# Proceedings of the Anatomical Society of Great Britain and Ireland

The Summer Meeting of the Anatomical Society of Great Britain and Ireland was held at the University of Glasgow from 13th to 15th July 1999. It included a symposium on 'Neurobiology of the basal ganglia'. The following are abstracts of communications and posters presented at the meeting.

## TALKS

**1 Skeletal changes in mandibular morphology following surgical correction of craniofacial microsomia**. By T. L. Cerajewska and G. D. Singh. *Dundee Dental Hospital and School, University of Dundee, UK.*

The purpose of this study was to determine skeletal changes in prepubertal children with unilateral craniofacial microsomia (CFM) treated with an inverted L osteotomy and autogenous bone graft of the mandible. After obtaining consent, pre-operative, early post-operative ($\approx 1$ y), and late post-operative ($\approx 3.5$ y) lateral cephalographs of 14 children (mean age $9 \pm 2$ y) were scanned. Nine mandibular homologous landmarks were digitised in triplicate for each cephalograph. Average mandibular geometries, scaled to an equivalent size, were generated using a Generalised Rotational Fit program (Procrustes superimposition) and subjected to statistical analysis. Digitisation errors were found to be not significant ($P > 0.05$). Euclidean distance matrix analysis indicated that there was a statistical difference ($P < 0.05$) between pre- and early post-operative, pre- and late post-operative, and between early and late post-operative mandibular configurations. The early post-operative form difference matrix showed increases in oblique length ($\approx 11\%$), ramus length ($\approx 26\%$), and ramus height ($\approx 18\%$), but the late post-operative form difference matrix showed fewer changes. Comparing pre- and post-operative transformations graphically, thin plate spline analysis indicated that both affine and nonaffine transformations contribute to the total spline (deformation) of the averaged mandibular forms. For all nonaffine transformations partial warp (PW) 6 had a high magnitude, indicating postero-inferior elongation of the mandible. In the transformation from early post- to late postoperative, PW5 had the largest magnitude, indicating a vertical elongation of the mandibular configuration. PW2 and PW3 also had magnitudes of reasonable size, indicating antero-posterior elongation in the infradentale to mental protuberance region. Thus an inverted L osteotomy acts to lengthen the body of the mandible and, postoperatively, the mandible continues to increase in length vertically. Mandibular skeletal morphology is improved significantly in CFM patients surgically treated with inverted L osteotomy, and this procedure appears to have little restriction on continued postoperative mandibular growth.

**2 Zinc supplementation and small intestinal crypt kinetics in CD-1 mice.** By M. Duff and R. R. Ettarh, *Department of Human Anatomy & Physiology, University College, Dublin*

Deficiency of zinc, an essential trace element which is necessary for normal growth and development, has been shown to alter the kinetic balance between cell destruction and renewal in the mucosal epithelium of the mammalian small intestine. While zinc replacement reverses the abnormal mucosal changes which occur in zinc deficiency the effects of zinc supplementation on cell kinetics has not been examined. Thirty 6 wk old male CD-1 mice were fed either a standard diet of pellets and tap water or a diet of pellets and zinc solution (0.3 mmol/l zinc sulphate in water). Animals were injected with vincristine after 14 d and killed by cervical dislocation in batches of 5 at 30 min intervals for up to 2 h. At laparotomy the small intestine was removed, its length measured, and then divided into 4 equal segments from which 2 midpoint samples were taken and fixed in Carnoy's fluid. Half of the samples were processed as tube preparations for wax histology and counts and measurements of crypt profiles obtained from 7 μm thick haematoxylin and eosin stained sections. The other half of the samples were stained with Schiff's reagent for the Feulgen reaction and crypts microdissected and evaluated for arrested metaphases. The results show that the small intestinal length and 'crypt-space' width in zinc fed animals did not differ significantly from the values obtained from control groups. Although the mean number of crypts in zinc fed animals was marginally higher in the distal small intestinal segments (fourth segment: controls $401692 \pm 21154$; zinc fed $424137 \pm 22349$ crypts; mean $\pm$ S.D.) than corresponding values in control animals this difference did not reach statistical significance. Similarly the difference in mean values between zinc fed and control groups in crypt cell production rate for the distal segments (third segment: controls $6.8 \pm 0.9$; zinc fed $9.8 \pm 2.2$ mitoses/h) was not statistically significant. The results indicate that zinc supplementation (in contrast to zinc replenishment) beyond normal dietary intake does not influence the rate of crypt cell production in the small intestinal mucosal epithelium.

**3 The effect of fructose-1,6-bisphosphate on the small intestine of the indomethacin treated CD-1 mouse.** By C. J. Tansey and R. R. Ettarh. *Department of Anatomy, University College, Dublin, Ireland.*

The administration of a high dose of indomethacin in mice is associated with tissue damage leading to ulceration as well

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as a reduction in the number of crypts of Lieberkuhn in the non-ulcerated part of the small intestine. Fructose-1,6-bisphosphate is a glycolytic intermediate that has been shown to have a protective effect against tissue damage caused by ischaemia and chemical agents. We proposed to examine the effect of fructose-1,6-bisphosphate on the small intestine of the indomethacin treated mouse. Male CD-1 mice were divided into 4 groups and given one of the following treatments: (1) a single dose of 0.9% saline intraperitoneally; (2) a single dose of indomethacin 85 mg/kg bodyweight intraperitoneally; (3) fructose-1,6-bisphosphate 1.5 g/kg bodyweight intraperitoneally twice a day for 4 d; (4) a single dose of indomethacin 85 mg/kg bodyweight intraperitoneally and fructose-1,6-bisphosphate 1.5 g/kg bodyweight intraperitoneally twice a day for 4 d. All animals were killed by cervical dislocation after 4 d. In all animals, the small intestine was removed, its length measured and then divided into 4 equal segments. Samples were taken from the midpoint of the fourth intestinal segment. Following fixation, wax embedding, sectioning and staining with haematoxylin and eosin, the number of crypt profiles per circumference of intestine was counted, the size of these crypt profiles was measured and the total number of whole crypts in each segment was calculated. Both indomethacin-treated groups showed a significant reduction in whole crypt numbers ($P < 0.01$, 2 way ANOVA). The magnitude of this reduction was 17% in the fructose-1,6-bisphosphate-treated animals compared with 32% in those that did not receive fructose-1,6-bisphosphate. These findings suggest that fructose-1,6-bisphosphate has a protective effect against indomethacin-induced small intestinal crypt loss.

**4 Further investigation of the bilateral cords of bipolar cells which appear in relation to developing somites in chick embryos (***Gallus gallus domesticus***).** By J. G. Gouda. *Department of Anatomy*, *University of Ahfad for Women*, *Sudan*.

Somite formation in chick embryos is a coordinated and multifactorial process. Factors influencing somite formation include the somite centres, Hensen's node, the neural folds and tube and the extracellular materials. Gouda & England (*J. Anat*. **184**, 1994) reported the existence of bilateral chains of bipolar cells running craniocaudally along the embryonic axis and present between the endoderm and axial structures at stage 6–13. We provided some evidence that these are neural in origin and critical to somite formation. It was hypothesised that the chains could be the endothelial lining of the aorta, or the lateral nephrogenic cord, or the neural crest and sympathetic chain (appearing at d 4–5, stages 22–25).

Over 100 chick embryos of stages 6–13 cultured in vitro were used in this study and examined by stereoscanning and transmission electron microscopy. Embryos were fixed in Bouin's or Karnovsky's solutions and divided into 3 groups. The first group was used to provide evidence that the chains are independent and discrete structures not previously described. The second group was used to prove that the chains are not the endothelial lining of the aorta by injecting the latter with dextran blue 2000 and FITC in PBS. The third group was used to study the effect of the surgical removal of the chain on somite formation.

The results suggest the following: (1) the chains of bipolar cells are discrete and cannot be confused with the nephrogenic cord, the neural tube or the aorta; (2) the chains play a role in the development of somites; (3) the chains may represent the previously unreported earliest stages in the development of the sympathetic chain.

**5 The formation of nephrogenic aggregates in the developing mouse kidney.** By W. Sellers, L. Sharp, A. Gordon and J. Bard. *Department of Biomedical Sciences*, *Edinburgh University*, *UK*.

Metanephros development in the mouse derives from a reciprocal inductive interaction between the ureteric bud and the metanephric mesenchyme (MM) on about E11; as a result of this the former gives rise to the collecting duct system and the latter the nephrons and stroma. In addition, the stem cells from which nephrogenic aggregates form become localised at the kidney periphery where they express Pax-2. The traditional view is that nephrons form in the following way: groups of MM cells aggregate, epithelialise, and form a comma-shaped, then an S-shaped body, one end of which develops into the glomerulus while the other fuses to the growing duct. However this view of nephrogenesis has some inconsistencies: the ureter will not induce across a filter and no inducer of MM has yet been found, while the origin of nephron polarity remains unknown. Furthermore it is not clear how the Pax2+ cells become localised at the periphery as the first populations of such cells to be seen are internalised within the tissue.

To try to clarify the situation, we have therefore re-examined MM morphogenesis using confocal microscopy with NCAM, laminin and Pax-2 markers. The data show first that aggregates form from cells within an NCAM+ and Pax2+ region of MM some 8–10 cell deep surrounding the duct tips, and second that these initial aggregates are in intimate contact with the duct epithelia. The aggregates then enlarge, probably by accretion, and rapidly epithelialise with laminin being expressed around them but being lost from the adjacent duct. Long after they epithelialise however, the duct cells continue to maintain the expression of NCAM, so allowing nephrogenic and duct epithelia to be distinguished. The expression of NCAM and Pax2 is rapidly lost from the MM immediately surrounding the aggregate but maintained in the MM surrounding the extending duct tip. This tip eventually meets the periphery of the kidney (E13 or so), so bringing Pax-2+ cells to the position that they will occupy for the remainder of nephrogenesis.

These and other digital image data not only provide a more coherent picture of early nephrogenesis, but yield quantitative assays against which to test future models of nephrogenesis.

**6 Fibroblast growth factor receptor signalling and the proliferation-differentiation balance in skull vault development.** By S. Iseki[1], A. O. M. Wilkie[2] and G. M. Morriss-Kay[1]. [1]*Department of Human Anatomy and Genetics and* [2]*Institute of Molecular Medicine*, *University of Oxford*, *UK*.

Fibroblast growth factor receptors (FGFRs) play major roles in skeletogenesis, and activating mutations of the human *FGFR1, FGFR2* and *FGFR3* genes cause premature

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fusion of the skull bones (craniosynostosis). We have investigated the patterns of expression of *Fgfrs1–3* in the fetal mouse head, with specific reference to their relationship to cell proliferation and differentiation in the frontal and parietal bones and in the coronal suture. *Fgfr2* is expressed only in proliferating osteoprogenitor cells; the onset of differentiation is preceded by down-regulation of *Fgfr2* and up-regulation of *Fgfr1*. *Fgfr3* is expressed in the cranial cartilage, including a plate of cartilage underlying the coronal suture, as well as in osteogenic cells, suggesting a dual role in skull development. The positional relationship between proliferation and differentiation of osteogenic cells is reflected in differential levels of FGF2 protein as detected by immunohistochemistry, levels being high in the differentiated region and low in the region of proliferation. Subcutaneous insertion of FGF2 soaked beads onto the coronal suture at E15 resulted in up-regulation of *Fgfr1* and of the differentiation marker *osteopontin* in the sutural mesenchyme, down-regulation of *Fgfr2* (and *Fgfr3*), and inhibition of cell proliferation, i.e. a shift from proliferation to differentiation, within 6 h. We suggest (a) that a gradient of FGF ligand, from high levels in the differentiated region to low levels in the environment of the sutural osteogenic stem cells, modulates differential expression of *Fgfrs 1* and *2*, and (b) that signalling through FGFR2 regulates sutural stem cell proliferation whereas signalling through FGFR1 regulates osteogenic differentiation.

**7 How different populations of myoblasts contribute to different fibre types during rat muscle development.** By P. M. Wigmore, G. F. Dunglison, J. F. Fulton and L. Y. L. Tan. *School of Biomedical Sciences*, *Queen's Medical Centre*, *Nottingham University*, *UK*.

Skeletal muscle is composed of different fibre types distinguished by their speed of contraction and metabolism. Individual muscles have particular proportions of fast and slow fibres distributed in a characteristic pattern within the muscle. The initial formation and distribution of different fibre types appears to be intrinsic to a muscle and does not require innervation or activity. In addition muscle fibres form in 2 successive waves which produce an early (primary) and a later (secondary) generation of fibres. It is possible that this diversity of fibre types and generations could be brought about by having several populations of myoblasts. Cells isolated from different stages of muscle development show different characteristics in vitro while individual clones of cells show commitment to form particular types of muscle fibre. This work directly tests the contributions of individual clones of myoblasts to different fibre types and generations in vivo.

Myoblasts were infected, in vivo, with replication deficient retroviruses carrying marker genes. Inheritance of the marker gene at cell division results in clones of marked myoblasts which subsequently fuse with the surrounding muscle fibres. We have analysed the fibre type and generation of these clusters of marked fibres derived from injection of retrovirus at embryonic day (E) 15 and 17. Animals were killed at E19. Clones of myoblasts derived from E15 injections only contributed to primary fibres. These were nearly all of a slow fibre type except for a few fast primary fibres found near the superficial regions of some muscles. These fast primaries occurred in clusters containing slow primaries are likely to be fibres which have converted from an initially slow phenotype. Clones derived from E17 injections fused with both slow primary and fast secondary fibres. These results indicate that at least 2 populations of myoblasts occur in vivo. An early 'embryonic' population which produces only slow primary fibres followed by a later 'fetal' populations which contributes to both primary and secondary fibres. There is no evidence of that clones of myoblasts are committed to fusing with particular fibre types. All operations were carried out under general anaesthetic (Hypnorm/midazolam) and animals were killed by cervical dislocation. All procedures were covered by Home Office licences.

**8 Caveolin-3 immunofluorescence forms a regular pattern in rabbit extensor digitorum longus skeletal muscle fibre membrane that is amenable to Fourier analysis**. By C. D. Ockleford[1], A. J. Rowe[2], S. Byrne[1], J. J. A. Scott[1] and H. Cairns[1]. [1]*Department of Pre-Clinical Sciences*, *School of Medicine and Biological Sciences*, *University of Leicester*; *and* [2]*Sutton Bonington Campus*, *Nottingham University*, *UK*.

Rabbit skeletal muscle fibre membrane exhibits a regular patterned array of caveolin 3 as revealed by indirect immunofluorescence confocal laser scanning microscopy. Fourier analysis of the pattern visualised in relaxed tissue by confocal laser scanning microscopy revealed a repeating intensity and included second order information. An average major spacing in the long axis of 1.48 μm was measured. This spacing was independently confirmed by direct measurement using confocal laser scanning immunofluorescence microscopy (n = 100, mean = 1.51 μm, S.D. = 0.17 μm). The major spacing coincides with the length of the A band indicating that the immunofluorescent intensities may overlie the junction between the A and I bands. Caveolae are known to be associated with relatively stiff membrane rafts rich in cholesterol and sphingolipid and with elements of the cytoskeleton. It is possible therefore to envisage a mechanism of rapid membrane shape change necessary to accommodate contraction and relaxation of the fibre. In this mechanism lipid is interposed (or interdigitates) between rafts of relatively stiff membrane increasing the lateral spacing on contraction as fibre girth increases and the fibre shortens. Relaxation in which the fibre membrane sleeve becomes longer and thinner may take place by the reverse process. The proposed mechanism is consistent with the observed greater variability in the lateral spacings between the bright immunofluorescence nodes than the long axis spacing.

**9 Evolution of the basal ganglia: new perspectives through a comparative approach**. By W. J. A. J. Smeets[1], O. Marin[2] and A. Gonzalez[2]. [1]*Department of Anatomy*, *Research Institute of Neurosciences*, *Amsterdam*, *The Netherlands*; *and* [2]*Departamento de Biologia Celular*, *Facultad de Biologia*, *Universidad Complutense*, *Madrid*, *Spain*.

The basal ganglia (BG) have received much attention during the last 3 decades mainly because of their clinical relevance. Our understanding of their structure, organisation and function in terms of chemoarchitecture, compart-

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mentalisation, connections (both macro- and micro-circuitry), and receptor localisation has increased equally. Most of the research has been focused on the mammalian BG, although a number of studies have been carried out in nonmammalian vertebrates. In particular the BG of reptiles and birds, which together with mammals constitute the amniotic vertebrates, have been thoroughly studied by means of tract tracing and immunohistochemical techniques. The terminology used for amniotic BG structures has been frequently adopted to indicate putative corresponding structures in the brain of anamniotes, i.e. amphibians and fishes, but data for such a comparison were, until recently, almost totally lacking.

It has been proposed several times that the occurrence of well developed BG structures probably constitutes a landmark in the anamniote-amniote transition. However our recent studies of connections, chemoarchitecture and development of the basal forebrain of amphibians have revealed that tetrapod vertebrates share a common pattern of BG organisation. This pattern includes the existence of dorsal and ventral striatopallidal systems, reciprocal connections between the striatopallidal complex and the diencephalic and mesencephalic basal plate (striatonigral and nigrostriatal projections), and descending pathways from the striatopallidal system to the midbrain tectum and reticular formation. The connectional similarities are paralleled by similarities in the distribution of chemical markers of striatal and pallidal structures such as dopamine, substance P and enkephalin, as well as by similarities in development and expression of homeobox genes. On the other hand, a major evolutionary trend is the progressive involvement of the cortex in the processing of the thalamic sensory information relayed to the BG of tetrapods.

By using the comparative approach new insights have been gained with respect to certain features of the BG of vertebrates in general, such as the segmental organisation of the midbrain dopaminergic cell groups, the occurrence of large numbers of dopaminergic cell bodies within the telencephalon itself and the variability in, among others, connectivity and chemoarchitecture. However the intriguing question whether the basal forebrain organization of non-tetrapods differs essentially from that observed in tetrapods still needs to be answered.

**10 New ideas on receptor localisation in the basal ganglia**. By Y. Smith, J. R. Hanson, A. Charara and A. I. Levey. *Yerkes Primate Center and Department of Neurology, Emory University, USA.*

Until the 1980s, it was thought that the effects of glutamate and GABA were mediated exclusively by activation of ligand gated ion channels. It is now well established that both neurotransmitters also interact with distinct types of G protein-coupled and metabotropic receptors which mediate their effects via activation of second messenger systems. The metabotropic glutamate receptors (mGluRs) family includes 8 major subtypes (mGluR1–8) pooled into 3 groups differentiated by amino acid sequence homologies, second messenger systems and agonist selectivities. Conversely the metabotropic effects of GABA are mediated by GABA-B receptors, which currently include 2 major subtypes named GABA-B-R1 and -R2. The role of GABA and glutamate in controlling the activity of basal ganglia output neurons via interactions with ionotropic receptors is well established, but the functions and localisation of metabotropic glutamate and GABA receptors in the basal ganglia are still poorly understood. This presentation focuses on recent findings on the subcellular and subsynaptic localization of Group I mGluRs (mGluR1a and mGluR5) and GABA-B-R1 receptors in the monkey pallidum as revealed by pre-embedding immunoperoxidase and immunogold techniques at the electron microscope level.

As with previous data on the hippocampus and cerebellum, mGluR1a and mGluR5 immunoreactivity is found at the edge of the postsynaptic specialisations of asymmetric synapses established by glutamatergic subthalamic-like boutons in both pallidal segments. However, a large proportion of gold particles are also seen in the main body of the postsynaptic specialisations of symmetric synapses formed by striatal GABAergic terminals. These data raise questions about the possible sources of activation and the potential roles of group I mGluRs in modulating GABAergic neurotransmission at striatopallidal synapses. Although both group I mGluRs are expressed in pallidal neurons, the proportion of membrane bound versus intracellular labelling is significantly different between mGluR1a and mGluR5. Whereas mGluR1a immunoreactivity is almost exclusively associated with the plasma membrane, mGluR5 immunolabelling is much more prominent in the cytoplasm of labelled elements. Whether this indicates a differential turnover rate or degree of internalisation between the 2 Group I mGluRs remains to be established.

To analyse the GABA-B receptor immunoreactivity we produced polyclonal antibodies that specifically recognize R1 receptor subunits. In both pallidal segments, neuronal perikarya and dendrites display moderate GABA-B-R1 immunoreactivity. Furthermore a large population of small unmyelinated axons and many putative glutamatergic terminals forming asymmetric synapses are labelled. Preliminary immunogold data indicate that postsynaptic GABA-B-R1 immunoreactivity is mostly extrasynaptic though perisynaptic labelling at symmetric and asymmetric postsynaptic specialisations was encountered. Some gold particles are also found in the main body of symmetric striatal synapses.

Our data demonstrate that metabotropic Group I glutamate receptors and GABA-B receptors are widely distributed and located to mediate both pre and postsynaptic effects in the monkey pallidum. Moreover, their association with subthalamic glutamatergic afferents make them potential candidates for the development of novel drug therapies in Parkinson's disease.

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**11 Synaptic organisation of the basal ganglia**. By J. P. Bolam. *MRC Anatomical Neuropharmacology Unit, Department of Pharmacology, University of Oxford, UK.*

The basal ganglia are a group of subcortical nuclei involved in a variety of processes including motor, cognitive and mnemonic functions. One of their major roles is to integrate sensorimotor, associative and limbic information in the production of context dependent behaviours. These roles are exemplified by the clinical manifestations of neurological

disorders of the basal ganglia. Recent advances in many fields, including pharmacology, anatomy, physiology and pathophysiology have provided converging data that has led to unifying hypotheses concerning the functional organisation of the basal ganglia in health and disease.

The major input to the basal ganglia is derived from the cortex. Virtually the whole of the cortical mantle projects in a topographical manner onto the striatum, this cortical information is 'processed' within the striatum and passed via the so-called direct and indirect pathways to the output nuclei of the basal ganglia, the internal segment of the globus pallidus and the substantia nigra pars reticulata. The basal ganglia influence movement by the projections of these output nuclei to the thalamus and thence back to the cortex, or to sub-cortical 'pre-motor' regions. Under resting conditions the basal ganglia tonically inhibit neurons in these target nuclei. Activity in the direct pathway leads to a disinhibition of neurons in the target nuclei and is associated with movement whereas increased activity in the indirect pathway leads to a greater inhibition and is associated with the attenuation of movement. An imbalance of activity in favour of the indirect pathway has been proposed to underlie the movement disorders of Parkinson's disease.

In this communication the synaptic organisation of the neuronal networks that underlie this functional organisation of the basal ganglia will be discussed. Particular emphasis will be placed on the synaptic organisation of the cortico-striatal projection, the organisation of neurons of the globus pallidus and possible sites of the synaptic integration, within the basal ganglia, of functionally diverse information derived from the cortex.

**12 Functional anatomy of movement disorders**. By A. R. Crossman. *School of Biological Sciences, University of Manchester, UK.*

There are 2 simple and robust conceptual models of movements disorders in basal ganglia disease, one describing the neural mechanisms underlying parkinsonian akinesia and the other the appearance of abnormal involuntary movements (dyskinesias). These are diametrically opposed mechanisms, at opposite ends of the pathophysiological spectrum. Whilst these models are crude approximations to the complexities of human functional anatomy, their validity is borne out by their predictive capacity and practical application in new neurosurgical approaches to the treatment of movement disorders, based upon manipulation of the globus pallidus and subthalamic nucleus.

The basal ganglia consist of the striatum and globus pallidus. The subthalamic nucleus (STN), substantia nigra (SN) and certain ventral thalamic nuclei are often included in a working definition, because of their close functional relationship with the basal ganglia. The striatum is composed of the caudate nucleus and the putamen. The striatum receives the majority of afferent connections to the basal ganglia from extrinsic sources. These include the dopaminergic nigrostriatal pathway, from the pars compacta of the substantia nigra (SNc), the glutamatergic corticostriatal projection from the cerebral cortex and a projection from the intralaminar nuclei of the thalamus. Nigrostriatal dopaminergic transmission appears to have a dual action in the striatum, with opposite effects upon the 2 types of intrinsic striatal cells which constitute its efferent projections (and which constitute the origins of the so-called 'direct' and 'indirect' pathways). Thus dopamine is inhibitory upon striatal neurons which project to the lateral segment of the globus pallidus and excitatory upon those which project to the medial segment.

Striatal cells project to 2 main targets, the globus pallidus (GP) and substantia nigra, pars reticulata (SNr). These neurons use the inhibitory transmitter, $\gamma$-aminobutyric acid (GABA). The globus pallidus consists of a lateral, or external, segment (GPI, Gpe) and a medial, or internal, segment (GPm, Gpi), which have different afferent and efferent connections. The GPm is regarded (together with the pars reticulata of the substantia nigra) as the principal output of the basal ganglia, since the majority of fibres projecting to other levels of the neuraxis originate there. Its principal afferent connection is the 'direct pathway' from the striatum. The neurons of the direct pathway synthesise the peptides substance P and dynorphin. Medial pallidal efferent fibres are GABAergic. The largest efferent projection from GPm is to the ventral anterior (VA), ventral lateral (VL) and intralaminar nuclei of the thalamus. The VA and VL project to motor cortical regions of the frontal lobe. GPm also sends a projection to the pedunculopontine nucleus (PPN) of the caudal midbrain. GPI, like GPm, receives a GABAergic projection from the striatum. This is the origin of the 'indirect pathway'. GPI projects across the internal capsule to the subthalamic nucleus and also establishes connections between the 2 pallidal segments. All these pathways are GABAergic.

The subthalamic nucleus is a major focus of GPI efferents and also receives input from the cerebral cortex and intralaminar thalamic nuclei. Its efferent projections are to the GP and SN, pars reticulata. These are excitatory and mediated by glutamate. These basal ganglia connections constitute one of several parallel circuits which interconnect striatal, thalamic and cortical levels. Activity in the indirect pathway, transmitted through the GPI-STN-GPm-thalamic-cortical circuit, is thought to be responsible for inhibiting unwanted or inappropriate movements or behaviours. Activity in the direct pathway is thought to facilitate and reinforce behaviourally relevant motor activity.

In Parkinson's disease, loss of striatal dopamine leads to abnormal overactivity of the 'indirect' striatopallidal projection to GPI, due to loss of inhibitory innervation. This causes excessive inhibition of GPI and relief of inhibition of STN. Overactivity of STN ensues and, because of the excitatory nature of the subthalamopallidal projection, this in turn causes overactivity of GPm neurons. A central feature of dyskinesias is abnormal underactivity of both the subthalamic nucleus and the medial globus pallidus.

**13 Dopamine and synaptic plasticity in the neostriatum**. By G. W. Arbuthnott[1], C. A. Ingham[1] and J. R. Wickens[2]. [1]*Centre for Neuroscience, Department of Preclinical Veterinary Sciences, University of Edinburgh, UK*; *and* [2]*Department of Anatomy and Structural Biology, University of Otago Medical School, New Zealand.*

After the unilateral destruction of the dopamine input to the neostriatum there are enduring changes in rat behaviour. These have been ascribed to the loss of dopamine and the

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animals are often referred to as 'hemiparkinsonian'. We have shown that in the denervated striatum, not only are the tyrosine hydroxylase positive boutons missing, but also the medium sized densely spiny output cells have fewer spines. Spines usually have asymmetric synapses on their heads. In a recent stereological study we were able to show that there is a loss of approximately 20% of asymmetric synapses in the lesioned striatum which is complete by a month after the lesion. Current experiments are trying to establish the specificity this loss. So far we have evidence suggesting that there is no obvious preferential loss of synapses from either D1 or D2 receptor immunostained dendrites in the striatum with damaged dopamine innervation. These experiments suggest that dopamine is somehow necessary for the maintenance of corticostriatal synapses in the neostriatum.

A different series of experiments has suggested that a similar effect may also be of interest in intact striatum. In slices of cortex and neostriatum maintained in vitro, in such a way as to preserve at least some of the corticostriatal connections, we have found that cortical stimulation results in robust excitatory postsynaptic potentials (EPSPs) recorded from inside striatal neurons. Using stimulation protocols derived from the experiments on hippocampal synaptic plasticity we have shown that the usual consequence of trains of high frequency stimulation of the cortex is the depression of the size of the EPSPs in the striatal cell. In agreement with similar experiments by others, the effect seems to be influenced by NMDA receptors since the unblocking of these receptors with low $Mg^{2+}$ in the perfusate uncovered a potentiation of the EPSPs after trains of stimulation. Dopamine applied in the perfusion fluid around the slices had no such effect but pulsatile application of dopamine, close to the striatal cell being recorded from, and in temporal association with the cortical trains, led to a similar LTP like effect.The reduction of $K^+$ channel conductance in the bath with TEA also had the effect of making cortical trains induce potentiation of corticostriatal transmission. TEA applied only to the cell being recorded from had no similar effect; the cortical stimulation again depressed the EPSP amplitude, so the site of action of TEA may well be presynaptic to the striatal cell.

Of course these 2 phenomena might not be related but it is tempting to suggest that dopamine protects some corticostriatal synapses by potentiating them but that in the absence of dopamine others simply disconnect and are no longer visible in the electron microscope.

**14 Striatal implantation and circuit reconstruction**. By S. B. Dunnett. *Centre for Brain Repair*, *Cambridge University*, *UK*.

Excitotoxic lesions of the neostriatum induce cognitive and motor deficits in experimental animals, and model both the neuropathology and symptoms of Huntington's disease.

Striatal grafts implanted into the denervated striatum survive, differentiate into both striatal- and nonstriatal-like neurons, restore input and output connections of the damaged striatum, and alleviate both motor and cognitive impairments in experimental rats and monkeys. Several lines of evidence suggest that the functional recovery is mediated by the grafts providing a reconstruction of the cortico-striato-pallidal circuitries of the host forebrain, including functional mapping of circuitry by immediate early gene induction, push-pull perfusion, microdialysis, electrophysiology, the lack of efficacy of pharmacological treatments, and the behavioural studies themselves.

In the light of the functional benefits in experimental studies, clinical trials of neural transplantation in Huntington's disease are now commencing in several centres worldwide. Although supported by the neurobiology, data are not yet available to determine whether improvement will be achieved in Huntington's patients to a similar degree to that demonstrated (at least under the best circumstances) in Parkinson's disease.

**15 Imaging basal ganglia function.** By D. J. Brooks. *MRC Cyclotron Unit*, *Imperial College School of Medicine*, *Hammersmith Hospital*, *London*, *UK*.

H2150 PET activation studies allow in vivo examination of brain systems involved in motor function. In normal subjects the caudate-dorsolateral prefrontal cortex loop is involved in selecting the direction and timing of movement, in learning novel sequences and in problem solving. The putamen-supplementary motor area (SMA) loop plays a primary role in preparing limb movements and facilitating prelearned sequences. When tasks are financially rewarded, orbitofrontal, temporal pole, and substantia nigra activation correlate with the level of reward achieved and $^{11}$C-raclopride PET reveals increased dopamine release occurs during performance. The role of the basal ganglia in facilitating these tasks is unclear but they may act to filter and focus frontally instigated motor programs.

Parkinson's disease (PD) patients are more impaired when performing freely selected actions (where there is underactivity of the basal ganglia and their projection areas SMA and dorsal prefrontal cortex) than visually cued movements (where there is compensatory overactivity of the lateral parietal and premotor cortex). The activation of SMA and prefrontal cortex can be restored in PD patients by giving them dopaminergic treatment such as apomorphine or striatal fetal implants. Loss of dopamine in Parkinson's disease leads to overactivity of internal pallidum and excessive inhibitory output to the thalamus and frontal areas. Posteroventral pallidotomy and subthalamic stimulation both act to improve bradykinesia and to restore levels of frontal activation.

Selected PD patients with focal limb dyskinesias but without head involvement showed increased rCBF during dyskinesias in lentiform nuclei, premotor and dorsal prefrontal cortex; pallidal rCBF levels correlate with dyskinesia severity. Activation induced by performance of paced joystick movements in freely chosen directions was also relatively increased in premotor and prefrontal areas when patients experienced dyskinesias. These findings suggest that basal ganglia-frontal projections become inappropriately overactive both at rest and during movement when patients are experiencing dyskinesias.

PET also provides a means of examining in vivo regional receptor availability using $^{11}$C-SCH23390, $^{11}$C-raclopride, and $^{11}$C-diprenorphine to measure dopamine D1, D2, and opioid receptor availability, and $^{11}$C-flumazenil to measure benzodiazepine binding at GABA-A sites. For dyskinetic and nondyskinetic PD our findings suggest that dyskinesias are unlikely to arise from a primary disturbance of dopamine

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receptor function in caudate or putamen. (It does not rule out, however, an imbalance of receptors in high and low agonist affinity conformations). Nor do $^{11}$C-flumazenil PET studies reveal GABA-A binding abnormalities in dyskinetic PD patients. Interestingly, patients who have received a clinically effective pallidotomy show decreased ventral thalamic $^{11}$C-flumazenil binding suggesting that internal globus pallidus (GPi) to thalamic GABA transmission is indeed interrupted by this surgical intervention.

Dyskinetic PD patients overexpress striatal opioid peptides and so exhibit an overactive direct pathway. Raised enkephalin levels may act to inhibit GABA release in GPe via presynaptic autoreceptors resulting, in turn, in inhibition of GPi. However when dyskinetic and nondyskinetic PD patients were studied with $^{11}$C-diprenorphine PET, the former showed a significant reduction in striatal, thalamic, and cingulate opioid site availability. This would be compatible with the presence of raised levels of endogenous opioid peptides, such as enkephalin and dynorphin, occupying a greater number of opioid sites. Reductions in putamen opioid binding correlated significantly with dyskinesia severity. These findings are in support of the above basal ganglia model to explain dyskinesias and suggest that selective opioids may have a future role as antidyskinesia agents.

**16 The genetics of basal ganglia disorders**. By W. G. Johnson. *UMDNJ-Robert Wood Johnson Medical School, New Jersey, USA.*

Recently, mutations of the á-synuclein gene were found to cause dominantly inherited Lewy-body Parkinson's disease (PD) and α-synuclein was found to be a major component of the Lewy body. However the cause of the common form of PD with the multifactorial rather than autosomal dominant inheritance pattern remains unknown.

α-synuclein precipitates slowly and apparently spontaneously at high concentration in solution and the mutations that cause PD accelerate precipitation. Other dominantly inherited late onset or adult onset dominantly inherited neurodegenerative diseases are associated with precipitation of proteins. In Alzheimer disease (AD), β-amyloid and tau abnormalities are found. In prion disorders, prion proteins are found. In Huntington disease (HD), a disorder with expanded CAG repeats, huntingtin precipitates are found. In dominantly inherited spinocerebellar ataxias (SCAs), also expanded CAG repeat disorders, the corresponding ataxin protein precipitates are found. In multiple system atrophy, MSA, α-synuclein precipitates are found. In progressive supranuclear palsy (PSP), tau precipitates are found. In familial ALS, a group of dominant disorders, SOD1 precipitates are found. Most of these disorders involve the basal ganglia in some way.

Since similar processes seem to affect neurons of adults or older individuals and since a relatively limited group of proteins seems to be involved, each producing a form of neurodegeneration, it is possible that certain common features are present that affect this group of proteins. Candidates include a conformational shift, as in prions, an abnormality of the ubiquitin-proteosome pathway, as seen in PD, or an abnormality of a pathway preventing precipitation (e.g. chaperonins), or potentiation of a pathway promoting precipitation (e.g. γ-glutamyl-transpeptidase.

Elucidation of the pathways causing this protein insolubilisation is the first step toward approaching prevention and reversal in these late onset neurodegenerative diseases.

**17 Morphometric studies in mice indicate that neuronal dysfunction may play a significant role in the early symptomatology of Huntington's disease.** By H. M. Johnston[1], M. Neilson[2], L. Kennedy[1], C.-M. Chen[1], M. Ennis[1] and P. F. Shelbourne[1] (introduced by A. P. Payne). [1]*Division of Molecular Genetics and Division of Neuroscience and* [2]*Biomedical Systems, Institute of Biomedical and Life Sciences, University of Glasgow, UK.*

Huntington's disease (HD) is a dominant, progressive disorder of humans characterised by motor abnormalities, psychiatric disturbances and cognitive decline. Postmortem brain tissue shows striking bilateral striatal atrophy, particularly in the caudate and putamen. The underlying cause of the disease is a genetic mutation that results in an abnormally long polyglutamine tract in a protein called huntingtin.

In an attempt to better understand how this mutant protein mediates the complex pathophysiological processes, we are investigating a mouse model of the disease that has been engineered to carry a mutation similar to that on human HD chromosomes, containing 80 CAG repeats. Although these mice show no signs of acute neurodegeneration on conventional neuropathological analysis, they display electrophysiological and behavioural abnormalities which we speculate could be the consequence of subtle neuronal loss or prolonged neuronal dysfunction.

In order to address this issue, we have instigated a morphometric study of the striatum using a Kontron 400 Image Analysis System and individually tailored software. Our current results confirm that mutant and control mice ($>$ 19 mo of age) show no significant differences in either the total number or density of cells within the striatum.

Given that the mice show changes that are reminiscent, at least, of early symptoms observed in the human disease, we would like to argue that some clinical features of HD may be caused by pathological processes such as neuronal dysfunction rather than acute cell death. If this proves to be the case, effective treatment of HD may well require an understanding and correction of these processes.

**18 Where cells die last: the AS/AGU rat as a model of impaired dopamine release and neurodegeneration.** By A. P. Payne, J. M. Campbell, G. Favor, R. W. Davies, R. G. Sutcliffe, D. Russell, T. W. Stone and D. P. Gilmore. *Institute of Biomedical and Life Sciences, Glasgow University, UK.*

The AS/AGU rat is a spontaneous recessive mutant which has arisen from Albino Swiss (AS) stock. These animals display disordered locomotion including ungainliness, whole body tremor, rigidity and an inability to initiate movement. To date no abnormalities of the cortex, cerebellum or alpha motor neurons have been reported. However there are deficits in the nigrostriatal dopaminergic

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system with depleted numbers of tyrosine hydroxylase-immunoreactive cells in the substantia nigra pars compacta from about 14 mo onwards. These decreased cell counts have not been recorded in younger animals despite the fact that (a) motor performance is impaired from an early age and (b) L-DOPA administration can improve motor performance. Furthermore whole tissue dopamine levels in the striatum – as measured by micropunch and high pressure liquid chromatography – do not decrease until after 6 mo of age. To resolve this, studies were carried out using striatal microdialysis in conscious, freely moving rats (using a cannula previously implanted under 2:1 Rompun:Vetelar anaesthesia, 1.1 ml/kg). These demonstrate marked reductions (~ 80%) in extracellular dopamine in the dorsal caudate-putamen of AS/AGU rats from 3 mo, the earliest age at which stereotaxic implantation can be reliably carried out. By contrast extracellular levels of dopamine metabolites such as DOPAC are greatly increased. Pharmacological studies using evoked release, reuptake blockade and monoamine oxidase inhibition demonstrate that the AS/AGU rat has impaired physiological release of dopamine and suggest that high levels of metabolites are probably due to excess dopamine in nonvesicular form being available within the terminals. If so this is of considerable interest since dopamine can act in an autotoxic manner, probably by the formation of free radicals. The AS/AGU rat demonstrates that cell loss itself may be one of the final events in a degenerative sequence, rather than the first. It also gives the possibility of an extended period prior to actual cell loss which can be used to examine the mechanisms of degeneration and/or to evaluate the efficacy of novel therapeutic treatments.

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**19 Fatigue and basal ganglia.** By A. CHAUDHURI and P. O. BEHAN (introduced by J. P. BENNETT). *Department of Neurology*, *University of Glasgow*, *Scotland.*

Basal ganglia are involved in the processing of information needed for planning and triggering self initiated movements. Fatigue, defined as a failure of endurance in motor activity and cognitive tasks, is an important symptom in the disorders affecting basal ganglia. Classically observed during the epidemic outbreak of post-encephalitic Parkinsonism (encephalitis lethargica) fatigue is common in patients with Parkinson's disease (PD) and striatonigral degeneration (multiple system atrophy or MSA). In both PD and MSA fatigue may antedate the development of motor or autonomic symptoms respectively. Basal ganglia are also implicated in the mechanism of fatigue in the postpolio syndrome. Recent research has drawn attention to the possible association between functional dopamine deficiency and fatigue in chronic fatigue syndrome. Both hypokinesia and fatigue respond well to levodopa therapy in PD. Dopamine agonists have been also used with modest success in the treatment of fatigue in other neurological disorders, e.g. amantadine in multiple sclerosis and bromocriptine in postpolio fatigue. Finally, fatigue is commonly found in depression and high frequency electrical stimulation of subthalamic nuclei has been recently reported to cause acute depression. Drugs that inhibit neuronal dopamine reuptake are potent antidepressants.

Despite these observations, the anatomical pathways and the chemical substrate for fatigue in the disorders of central nervous system are not fully understood. There is extensive, bidirectional linkage between the basal ganglia and cerebral cortex that includes several functional and anatomically distinct circuits regulating motor activity and more complex cognitive behaviours. The major neocortical projections of basal ganglia travel via thalamus and thalamic diseases can modify the feedback circuits between the basal ganglia and the cerebral cortex. Basal ganglia are involved in the higher order, cognitive aspects of motor control and these neurons also influence many other functions through their extensive connections with the association cortex and limbic structures.

It is important to recognise fatigue as a symptom of neurological disease that may suggest a structural or functional disorder of basal ganglia or their pathways. A better understanding of the anatomy and neurotransmission in basal ganglia projections would be extremely important in the management of fatigue.

**20 The effects of dopamine replacement therapy on striatal NMDA receptor subunit expression in rat models of Parkinson's disease.** By P. RAVENSCROFT, B. HENRY, A. R. CROSSMAN and J. M. BROTCHIE. *Manchester Movement Disorder Laboratory*, *School of Biological Sciences*, *University of Manchester*, *UK.*

Parkinson's disease is characterised by reduced levels of striatal dopamine, caused by degeneration of dopaminergic nigrostriatal neurons. The dopamine precursor L-DOPA remains the most widely used treatment for Parkinson's disease but is plagued by debilitating dyskinetic side effects following repeated administration. Enhanced activation of glutamatergic NMDA receptors localised on the striatal output pathway is an important feature of the neural mechanisms underlying the generation of L-DOPA induced dyskinesia. Some dopamine receptor agonists, such as bromocriptine, are able to alleviate symptoms of Parkinson's disease with a reduced incidence of dyskinesias compared with L-DOPA. We hypothesise that enhanced nMDA receptor subunit expression may accompany repeated L-DOPA, but not bromocriptine, treatment. NMDA receptors are a heteromeric protein complex of subunits from at least 2 families, NR1(a-h) and NR2(A-D). In this study, the topographic changes in striatal nMDA receptor subunit expression in the 6-hydroxydopamine Lesioned rat model of Parkinson's disease were assessed using in situ hybridisation, following treatment with vehicle, L-DOPA (6.5mg/kg) or bromocriptine (5 mg/kg) twice daily by intraperitoneal injection for 21 d.

Following L-DOPA treatment, NR1 and nR2B subunit expression in the rostral striatum was elevated by 36% ($P < 0.05$) and 23% ($P < 0.05$), respectively, compared with vehicle treated animals. No significant changes in subunit expression were seen following bromocriptine administration.

Upregulation of nMDA receptor subunit expression may contribute to the neural mechanisms underlying L-DOPA induced dyskinesia. Co-administration of L-DOPA with nR2B subunit selective nMDA receptor antagonists may be beneficial in treating the symptoms of Parkinson's disease without eliciting dyskinesia.

**21 Topographical variations in NMDA-adenosine-cAMP signalling in rat striatum: implications in Parkinson's disease.** By J. E. NASH and J. M. BROTCHIE. *Manchester Movement Disorder Laboratory, School of Biological Sciences, University of Manchester, UK.*

The neural mechanisms underlying the generation of symptoms in Parkinson's disease involve overactive transmission at both NMDA and adenosine $A_{2a}$ receptors in the striatum. Blockade of NR2B-containing NMDA receptors or adenosine $A_{2a}$ receptors alleviates parkinsonian symptoms in rats and primates in a similar manner. We thus hypothesised a common mechanism of action for these classes of antiparkinsonian agents. In striatal slices prepared from the 6-hydroxydopamine lesioned rat model of Parkinson's disease, NMDA-induced increases in cAMP were significantly higher, being $449 \pm 68\,\%$ (mean $\pm$ SEM, $n = 6$) of basal levels. Adenosine $A_{2a}$ receptor activation using the agonist CPCA (3 μM) led to elevation of cAMP levels. In the striatal slices prepared from the rat, NMDA receptor activation also elevated cAMP levels to $200 \pm 21\,\%$ of basal. The adenosine $A_{2a}$ receptor antagonist DMPX (100 μM) blocked the increase in cAMP evoked by 100 μM NMDA, suggesting that NMDA induced elevation of cAMP involves stimulation of adenosine $A_{2a}$ receptors. The antiparkinsonian actions of NR2B-selective NMDA receptor antagonists are mediated in the rostral striatum. Furthermore, NMDA induced increases in cAMP were greater in the rostral striatum than in caudal regions of the striatum. We propose that in animal models of Parkinson's disease, increased NMDA induced cAMP levels in the rostral striatum may be responsible for the production of parkinsonian symptoms. Therefore either NR2B-selective NMDA receptor antagonists or adenosine $A_{2a}$ receptor antagonists may mediate their antiparkinsonian actions via a mechanism involving reductions of cAMP levels in the rostral striatum.

**22 The neuropathology of mice carrying mutant APP and PS-1 transgenes: an EM study.** By M. A. KURT[1,2], D. C. DAVIES[1], M. KIDD[1], K. DUFF[3], K. H. JENNINGS[4], E. H. KARRAN[4] and S. J. NEWMAN[4] [1]*St George's Hospital Medical School, London, UK*; [2]*University of Uludag School of Medicine, Turkey*; [3]*Nathan Kline Institute, Orangeburg, USA*; *and* [4]*Smithkline Beecham Pharmaceuticals, Harlow, UK.*

APP and PS-1 mutations lead to an increase in beta amyloid (Aβ) production. Despite the fact that a number of transgenic mice develop cerebral Aβ plaques, few have been subjected to ultrastructural investigation. We therefore, investigated the doubly transgenic (mutant human $APP_{K670N,M671L}$ mutant human $PS1_{M146L}$) mouse which develops Aβ deposits much earlier than singly transgenic littermates. Widespread Aβ plaques with or without a distinct core were found in grey matter. Aβ plaques were also present in white matter as were cerebrovascular Aβ deposits. Astrocytosis was greater around grey matter than white matter plaques. In some plaques, Aβ cores were associated with cellular profiles containing prominent endoplasmic reticulum and a homogenous cytoplasm that appeared to be neuronal. Some of these profiles in grey matter contained large dense vesicles. The morphology and location of other profiles indicated them to be microglia or oligodendocytes. Some Aβ fibrils appeared to lie within these profiles but they may have been simply surrounded by the cell profile since the profile membrane was not always visible. Dark atrophic neurons were present around grey matter plaques. Interestingly, filamentous structures reminiscent of the PHFs were found inside one atrophic neuron. Thus the neuropathology observed in PS1/APP mouse brain is similar to that in AD and they appear to be the best model of AD pathology currently available.

**23 Neuronal nitric oxide synthase in the hypothalamic osmotic control system: dissociation of changes in the input osmosensitive and output magnocellular nuclei**. By H. WANG, J. F. MORRIS and D. MA. *Department of Human Anatomy and Genetics, University of Oxford, UK.*

The hypothalamic osmoregulatory system comprises several input nuclei including the subfornical organ (SFO), median nucleus, and organum vasculosum of the lamina terminalis (OVLT) and the output vasopressin-secreting magnocellular neurons of the paraventricular and supraoptic nuclei, which are also osmosensitive. Nitric oxide synthase activity (nNOS) in the magnocellular neurons is known to be enhanced by acute osmotic stimulation and appears to act as a local negative feedback. Our comparison of 2 mutants – Brattleboro (BB) rats which cannot secrete vasopressin and renal diabetes insipidus (*di/di*) mice which cannot respond to vasopressin – indicated that long term nNOS is also regulated by osmotic stimuli. The immediate early gene product Fos is a marker of the activation of neuronal pathways. To determine whether the input and output nuclei are regulated coordinately, we have investigated nNOS and Fos in the input and output nuclei in the 2 mutants during treatment with desamino D-arginine vasopressin (dDAVP) which corrects the diabetes insipidus in the BB rats, but not the *di/di* mice.

Pairs of BB rats and *di/di* mice drank either normal water or water containing 4 mg/l dDAVP for 4 or 14 d. The animals were then deeply anaesthetised with sodium pentobarbital, fixed by perfusion and the hypothalami studied by either in situ hybridization for nNOS (after 4 d dDAVP) or immunocytochemistry for nNOS or Fos (after 14 d dDAVP). nNOS mRNA was also compared in BB and wild-type Long Evans rats.

In magnocellular neurons of the mutant animals, nNOS was increased in both the cell bodies and dendrites, and extended further into the dendrites of BB rats. nNOS- and Fos-immunoreactive neurons were present in both the input osmosensitive and magnocellular nuclei. Administration of dDAVP, which corrected the diabetes insipidus in the BB rats reduced the nNOS mRNA expression and the numbers of nNOS- and Fos-immunoreactive neurons in the magnocellular nuclei, but did not affect either nNOS or Fos in the input osmoregulatory centres. dDAVP treatment had no effect on nNOS in any of the nuclei studied in *di/di* mice.

The results show that chronic osmotic status regulates nNOS and Fos in magnocellular neurosecretory nuclei, but not in the upstream osmoregulatory centres. Regulatory feedback via V2 receptors is also unlikely given the lack of blood–brain barrier in the OVLT. Parallelism of Fos and

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nNOS expression in all parts of the system suggests a functional link between these 2 regulators.

**24 Function and potential of hammerhead ribozymes against the neuronal nitric oxide synthase mRNA.** By D. MANIOTIS[1], H. M. CHARLTON[1] and L. A. PHYLACTOU[2]. [1]*Department of Human Anatomy and Genetics, University of Oxford, UK; and* [2]*Department of Molecular Genetics, The Cyprus Institute of Neurology and Genetics, Nicosia, Cyprus.*

Catalytic RNA molecules (ribozymes) have been widely used specifically to suppress gene expression. The hammerhead ribozyme (< 40 ribonucleotides) can be designed to bind and cleave, theoretically, any RNA molecule. Neuronal nitric oxide synthase (nNOS) is an important molecule in the central nervous system involved in healthy (e.g. vasodilation, neurotransmission) and disease conditions (e.g. oxidative stress). Therefore, downregulation of nNOS gene expression by hammerhead ribozymes may offer new insights into its function and form the basis of therapeutic strategies against neurological diseases.

Two antisense ribozymes, nNOS-RZ1 and nNOS-RZ2, have been designed and constructed against the 5′ end and the middle part of the nNOS mRNA, respectively. In vitro (cell-free) experiments demonstrated the ability of both ribozymes to cleave shorter versions of the nNOS RNA targets as detected by autoradiography. The efficacy of both ribozymes on the endogenous nNOS mRNA targets was determined in human TGW-I-nu neuroblastoma cells. Cells were transfected with presynthesised ribozymes, or ribozyme-containing constructs. Downregulation of the endogenous nNOS gene expression from ribozyme-transfected cells was more evident in the cells treated with nNOS-RZ2 ribozyme. Ribozyme-adenoviral vectors have also been constructed. Further experiments will demonstrate the ability of adenoviruses to deliver and express anti-nNOS ribozymes in cell culture and in vivo.

The above results suggest that hammerhead ribozymes can be important tools for the regulation of nNOS gene expression and the development of gene therapy protocols for neurological disease. (Supported by the Anatomical Society of Great Britain and Ireland, UK)

**25 Quantitative analysis of contacts formed by serotoninergic and noradrenergic axons with premotor spinal interneurons in cats**. By D. J. MAXWELL[1], J. S. RIDDELL[1] and E. JANKOWSKA[2] (introduced by A. P. PAYNE). [1]*Institute of Biomedical and Life Sciences, University of Glasgow, Glasgow, UK; and* [2]*Department of Physiology and Pharmacology, Göteborg University, Sweden.*

Bulbospinal monoaminergic systems have powerful modulatory effects upon spinal reflex pathways but the mechanisms which underlie this modulation have not yet been established. We investigated the possibility that monoaminergic axons make contacts with a group of spinal interneurons which project to motor nuclei and are monosynaptically activated by group II muscle afferents (Cavallari et al. *J. Physiol.* **389**, 1987).

Interneurons in L4-L5 spinal segments of deeply anaesthetised adult cats were electrophysiologically characterised and intracellularly labelled with tetramethylrhodamine dextran (see Carr et al. *Brain. Res. Bull.* **34**, 1994). Animals were fixed by perfusion and serotoninergic and noradrenergic axons were identified with immunofluorescence in sections containing labelled cells.

Contacts between immunofluorescent axons and rhodamine dextran-labelled interneurons (n = 5) were investigated with a 3 channel confocal laser scanning microscope (BioRad MRC1024) and analysed quantitatively with Neurolucida for Confocal (MicroBrightField Inc.). The 5 cells were reconstructed and contacts were plotted on the reconstructions. The average number of contacts formed by serotoninergic axons for each cell was $140 \pm 28$ (mean $\pm$ S.D.) and the average number of noradrenergic contacts was $38 \pm 13$. Both types of axon were found in apposition to cell bodies and dendrites but 95% of contacts were formed with dendrites. Sholl analysis indicated that contacts were distributed on proximal, intermediate and distal dendrites but there was a gradual decrease of numbers of contacts with distance.

These results show that putative synaptic contacts are made by serotoninergic and noradrenergic fibres with the whole surface of premotor spinal interneurons in pathways from group II muscle afferents. This provides a morphological basis for the modulatory actions of monoamines on this pathway.

This work was supported by The Wellcome Trust, the Robertson Trust and the Swedish Medical Research Council.

## POSTERS

**P1 Ultrastructural study of glomerular sclerosis in Lewis and Dwarf rats subjected to subtotal nephrectomy and exogenous growth hormone treatment.** By G. H. COPE[1] and M. SOLEIMANI[2]. [1]*Department of Biomedical Science, University of Sheffield and* [2]*Department of Biology, Arak University, Islamic Republic of Iran.*

The objective was to obtain structural information concerning glomerular hypertrophy and sclerosis of the remnant kidney that occurs following subtotal nephrectomy and to investigate the possible effects of exogenous growth hormone, particularly on Dwarf (growth hormone deficient) rats. For this 16 wk old male Lewis and Dwarf Lewis rats were subjected to 5/6 nephrectomy (SNX) or a sham operation under deep anaesthesia with Sagatal. The rats were then allowed to recover and 1 mo later were injected twice daily with either recombinant human growth hormone (1.6 IU/d) or saline for 30 consecutive days before being killed 120 d after operation by exsanguination under Sagatal anaesthesia. Tissues were fixed with buffered 1% glutaraldehyde followed by 1% osmium tetroxide and embedded in Araldite.

Treatment with growth hormone alone caused focal thickening of the basement membrane of the Bowman's capsule and thickening of the filtration membrane of the glomerular tuft. SNX caused considerable enlargement of the remnant glomeruli, hypertrophy of the parietal cells and fibrosis of the capsule. Expansion of the mesangial cells and the deposition of increased amounts of amorphous material within the glomerular tuft was also evident. Podocytes became enlarged and their processes developed a paucity of

organelles. Often the pattern of foot processes became disrupted either by the development of pseudocysts or the collapse of the podocytes on to the filtration membrane. In some cases crescents formed as the podocytes adhered to the parietal layer. In some instances the extent of the damage was more pronounced in the dwarf strain and all of these effects appeared more pronounced in the nephrectomised rats that were treated with growth hormone. We conclude that growth hormone has a slight potentiating effect on these processes.

**P2 Ultrastructural study of the effects of recombinant human growth hormone on tubulo-interstitial fibrosis in Lewis and Dwarf rats following subtotal nephrectomy**. By M. Soleimani[1] and G. H. Cope[2]. [1]*Department of Biology*, *Arak University*, *Islamic Republic of Iran*; *and* [2]*Department of Biomedical Science*, *University of Sheffield*, *UK*.

The objective was to obtain detailed ultrastructural information concerning the pathological changes to the tubules and cortical interstitium of the remnant kidney that occur following subtotal nephrectomy and to investigate the possible effects of exogenous growth hormone, particularly on Dwarf (growth hormone deficient) rats. For this, 16 wk old male Lewis and Dwarf Lewis rats were subjected to 5/6 nephrectomy (SNX) or a sham operation under deep anaesthesia with Sagatal. The rats were then allowed to recover and 1 mo later were injected twice-daily with either recombinant human growth hormone (1.6 IU/d) or saline for 30 consecutive days before being killed 120 d after operation by exsanguination under Sagatal anaesthesia. Tissues were fixed with buffered 1% glutaraldehyde followed by 1% osmium tetroxide and embedded in Araldite.

Thickening of basement membranes was encountered in sham operated rats treated with growth hormone and SNX caused duplication of basement membranes. Considerable tubule damage was evident after SNX including loss of microvilli, and the blebbing of the cytoplasm into the tubule lumen. Mitochondria often appeared enlarged or damaged and accumulations of lipid droplets and increased numbers of lysosomes were frequent observations. After SNX the interstitium became infiltrated with lymphocytes, plasma cells and mast cells. Fibroblasts became enlarged and occasional myofibroblasts were encountered. Focal accumulations of extracellular fibres and amorphous extracellular material became apparent. These effects appeared to be exacerbated by growth hormone treatment although overall, there seemed to be no difference between the responses of the Lewis and Dwarf strains.

**P3 Effects of mouse pre-Sertoli cells on female germ cell entry to meiosis in vitro**. By S. Mackay, N. J. S. Henderson and R. A. Smith. *Laboratory of Human Anatomy/IBLS*, *University of Glasgow*, *UK*.

Great advances have been made in the field of gonadal differentiation in recent years with the identification of the testis determining gene in both man and the mouse. This master gene on the Y chromosome, *SRY* in the human and *Sry* in the mouse, is thought to act in a cell-autonomous fashion to determine that cells in the somatic population develop as pre-Sertoli cells. Triggering of somatic cell differentiation along the Sertoli cell pathway is therefore a key event and further steps in gonadal differentiation follow on in a developmental cascade. Despite the key role of pre-Sertoli cells in testis differentiation, their cell biology remains sketchy.

In vivo adult Sertoli cells are supported by a basement membrane of extracellular matrix (ECM). As ECM has been shown to be critical for normal morphogenesis and differentiation, ECM components and reconstituted basement membrane (RBM) have been widely used to study adult Sertoli cell differentiation in vitro. We have recently developed the application of similar culture techniques to investigate the development of mouse pre-Sertoli cells (Mackay et al., *J. Electron Microsc.* **48**, 1999) with encouraging results.

It has been suggested that pre-Sertoli cells prevent entry to meiosis in the male (McLaren & Southee, *Dev. Biol.* **187**, 1997). We have used a coculture technique to assess possible effects of mouse pre-Sertoli cells on female germ cell entry to meiosis in E12.5 and E13.5 ovaries. A total of 12 ovaries were added to pre-Sertoli feeder layers established from testes of equivalent stage in at least 3 repeat experiments. Controls included stage matched ovarian explants cultured without Sertoli cells. After 3 d in culture, material was fixed and processed for light and transmission electron microscopy. Entry to meiosis was unaffected at E13.5; however, when ovaries were explanted at the earlier stage of E12.5, just prior to entry to meiosis, most germ cells disappeared over the culture period. These results suggest that the critical period during which pre-Sertoli cells exert their effects on germ cell meiosis is just prior to the time of entry.

**P4 A novel approach for retina transfection: in vitro and in vivo electroporation in chick using green fluorescent protein as a reporter gene.** By D. Le Rouëdec[1], M. Abu-Elmagd[1], P. M. Wigmore[2] and P. J. Scotti Ng[1]. [1]*Institute of Genetics and* [2]*School of Biomedical Sciences*, *Queen's Medical Centre*, *Nottingham University*, *UK*.

Transcription factors play a key role in the process of cell maturation during development of the nervous system. The purpose of this study is to establish an efficient technique for transfection in order to investigate the role of such transcription factors during development of the retina, a tissue whose development has been extensively studied. The method uses a new approach of in vivo and in vitro electroporation in order to obtain constitutive expression of Sox and basic helix-loop-helix genes, 2 kinds of transcription factors known to be important during cell maturation in the retina.

The genes of interest, cSox11 and ngn2, were first cloned into a vector containing the green fluorescent protein (GFP) reporter gene. This made it possible to detect their presence and expression of transfected genes in the transfected cells. The technique of electroporation was developed by transfecting DNA into retina cells of chicken *Gallus gallus*. In vivo expression was achieved by injecting DNA directly into the eye or neural tube at different stages of development (from d 1 to d 3 of incubation). This was followed by

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electroporation using a square pulse electroporator. The embryos were then incubated further, killed by decapitation, and processed for frozen sectioning or whole mount in situ hybridisation. In vitro electroporation was performed on 8 d old embryonic retina, cultured for 3 d before being processed in the same way as the in vivo tissue. In vitro results showed a high rate of transfection, while the in vivo technique had a more limited success as few scattered GFP expressing cells were observed.

The development of this technique has proven to be successful, as both in vivo and in vitro techniques can be used for various studies. The investigation of ectopic expression of transcription factors is now being carried out. It is anticipated that the genes used might interfere with cell migration and/or differentiation, and in situ analysis and immunostaining will soon reveal more details in their role and highlight the co-ordination of transcription factors during neuronal development.

This work was supported by a research studentship from the Anatomical Society of Great Britain and Ireland.

**P5 Identification of Sox genes in mouse developing limb and muscle.** By S. Pinto Cardoso[1], M. Cheung[2], P. J. Scotting[2] and P. M. Wigmore[1]. [1]*School of Biomedical Sciences and* [2]*Institute of Genetics, Queen's Medical Centre, Nottingham University, UK.*

Sox genes are members of a recently described family of transcription factors. They are characterised by the inclusion of a 230 bp High Mobility Group (HMG) box, responsible for the sequence specific DNA binding. Sox genes have been classified into 7 subgroups according to their amino acid identity within the HMG box. Subgroup F contains the only Sox genes (Sox 7 and 18) which have been described as being expressed in adult skeletal muscle.

To identify Sox gene expression in the developing limb and muscle, RT-PCR was performed using degenerate primers which anneal to the 5′ and 3′ ends of the HMG box. In addition a degenerate 5′ primer, specific for subgroup F, was used in separate reactions. Total RNA was isolated from muscle tissue derived from embryonic day (E) 17 and 19 embryos and from newborn mice. RT-PCR was performed using both sets of degenerate primers and PCR products cloned and sequenced. Expression of Sox4 and L-Sox5 HMG boxes was identified using the degenerate 5′ and 3′ primers. The expression pattern of Sox4 was determined by in situ hybridisation in whole mouse E13.5 sections. This showed that Sox4 is expressed in the connective tissue within the limb. No Sox4 expression was found in the developing muscle in any region of the embryo. L-Sox 5 is expressed in chondrocytes and its presence here is possibly due to the inclusion of cartilage in the samples. Using primers specific to Sox subgroup F, the HMG box of Sox7 was identified. Since this is a subgroup F Sox gene it is likely that this gene is expressed in developing skeletal muscle. Using embryonic tissue it is difficult to obtain completely pure muscle tissue. For this reason, the C2C12 mouse myoblast cell line was used for RT-PCR using both sets of primers. C2C12 cells were grown in proliferation and differentiation media. A PCR product was identified from proliferating C2C12 cell mRNA using degenerate 5′ and 3′ primers. This PCR product is currently being cloned.

**P6 BRE, A new modulator of TNF-α action, is involved in the regulation of apoptosis in the developing mouse embryo.** By A. K. C. Leung[1], R. W. M. Tong[1], M. K. Tang[1], J. Y. H. Chan[2] and K. K. H. Lee[1] (introduced by J. Dixon). [1]*Department of Anatomy and* [2]*Department of Clinical Oncology, Chinese University of Hong Kong, Hong Kong.*

We have previously identified a stress responsive gene *BRE* that is *b*rain and *r*eproductive organs *e*xpressed. *BRE* has recently been found to interact with the juxtamembrane region of p55 tumour necrosis factor receptor-1 (TNFR1) and inhibits the activation of the transcriptional factor NF-κB induced by TNF-α. To elucidate the role of *BRE* during development the expression patterns of BRE, NF-κB and TNFR1 were analysed in mouse embryos by immunohistochemical staining and in situ hybridisation. In the embryo BRE, NF-κB and TNFR1 were found co-expressed in the heart, brain and neural tube, as well as the intervertebral and interdigital tissues which normally undergo apoptosis. In addition, examination of BRE expression in the developing limb suggests that it may be involved in chondrogenesis and myogenesis. To assess the ability of BRE to induce apoptosis, embryonic limb cells were transfected with a GFP reporter construct carrying the *BRE* gene. Overexpression of *BRE* induced cell death in a majority of limb mesenchymal and interdigital cells. However there were also *BRE*-transfected limb myogenic cells that appeared morphologically normal. The differential expression of *BRE* in mouse embryos and its effect on cellular apoptosis, differentiation and maturation indicated that *BRE* might play an important role in development, by modulating the TNF transduction pathway.

**P7 Ten curiosities of the Cleland Collection.** By A. Heffernan and S. W. McDonald. *Laboratory of Human Anatomy, University of Glasgow, UK.*

This demonstration focuses on 10 crania collected by John Cleland, Regius Professor at Glasgow 1877–1910. Each has features of interest.

(1) *Cast of cranium of Robert Burns*. A mould of Burns' skull was made by George Combe when the mausoleum in Dumfries was opened for Mrs Burns' funeral in 1834. (2) *Cast of skull of Robert the Bruce*. The eroded appearance of the anterior nasal aperture and superior alveolus suggest leprosy and supports the testimony of mediaeval documents. (3) *Large brachycephalic male skull donated by an old student's friends*. Occasional annotations show the skull's use as a learning aid. An excised zygomatic arch suggests that it came from the dissecting room. (4) *Skull showing phrenological territories*. The words and numbers reflect early and later phrenological systems. The annotations are in French. (5) *French sailor who killed himself by pistol shot in the harbour*. Acquired by Professor Allen Thomson in 1851, the phrase 'in the harbour' suggests the Glasgow docks. The Glasgow Herald of 31st January 1851 reports an unclaimed body of a sailor in the Clyde. Why someone who shot himself should be in the water is not explained. (6) *Skull of a Russian soldier shot at Balaclava*, 1854. A round hole with chipped edges externally, lies immediately posterior to the left frontal eminence. Half of a likely entrance hole lies where the right parietal bone met the now lost temporal

bone. (7) *Outer table of left side of vault filed away*. A teaching aid showing the diploic veins. (8) *Adult hydrocephalus*. A round, smooth edged hole at the confluence of sinuses was probably due to a small meningocele rather than paracentesis which would have been carried out away from the venous sinuses. (9) *Skull of young person*. Thin and delicate skull consistent with a child of about 12–13 y. A round 'moth-eaten' lesion on the left frontal bone suggests cranial tuberculosis. (10) *Female, old and toothless*. An asymmetric skull showing features of old age and a class III malocclusion.

**P8 Health care professionals all get the same anatomy, don't they?** By A. L. STAKER[1] and P. COLLINS[2]. [1]*Anglo-European College of Chiropractic*; *and* [2]*University of Southampton, UK.*

The education of health professionals is centred on providing a solid anatomical basis which acts as a foundation for the application of diagnostic and therapeutic disciplines (Educational Affairs Committee (AACA), *Clin. Anat*. **9**, 1996). The Commission on Alternative Medicine in Sweden, 1987, reported that 'Doctors of chiropractic follow a 4 to 5 y course of university level training…in its preclinical parts found to be the equivalent of Swedish medical training'. Despite a similar standard of education in chiropractic and medical undergraduate programmes today, there does exist a number of fundamental differences, one of which is evident in the curriculum for clinical anatomy. Current chiropractic theory is dependent upon an integral knowledge of the anatomy and physiology of the spine and its neural elements, the biomechanical relationship between vertebrae and the pathophysiology of various spinal structures (Haldemann, '*Principles and Practice of Chiropractic*', 1992). Thus the anatomy curriculum of the chiropractic undergraduate degree has differences in emphasis and content, when compared with medicine, that reflect the different scope of practice of chiropractic and ensures that the chiropractor is competent in neuromusculoskeletal diagnosis and the employment of manual treatment skills (Chapman-Smith, *The Chiro. Report* **8**, 1994). This paper focuses on the similarities and differences between the teaching of anatomy as a foundation subject for chiropractic students at the Anglo-European College of Chiropractic and medical students studying at the University of Southampton. Such a comparison highlights the differing needs in the anatomical education of health professionals to ensure that their undergraduate education provides a suitable basis for their chosen field of clinical training and practice.

**P9 An anatomoclinical study regarding the anastomoses of coronary arteries.** By A. HASANOVIČ. *Department of Anatomy, University of Sarajevo, Bosnia and Herzegovina.*

The purpose of this study is to examine the existence and clinical importance of anastomoses of the human coronary arteries, especially when the coronary circulation is damaged. The investigations were carried out using 30 human cadaveric hearts from the Department of Anatomy. Additional data on 30 hearts with occlusion of coronary arteries were obtained in cooperation with the Cardiology Department and the Radiology and Oncology Institute of the Clinical Centre in Sarajevo.

Using different methods (dissection, injection-corrosion casts, in vivo coronary angiography) we established the existence of anastomoses of the coronary arteries on our material, and on the human hearts with signs of coronary occlusion we found collaterals which were intracoronary (or homocoronary) and intercoronary. The most frequently occurring collateral pathways are: (1) left to right from the anterior descending to the posterior descending; (2) left to right from the distal circumflex to the distal right coronary; (3) intracoronary collaterals between 2 or more branches of the left coronary artery; (4) right to left interconal. We also found that the collaterals were more numerous with a stronger degree of occlusion, and that there was not always a correlation between the rate of anatomical change and the clinical image.

These investigations are important because contrasting opinions are given in the literature regarding the anastomoses of coronary arteries and their role in collateral circulation. The results should have importance in clinical medicine, especially in the diagnosis and therapy of coronary disease.

**P10 Cells expressing the NG2 chondroitin sulphate proteoglycan: response to axon loss in the developing rat optic nerve.** By K. GREENWOOD, M. BERRY and A. M. BUTT. *Neuroscience Research Centre, GKT School of Biomedical Sciences, King's College, London, UK.*

Antibodies to NG2, a membrane embedded chondroitin sulphate glycoprotein, label significant numbers of cells in the developing and adult rat optic nerve. In culture, NG2 labels O-2A progenitors, which have the capacity to develop into either oligodendrocytes (OLs) or type 2 astrocytes depending on culture conditions. In both adult and developing CNS, NG2+ cells have an oligodendrocyte progenitor cells (OPC) phenotype, co-expressing PDGF$\alpha$R and O4, but not conventional glial markers, such as GFAP, Rip and lectin. In the adult optic nerve, NG2 cells extend processes to subserve nodes of Ranvier, suggesting to us that they may be a specialised mature glial phenotype. Thus, we wished to test the hypothesis that NG2+ cells in the developing optic nerve are OPCs that may develop into OLs or novel adult NG2 cells. Since OPCs are believed to depend on axons for their survival, we investigated the response of NG2 cells to axonal degeneration. Rat pups at postnatal day (P) 1 were deeply anaesthetised using halothane and unilateral enucleation was performed by retinal ablation. Optic nerves were sampled at P4 and P16 for immunohistochemical analysis. There was no loss of NG2 immunolabelling following axonal degeneration, but instead there was an increase in NG2 expression, compared with contralateral nerves, which served as controls. NG2 cells appeared reactive and did not express GFAP or MBP, and were not astrocytes or oligodendrocytes; nor did NG2 cells have the morphological appearance of ED1 labelled microglia or macrophages. The results demonstrate that NG2 cells do not depend on axons for their survival, but rather that they reacted rapidly to axonal loss, by increased NG2 expression and, possibly, increased numbers of NG2 cells. In con-

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clusion, this study indicates either that NG2+ cells are not OPCs or that the latter do not depend on axons for their survival, in contradiction to previous studies. We are currently testing whether NG2 cells express PDGFαR or bind O4, considered diagnostic for OPCs in vivo. One possible explanation for our findings is that NG2 cells and OPCs derive from a common stem cell which diverges in the neonatal brain, the former persisting as adult NG2 cells, whilst the latter become OLs.

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**P11 Sensory peptidergic innervation of the lower urinary tract in young adult and aged rats.** By H. Mohammed and R. M. Santer. *Anatomy Unit, Cardiff School of Biosciences, University of Wales, Cardiff, UK.*

Previous studies have shown that the urinary bladder and ureter of rats has a dense sensory innervation. Sensory neuropeptides such as calcitonin gene related peptide (CGRP) and substance P (SP) have been used as markers for sensory nerves in the lower urinary tract. The aim of this study was to examine the effects of ageing upon sensory nerve densities in the rat urinary bladder and distal ureter. Samples of freshly excised urinary bladder and ureter from 14 terminally anaesthetised Wistar rats, aged 12 wk and 24 mo were used in this study. Immunostaining for CGRP and SP was performed using indirect immunofluorescence. Semiquantitative estimations of nerve densities were made of fibres innervating the urothelium, smooth musculature and blood vessels of the ureter and different regions of the urinary bladder (base, body and dome). The base of the urinary bladder in all rats showed a greater density of innervation compared with the body and dome. CGRP-immunoreactivity (IR) of different regions of rat urinary bladder was unchanged in the aged rats except in the muscle of the base, in which a decrease in nerve density was observed. It was also observed that aged rat bladder had long lengths of CGRP-IR axons in the subepithelial region of the base, but that they were shorter and less dense in the muscle layers. In addition no changes in CGRP-IR nerve density between young and aged rat were observed in the ureter. This study has also revealed that SP-IR axons were smaller and less numerous than CGRP-IR axons but that they had the same overall distribution as CGRP-IR fibres. SP-IR in different regions of the bladder was also affected by age: there were no SP-IR axons observed in the aged urothelial tissue and there was also a reduction in subepithelial varicosities in both the body and the dome of the urinary bladder of aged rats. Moreover, the subepithelial and muscle in aged rat ureters had less immunostaining for SP than in young adult rat ureters. This study has demonstrated that there are differences in sensory innervation of urinary bladder and ureter between young and aged rats. This suggests that the proper functioning of the afferent limb of the micturition reflex may be compromised in old age.

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**P12 No alteration in rat brain neostriatal neuronal densities after 6-hydroxydopamine lesions of the dopamine-containing input fibres.** By A. K. Wright and G. W. Arbuthnott (introduced by A. P. Payne). *University of Edinburgh Centre for Neuroscience, Department of Preclinical Veterinary Sciences, University of Edinburgh, UK.*

When the dopamine input to the neostriatum is destroyed by the application of 6-hydroxydopamine to the medial forebrain bundle of the rat brain, one consequence is a loss of asymmetric synapses on the medium sized densely spiny neurons on the side of the lesion (Ingham et al. *J Neurosci* **18**, 1989). After the lesion striatal neurons also show a loss of spines, which carry the majority of asymmetric synapses, and this parallels the loss of these synapses almost exactly (Ingham et al. *Exp Brain Res* **93**, 1993). However, it has been shown that depletion of dopamine leads to signs of apoptosis in neurons in the striatum (Mitchell et al. *Neurosci* **63**, 1994). Our results cannot distinguish reliably between synaptic loss as a result of remodelling or from cell death. Nevertheless neuronal loss as well as synaptic loss would make a major difference in the therapeutic strategies that are relevant to Parkinson's disease. We therefore undertook to count neurons by unbiased stereological methods in the same area of the striatum in which we had previously counted synapses.

Six animals successfully lesioned with 6-hydroxydopamine injections into the medial forebrain bundle were perfused with 4% paraformaldehyde and 0.5% glutaraldehyde in 0.1 M phosphate buffer at pH 7.4, 26 d after the lesion. Sections were cut through the striatum on a freezing microtome and a full serial series of 60 40 μm sections placed in a multiwell tray. From those sections a set of 10 were chosen in a systematic random fashion and stained for neuronal nuclei with the antibody A60 which recognises a neuronal protein neuN. The total number of neurons in the area of the striatum from which we previously calculated synaptic number was then estimated using the optical dissector on a Neurolucida computer controlled microscopy system. We also estimated the total volume of the striatal area from which the counts came using the Cavalieri method on the same system. The mean count for the cell numbers was $1.91 \times 10^3$ ($\pm 0.12$ S.D.) on the control side and $1.79 \times 10^3$ ($\pm 0.14$) on the lesion side. The small ($\sim 6\%$) difference in total is not statistically significant, and when the cell densities were calculated allowing for the changes in striatal volume which often accompany the lesion, the difference ($\sim 0.7\%$) is even smaller ($297.9 \pm 28.3$ neurons/100 mm$^3$ on the lesioned side versus $310.0 \pm 21.4$ on the control side).

We conclude that cell loss in the lesioned striatum cannot explain the 20% loss of asymmetric synapses that we see after 6-hydroxydopamine destruction of the ascending nigrostriatal dopamine fibres in the rat brain.

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