcardially perfused 2–5 d later under pentobarbitone induced terminal anaesthesia, with buffered paraformaldehyde and picric acid fixative, depending on the

https://doi.org/10.1046/j.1469-7580.1998.19230447.x Published online by Cambridge University Press

animal's age. Frozen sections of cervical cord were cut, divided into parallel groups and immunoperoxidase stained for either (1) CT-B to reveal muscle sensory afferents and EDC motoneurons, including their dendritic trees (2) parvalbumin (PV) a Ca^{2+} binding protein expressed by all large diameter sensory afferents as well as other axons and neuronal cell bodies, and (3) calcitonin gene related peptide (CGRP) expressed by nociceptive sensory afferents and most motoneurons. At P7 and P14 PV positive afferents could clearly be seen projecting from the dorsal root and cuneate fasciculus into the ventral horn and the motoneuron pools in particular. By P28 and P70 PV expression in sensory afferents seemed largely confined to the cuneate fasciculus. At P7 and P14 CT-B labelling revealed muscle afferents innervating the dorsolateral motoneuron pools as well as regions of the dorsal horn and intermediate grey matter. Contacts could be seen between axons and EDC motoneuron proximal dendrites. Muscle afferents also innervated flexor motoneuron pools. At P28 and P70, CB-T labelled sensory axons terminated in the intermediate grey matter and made contact with distal motoneuron dendrites only. At all ages, CGRP fibres were predominantly located in the dorsal horn but at P7 and P14 very fine varicose fibres could be seen in the ventral horn, some recognisable as branches of larger axons in the deep dorsal horn. However, a population of CGRP positive neurons in the medial ventral horn, distinct from motoneurons, was also observed only in younger animals and could have contributed to ventral horn fibre staining. We conclude that there is a pruning of sensory afferents projecting to the ventral horn during late development.

D. 17 Acetylcholinesterase expression and motoneuron growth in human cervical spinal cord gestational age 25–42 wk. By G. ARNOTT, C. WRIGHT* and G. J. CLOWRY. *Departments of Child Health and Pathology**, *University of Newcastle upon Tyne*

Perinatal lesions to corticospinal pathways in turn disrupt spinal cord development such that cerebral palsy sufferers have a double impairment of impaired voluntary control coupled with disordered reflex pathways. Increasingly, cerebral palsy is seen to result from damage to the internal capsule in premature babies born at less than 30 wk gestation. We are studying the normal development of the spinal cord in the perinatal human in order to understand which events may be perturbed by descending pathway lesions at this time. Cervical spinal cord was taken 12–48 h postmortem with permission of the parents from neurologically normal babies who had died soon after birth usually of complications arising from prematurity. The spinal cord was immersion fixed in phosphate buffered 4% paraformaldehyde for 3–7 d. The cord was then divided into spinal segments, immersed in buffered 30% sucrose overnight and 50 mm frozen sections cut. Sections were histochemically stained for acetylcholinesterase by a modified Karnovsky and Roots procedure. The cell bodies and proximal dendrites of motoneurons were stained along with isolated neurons in the intermediate grey matter and dorsal horn. Axonal staining was weak and diffuse. Staining was stronger with increasing age and shorter fixation times. The cross sectional areas of identified motoneurons from the lateral columns of cervical segment C6 were measured using the neurolucida software package. One hundred motoneurons consisting of 25 nearest neighbours each from both sides of the cord in 2 sections were measured from 8 spinal cords ranging in age from 25–42 wk of gestation. Median cross sectional area of motoneurons increased linearly with age ($R^2 = 0.9302$) from 663 μm^2 at 25 wk to 1077 μm^2 at 42 wk. Frequency distribution histograms showed that the range of motoneuron sizes broadened with age, but without producing the bimodal distribution seen in studies of mature motoneuron size. A bimodal distribution reflects the different sizes of alpha and gamma motoneurons. The unimodal distributions observed here are, however, characteristic of populations of developing motoneurons. In conclusion, perinatal corticospinal pathway damage would coincide with, and could disrupt, a period of motoneuron development.

Supported by the Wellcome Trust and the Leech Charitable Trust.

D. 18 Distribution of glycinergic synapses on the surface of forelimb flexor motoneurons in the developing rat. By Z. FALLAH, T. BOOTH* and G. J. CLOWRY. *Department of Child Health and the Biomedical EM Unit**, *University of Newcastle upon Tyne*

Increasing influence of inhibitory pathways over motoneurons could underlie the development of fine motor control. This study investigated possible changes in inhibitory input to motoneurons during the maturation of locomotion and onset of forepaw use. Flexor digitorum profundus motoneurons were retrogradely labelled from the muscle with cholera toxin B subunit (CT-B). A 0.5% solution was injected into muscle under Halothane anaesthesia. The animals survived 2–3 d postoperatively and were then anaesthetised and killed by transcardiac perfusion with fixative at ages postnatal day P7, P14 and P30, 4 animals at each age. Frozen sections of lower cervical spinal cord were immunofluorescently double stained for CB-T and for gephyrin, a component of the synaptic inhibitory glycine receptor, and analysed in a dual channel laser scanning confocal microscope. Motoneuron cell bodies, proximal and higher order dendrites were observed at one wavelength, and gephyrin clusters at inhibitory synapses at another. Using merged images the number of clusters per 100 mm of cell membrane was calculated for lengths of somatic, proximal dendritic and distal dendritic membrane. Similar lengths of membrane for each compartment from each animal were sampled from individual and spatially separated thin optical sections.

n = 4 per age	P7			P14			P28		
	Soma	Prox	Dist	Soma	Prox	Dist	Soma	Prox	Dist
Mean Cluster 100 µm	41.3 S.D. 8.9	48.7 S.D. 5.4	45.0 S.D. 5.0	48.7 S.D. 9.9	48.8 S.D. 11.4	46.6 S.D. 10.9	51.9 S.D. 7.6	52.2 S.D. 6.0	50.9 S.D. 3.4

All compartments show a slight but not statistically significant increase in inhibitory synapse density with age.

https://doi.org/10.1046/j.1469-7580.1998.19230447.x Published online by Cambridge University Press

We did sample some small cell bodies at P7 with none or few gephyrin clusters. We were surprised to see such a high density on more distal dendrites. Immunoelectron microscopy, using CT-BHRP as a retrograde tracer, qualitatively confirmed the existence of gephyrin positive synapses in high densities on distal dendrites and the existence of some small neurons without somatic gephyrin clusters. Our studies reveal that motoneuronal inhibitory synapse distribution is largely determined early in development and that inhibitory synapses may have a greater role on distal dendrites than is generally recognised.

D. 19 Motoneurons induced to sprout remain susceptible to nerve injury in adult transgenic mice overexpressing GAP-43. By D. I. HARDING, L. GREENSMITH, P. N. ANDERSON and G. VRBOVÁ. *Department of Anatomy and Developmental Biology*, *University College London*

In the adult, motoneurons survive axotomy, while in neonatal animals axotomy causes motoneuron death. The reason for this disparate response of motoneurons to axotomy is not clear, but may depend on the different phenotype of young and adult motoneurons. One such difference is that neonatal motoneurons are still growing, while adult neurons have stopped growing and are now specialised for transmitting messages to their target. Whether this transition in phenotype is important for the survival of motoneurons was examined in this study. We tested the possibility that adult motoneurons induced to grow, by encouraging sprouting, would respond in a similar manner to neonatal motoneurons and die following nerve injury. A model was used where the neurons of adult transgenic mice overexpress the chick growth-associated protein GAP-43. In these animals extensive nerve sprouting at the neuromuscular junction occurs (see Aigner et al. *Cell*, **83**, 1995). Under Halothane anaesthesia and sterile conditions unilateral sciatic nerve crush was performed in both transgenic and wild type mice at 1 mo of age. When the mice were 5 mo old they were reanaesthstised and HRP was injected into the soleus muscle of both legs using a fine Hamilton syringe. 24 h later the mice were anaesthetised with chloral hydrate (1 ml/100 g body wt, ip) and prepared for isometric tension recordings. Tetanic contractions of the tibialis anterior (TA) muscle of both legs were recorded to determine muscle force. The mice were then perfused and the spinal cords removed and processed for HRP histochemistry. The number of retrogradely labelled motoneurons in the ventral horn of the spinal cords was counted. In the cords of the wild type mice there were 36 ($\pm$1.29 S.E.M.., n = 6) motoneurons supplying the soleus muscle on the control side, and 36.7 ($\pm$1.23 S.E.M., n = 6) motoneurons on the operated side. Nerve crush therefore did not result in motoneuron death in the wild type mice. This was reflected in the tension recordings which showed that the reinnervated TA muscles were not significantly weaker than the control muscles, producing 88.9% ($\pm$2.28 S.E.M., n = 5) of the tension of TA muscles from the control side. The spinal cords of transgenic mice (which overexpressed GAP-43) contained 30 ($\pm$1.68 S.E.M.., n = 4) motoneurons supplying the soleus muscle on the control side and only 12.3 ($\pm$0.48 S.E.M., n = 4) motoneurons on the operated side. This loss of motoneurons following adult sciatic nerve crush was reflected by a decrease in weight and force production of the reinnervated TA muscles compared to that on the control side. The reinnervated TA muscles only produced 51.1% ($\pm$3.61 S.E.M., n = 4) of the tension of TA muscles from the control sides. Thus it appears that inducing adult motoneurons to sprout in transgenic mice does indeed render them susceptible to nerve injury. These results suggest that the transition of the motoneuron from a growing cell to a mature transmitting cell is essential for it to survive nerve injury.

D. 20 Expression of CHL1, a cell recognition molecule closely related to L1, in adult rat brain following the implantation of a peripheral nerve graft. By V. CHAISUKSUNT, Y. ZHANG, M. SCHACHNER*, P. N. ANDERSON and A. R. LIEBERMAN. *Department of Anatomy and Developmental Biology*, *University College London and *Zentrum für Molekulare Neurobiologie*, *Hamburg*

The expression of the close homologue of L1 (CHL1), a recently identified member of the L1 family of neural recognition molecules, has been investigated in the brain of adult rats following the implantation of a peripheral nerve graft. Adult female Sprague-Dawley rats were deeply anaesthetised with Halothane and a piece of tibial nerve removed from the left thigh and implanted through a craniotomy into the neostriatum, thalamus or cerebellum. Two days to 2 wk after grafting, the animals were reanaesthetised and killed by decapitation. The expression of CHL1 mRNA was detected by in situ hybridisation of sections through the brain with a digoxygenin-labelled riboprobe. Following grafting into the striatum, strong expression of CHL1 mRNA was detected in cells located close to the grafts whereas there was no significant signal in the striatum on the nongrafted side. The CHL1 positive cells varied in size: the small cells resembled glia while the largest were 20–30 mm in diameter and were probably neurons. Following grafting into the thalamus, cells in the thalamic reticular nucleus close to the graft were found to have upregulated CHL1 mRNA. Similarly, following grafting into the cerebellum, neurons within the deep nuclei, but not Purkinje cells or other neurons in the cerebellar cortex, were found to have upregulated CHL1 mRNA. These findings indicate that some CNS neurons upregulate CHL1 mRNA in response to a peripheral nerve graft. These are the same groups of neurons which we have previously shown to regenerate axons into the grafts. Although the function of CHL1 remains unclear, it is known to be a strong promoter of neurite outgrowth in culture and may play a role in the growth of CNS axons along the Schwann cell columns in the grafts.

D. 21 Modelling prenatal development of the cat retinogeniculate pathway. By S. J. EGLEN (introduced by S. MILLER). *Department of Cognitive Science*, *University of Edinburgh*

The feline lateral geniculate nucleus (LGN) is the primary destination for retinal axons from both eyes. The mature

https://doi.org/10.1046/j.1469-7580.1998.19230447.x Published online by Cambridge University Press

LGN is organised into multiple topographic maps of retinal space, segregated according to the eye of origin. During prenatal development, spontaneous waves of neural activity travel across each retina. Blockade of this activity with tetrodotoxin prevents development of the eye-specific laminae in the LGN. A popular hypothesis is that these correlated waves drive activity-dependent processes to refine development of LGN topography and ocularity. To investigate this hypothesis, a neural network computer simulation of 2 retinae innervating one LGN is presented, based upon earlier work (Keesing et al. NIPS 4, 1992). Synaptic connections between retinal and LGN units are represented by weighted values which are adapted during the simulation according to local rules. A form of Hebbian adaptation is used to adjust weights in proportion to the correlation in activity of the retinal and LGN units. Normalisation of synaptic strength from each retinal unit and to each LGN unit is used to ensure that weights do not increase without bounds. The initial conditions of the network include an overall topographic bias and an advantage for the contralateral eye to innervate higher into the LGN. This model replicates many aspects of cat retinogeniculate development. First, the model segregates LGN units into 2 eye-specific layers, matching the formation of layer A and layer A1. Secondly, each horizontal slice of the LGN forms a retinotopic map of visual space, so that neighbouring LGN units respond to neighbouring retinal units. Thirdly, these topographic maps are aligned so that LGN units within a vertical slice respond to the same subset of retinal units, thus forming the projection columns also found in the cat LGN. By varying the spatiotemporal properties of the retinal waves, the model can also predict development under abnormal conditions. We find that as retinal waves get broader, the size of receptive fields of LGN units increases. Reducing the probability of waves being present in one retina gives the other retina a competitive advantage to innervate more LGN units. In the limit when one eye never produces any retinal waves, that eye disconnects from the LGN completely. This can be empirically tested by selective activity blockade in one eye.