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THEME THREE PLENARY

Symposium Honouring Dr David Graham

0 - 30
RELATING IN VIVO PATHOPHYSIOLOGY TO NEUROPATHOLOGY AFTER HUMAN HEAD INJURY
JD Pickard, (*Cambridge UK*)

The meticulous clinico-pathological studies undertaken by the Glasgow School of Neuropathology have indicated that various processes may prove lethal or be associated with severe disability and yet it has been difficult to define those processes in life. Examples include cerebral ischaemia, cholinergic dysfunction and the different patterns of neuropathological damage associated with the vegetative state. This problem of linking neuropathology to processes in life is almost certainly a result of the historical limitations of technology available for clinical studies. Recent developments including continuous computerised multi-modality bedside monitoring, functional brain imaging (PET and MR), neuropsychological profiling and *in vivo* 'neuropathology' using voxel based MR morphometry are beginning to bridge the gap between *in vivo* pathophysiology and neuropathology.

0 - 31
THE CONCEPT OF SECONDARY AXOTOMY: THE BASIC SCIENCES.
WL Maxwell (*Glasgow, UK*)

In terms of treatment of patients after blunt head-injury, perhaps the most significant finding over the last decade has been that the majority of injured axons enter a pathological cascade of post-traumatic events which occur over hours or days after the initial insult. A discrete and characteristic series of axonal pathologies leading to what is now termed "secondary axotomy" have been documented. These will be reviewed and a model of the effects of injury to the axolemma developed. Recognition that there is a time course extending over several hours before secondary axotomy occurs allows hope for development of therapies which may ameliorate the severity of pathology and improve outcome for the patient. An assessment of animal experiments designed to investigate potential therapeutic targets will be undertaken and their relevance and/or efficacy assessed. Recent work has indicated that there are ongoing pathologies within the brain of patients who survive blunt head-injury. These will be reviewed and an improved experimental plan for the study of either patients or experimental animals after traumatic brain injury suggested.

Dr Graham has been a key contributor to the development of the ideas indicated above. His contribution will be illustrated thoughout the presentation.

SESSION 3.1*:* Inflicted Head Injury in Infants

0 - 32
INFANT HEAD INJURY - WHAT'S NEW?
H Whitwell (*Sheffield, England*)

Neuropathological literature on head injury in infants has been scant. Many textbook reviews include older papers with relatively few cases, poor clinical information and limited neuropathological investigation. The advent of new markers to identify axonal injury in the 1990s have led to a better neuropathological understanding of the processes involved in traumatic brain injury including that of infants. In 2001, the largest series of fatal cases of inflicted head injury in infants and children was published. Age related patterns of brain injury were demonstrated and hypoxic-ischaemic injury was the major pathology rather than diffuse traumatic axonal injury as had previously been thought. Of particular significance was the finding of focal cranio-cervical injury within the brain stem sug-

gestive of stretch injury to the neuro-axis, which could have the potential of causing the apnoea.Within this series of cases was a small number of young infants who showed no evidence of impact, in particular, no skull fractures or scalp bruising with a common presentation of collapse, brain swelling with hypoxic-ischaemic changes and thin film subdural haematomas. Further study to look at the pathology in some of these cases has raised the issue of the aetiology of thin film subdurals in the presence of severe brain swelling with hypoxic damage and question whether or not in the young infant these are necessarily traumatic in nature.

0 - 33
INFLICTED HEAD TRAUMA IN INFANTS AND YOUNG CHILDREN - MEDICOLEGAL ASPECTS
RW Byard (*Adelaide, Australia*)

The evaluation of inflicted head trauma in infants and young children is often complex and highly contentious. Difficulties arise at all levels and may include inadequate initial investigations, poorly documented autopsy findings and contradictory interpretations of lesions when found. Head injuries in infants are particularly difficult to evaluate given a paucity of external findings, unique anatomy and poorly understood mechanisms of initial injury. Double blind trials involving head injury infants are not possible, and extrapolation from animal and mechanical models has been criticised. The older literature often relied on uncorroborated histories of injury, and extrapolation is often made from small numbers of cases. The role of the expert in court is unenviable. Opinions are requested on the degree of force required to cause injuries and on possible mechanisms. Particular constellations of injuries including retinal haemorrhages and subdural haematomas may be used to support a diagnosis of shaking, however, secondary hypoxic damage and immunization have been cited as possible causes of similar lesions. This often results in protracted courtroom debate over possible contributions of factors to the fatal episode. If an infant or young child has died from a head injury that is not explainable by carers or by a close analysis of the environment where the child was injured, inflicted injury must be strongly suspected. A practical approach for pathologists called before the courts is to use the general term 'blunt craniocerebral trauma' and to avoid as much as possible the use of speculative theories or hypotheses in evolution.

0 - 34
CLINICAL DIAGNOSIS AND MANAGMENT OF INFLICTED CHILDHOOD NEUROTRAUMA: NAVIGATING THE CONTROVERSIES.
AC Duhaime (*Lebanon, USA*)

The understanding of non-accidental head injuries in infants and young children has evolved over the past decades, but still is incomplete. Clinicians and families find themselves in an emotionally charged arena in which opinions may be strongly held and the medicolegal implications are enormous. This talk will review the history and science behind some of the prevailing theories of differential diagnosis, management, and outcome, with an emphasis on head injury biomechanics, the evidence for age-dependent differences in injury response, and clinical data regarding children with accidental and non-accidental trauma. What has been clearly delineated will be contrasted to areas which are still largely untested. A practical scheme for approaching this area in the clinical sphere will be provided.

0 - 35
EXPERIMENTAL MODELS OF REPEAT HEAD INJURY IN THE DEVELOPING BRAIN
M Prins (*Los Angeles, USA*)

It is unknown what proportion of head injuries are repeat brain injuries, but most reported cases involve male adolescents (Cantu and Voy, 1995), making it a relevant pediatric subpopulation. While nation wide incidence of repeat head injuries is unknown, smaller scaled studies suggest that among high school football players alone, 34.9% experienced 1 previous concussion and 20% 2 or more concussions (Collins et al., 1999; Langburt et al., 2001). The majority of reported injury cases are sports related (Collins et al., 1999; Langburt et al., 2001; Proctor and Cantu, 2000; MMWR, 1997; Saunders and Harbaugh, 1984), presenting a real threat to children and young adults. A unique type of repeat head injury found among only the youngest victims is Shaken baby syndrome. Physical abuse is the most common cause head injury among children under 1 year of age in 1985 (Billmire and Myers, 1985) and is the leading cause of severe traumatic brain injury in children under 2 years of age (American Academy of Pediatrics, 2001). This review explores the current experimental repeat head injury models designed to address issues of pediatric head injury and the progress made towards neuroprotection.

SESSION 3.2*:* Axonal Injury

0 - 36
A REVIEW OF THE COURSE AND CONSEQUENCES OF TRAUMATIC AXONAL INJURY
JT Povlishock (*Richmond, USA*)

Axonal injury is a common feature of traumatic brain injury in animals and man. Although it was initially thought that the axons were torn at the moment of impact, more contemporary research has shown otherwise. Animal and human studies have shown a complex repertoire of intraaxonal pathology that leads to axonal damage and disconnection over periods ranging from minutes to several hours postinjury. Some axons reveal overt cytoskeletal and axolemmal abnormalities that elicit local axonal failure without the formation of reactive axonal swelling. Others reveal subtle cytoskeletal changes that lead to impaired axonal transport, local swelling, and detachment. Lastly, some axons show even more subtle and transient perturbation associated with recovery. These different forms of intraaxonal pathology are most likely correlated with fiber size, degree of myelination, and injury type. These structural changes are associated with electrophysiological abnormalities linked to abnormal compound action potentials in both non-myelinated and myelinated fibers. Following axonal disconnection, anterograde, Wallerian degeneration, and deafferentation occur, and they precipitate various adaptive or maladaptive neuroplastic changes that are paralleled by specific structural, functional, and behavioral responses. Retrograde to the axonal injury, the sustaining soma shows transient cellular perturbation reflected in the upregulation of stress related proteins and the subsequent alteration of protein translation. These cellular responses do not necessarily progress to overt atrophy and/or apoptotic/necrotic cell death. This presentation will provide an overview of these injury-induced axonal events, as well as their related anterograde and retrograde responses, while also discussing their therapeutic modulation. (Supported by NIH grant NS-20193)

0 - 37
AXON DEGENERATION IN A MODEL OF WHITE MATTER ISCHEMIA
VH Perry (*Southampton, UK*)

Traumatic and ischemic injury of the central nervous system result in significant amounts of damage to the fibre tracts of the CNS. The molecular events that underlie axon degeneration resulting from such injuries are poorly understood. The discovery of the mutant Wld mouse, in which Wallerian degeneration is dramatically delayed, has shown us that axon degeneration in wild-type mammals is an active auto-destructive process akin to programmed-cell-death. The mutation in the Wld mouse involves a component of the ubiquitin-proteosome pathway implicating this pathway in axon degeneration. To further study the molecular pathways involved in axon degeneration in ischemia we have developed a new *in vivo* model. The focal injection of endothelin-1 into the spinal cord of the rat generates a local ischemic region around axons distant from their cell bodies of origin and the axon terminals. This ischemic lesion in the white matter results in a minor inflammatory infiltrate when compared to that seen in adjacent grey matter. Of particular interest is that axon injury, as revealed by immunocytochemistry for APP, is dramatically delayed relative to the degeneration of adjacent neuronal cell bodies. Further study of the processes of ischemia induced axon degeneration offers new therapeutic opportunities with a potentially longer therapeutic window relative to neuroprotection at the cell soma.

0 - 38
FINITE ELEMENT MODELLING OF IMPACT-INDUCED AXONAL INJURY IN SHEEP
R Anderson (*Adelaide, Australia*)

This paper describes a numerical study of axonal injury in the anaesthetised sheep. Sheep were subjected to an impact to the left lateral region of the skull and were allowed to survive for four hours after the impact. The axonal injury was identified using immunohistological methods and was mapped and quantified. Axonal injury was produced consistently in all animals. Commonly injured regions included the sub-cortical and deep white matter, the hippocampi and the margins of the lateral ventricles. The degree of injury was closely related to the peak impact force and to kinematic measurements, particularly the peak change in linear and angular velocity. There was significantly more injury in animals receiving fractures. A three-dimensional finite element model of the sheep skull and brain was constructed to simulate the dynamics of the brain and skull during the impact. The model was used to investigate different regimes of material properties and boundary conditions, in an effort to produce a re-

alistic model of the skull and brain. Model validation was attempted by comparing pressure measurements in the experiment with those calculated by the model. The distribution of axonal injury was then compared with the output of the finite element model. The finite element model was able to account for approximately thirty per cent of the variation in the distribution and extent of axonal injury, using von Mises stress as the predictive variable. Logistic regression techniques were used to construct sets of curves that relate the extent of injury, to the predictions of the finite element model, on a regional basis.

0 - 39
RELATIONSHIP OF DECREASED BRAIN TISSUE VISCOELASTICITY TO TRAUMATIC AXONAL INJURY FOLLOWING TRAUMATIC BRAIN INJURY
K Darvish, J Stone (*Richmond, USA*)

Traumatic axonal injury (TAI) contributes to the morbidity and mortality following TBI. TAI is thought to involve initial mechanical perturbation of axons, leading to local degeneration and delayed axotomy. Of interest in studying TAI is the ability to assess quantifiable measures of mechanical tissue disruption in relation to TAI. One such measure, known as tissue viscoelasticity (TVE), reflects the intrinsic composition and structure of tissue. In the current investigation, we explore whether TVE decreases following TBI, and whether alteration in TVE is found in relation to TAI. Male Sprague-Dawley rats underwent impact acceleration TBI. One group of animals were immediately euthanized and TVE was determined by step and hold indentation technique with a 0.78 mm flat indentor. Indentation occurred to a depth of 2 mm over 50 milliseconds and was performed at the brainstem pontomedullary junction (PmJ) and pyramidal decussation (PDx). Least square fit of whole loading time history to a non-linear viscoelastic model analysis was performed. A separate group of animals survived 3 hours post-injury, with APP immunostaining performed to identify TAI. In TBI-injured rats, the instantaneous elastic response was reduced 70% at the PDx and 40% at the PmJ, with a uniform 30% reduction in relaxation compared to SHAM injured controls. A 10-fold increase in TAI occurred at the PDx vs. the PmJ. The current investigation reveals brain TVE is altered following TBI in relation to sites of TAI. This study is the first step in developing a finite element model of TBI that incorporates pathobiologically meaningful datasets.

0 - 40
TRAUMATIC BRAIN INJURY EVOKES DIFFUSE AXONAL INJURY IN THE SPINAL CORD
A Büki, D Szellár, A Zsombok, J Lückl, J Pál, T Dóczi, JT Povlishock (*Pecs, Hungary; Richmond, USA*)

While the bulk of evidence demonstrates that traumatic brain injury (TBI) evokes diffuse axonal injury (DAI) in the brain, the occurrence of DAI in the spinal cord (SC) following moderate/severe TBI has been less extensively investigated. In this study, we assessed DAI in the cranio-cervical junction (CCJ), the cervico-thoracic (C-T) and the thoraco-lumbar (T-L) SC in a rodent model of impact acceleration TBI. Rats were transcardially fixed with aldehydes at 2, 6, and 24 hours post-injury ($n = 30$). Vibratome sections were reacted with antibodies against the calpain and caspase-mediated breakdown products of brain spectrin as well as classical markers of DAI such as RMO-14 and APP that target cytoskeletal abnormality and impaired axonal transport respectively. Consistent with previous observations in this model, the CCJ demonstrated numerous diffusely injured axons in the cerebrum and brain stem. However, an unanticipated finding was the presence of DAI in the SC down to the TH-L level. The damaged axons appeared vacuolated and swollen and at 24 hours, most axonal bulbs were disconnected. These observations demonstrate that acceleration-deceleration head injury evokes widespread DAI also involving remote regions of the SC. These findings may be of relevance to human TBI, mandating their evaluation in both the adult and pediatric population. Further, in animal investigations of TBI, the current study also suggests that SC damage should be considered when evaluating post TBI functional recovery. Lastly, this study begs the question whether repeated minor axonal injury can also play a role in the development of spondylotic myelopathy.

SESSION 3.3*:* Trauma and Neurodegeneration

0 - 41
THE LINK BETWEEN CELLULAR CHANGES ASSOCIATED WITH AXONAL INJURY AND ALZHEIMER'S DISEASE
JC Vickers, TC Dickson, JA Chuckowree, RS Chung, AK West, MI Chuah (*Hobart, Australia*)

The link between ß-amyloid plaque pathology and neuronal degeneration is a central issue in Alzheimer's

disease research. We have proposed that ß-amyloid plaque formation causes structural perturbation of axons and that it is the aberrant regenerative response of neurons to injury that ultimately leads to neurodegeneration. In order to understand how nerve cells respond to injury, we have examined both *in vivo* and *in vitro* models of neural trauma. The *in vivo* model involves structural injury to the mature rat brain induced by insertion of a 25 gauge needle. The *in vitro* model utilises transection of axonal bundles of rat cortical neurons maintained in long term culture (21 days *in vitro*). Short term changes following injury involved stereotypical 'reactive' alterations in axons, leading to the disruption of microtubules as well as ring- and bulb-like neurofilamentous accumulations. Identical axonal pathology was associated with plaque formation in the earliest stages of Alzheimer's disease. Study of the experimental models indicated that the reactive phase of the axonal response to injury is subsequently followed by substantial local neurite sprouting, involving key proteins associated with initial neurite development. This may indicate why aberrant regenerative sprouting is a feature of brain pathology in the later stages of Alzheimer's disease. The experimental models are being utilised to investigate agents which either promote or inhibit the intrinsic neuronal response to injury, a potential therapeutic approach for neurodegenerative disease as well as acquired forms of CNS injury.

0 - 42

APOLIPOPROTEIN E AND BRAIN INJURY: GENETICS, BIOLOGICAL MECHANISMS AND POTENTIAL FOR THERAPY.

JAR Nicoll (*Southampton, UK*)

Clinical studies have provided evidence that genetic factors may be responsible for some of the previously unexplained variability in outcome after acute brain injury. Specifically, the e4 allele of the apolipoprotien E (APOE) gene is associated with poor outcome after traumatic brain injury, spontaneous intracerebral haemorrhage, cardiac bypass surgery, cerebral ischaemia after cardiopulmonary resuscitation and in boxing; in subarachnoid haemorrhage the evidence is conflicting; the effect appears not to influence outcome from ischaemic stroke. Multidisciplinary approaches including studies of human neuropathology and genetically modified mice have provided evidence of isoform specific differences in various functions in which apoE participates. These include differences in neuronal protection, neuroinflammation, repair and remodeling, as well as vascular and haematological factors. Clearer understanding of these processes may lead to better prediction of outcome in patients with brain injury and might possibly identify targets for therapy to reduce the severity of brain damage or to promote the capacity of the brain for repair.

0 - 43

NEUROPROTECTIVE EFFECTS OF SAPP? ADMINISTRATION FOLLOWING TBI

C Van Den Heuvel, E Thornton, P Blumbergs, R Vink (*Adelaide, Australia*)

Amyloid precursor protein (APP) has previously been shown to increase following traumatic brain injury (TBI). While a number of investigators assume that this may be deleterious to outcome, soluble amyloid precursor protein alpha (sAPPα) is a product of the non-amyloidogenic cleavage of amyloid precursor protein (APP) that has previously been shown *in vitro* to have many neuroprotective and neurotrophic functions. However, no study to date has addressed whether sAPPα may be neuroprotective *in vivo*. The present study examined the effects of *in vivo*, post-traumatic sAPPα administration (icv) on neurological outcome, cellular apoptosis and axonal injury following impact/acceleration TBI in rats. Treatment with sAPPα significantly improved motor outcome compared to vehicle treated controls as assessed using the rotarod task. Immunohistochemical analysis using antibodies directed toward caspase 3 showed that post-traumatic treatment with sAPPα significantly reduced the number of apoptotic neurones within the hippocampal CA3 region and within the cortex 3 days after injury compared to vehicle treated animals. There was no significant difference in the numbers of apoptotic neurons at this time point between the sham and sAPPα treated groups. All vehicle treated animals demonstrated axonal injury (AI) within the corpus callosum. In contrast, sAPPα treated animals demonstrated a significant reduction in axonal injury within the corpus callosum between 1 and 7 days post-injury. Our results demonstrate that administration of sAPPα *in vivo* improves functional outcome and reduces neuronal cell loss following severe diffuse traumatic brain injury in rats.

0 - 44

INFLUENCE OF APOE GENOTYPE ON SECONDARY INSULTS AFTER TRAUMATIC BRAIN INJURY

I Liaquat, I R Piper, LT Dunn, G Murray GM Teasdale (*Glasgow; Edinburgh UK*)

Background: The $\varepsilon 4$ allele is associated with poor outcome after TBI. Previous studies have suggested that it may also be associated with larger traumatic intracerebral haematomas and more frequent hypotensive insult (1, 2). We have investigated the effects of APOE genotype on secondary insults after TBI.

Methods: All patients with data on APOE genotype, GOS at 6 months, minute-by-minute monitoring of arterial pressure, CPP, ICP and SaO2 were included in the study. Edinburgh Browser Software was used to quantify the duration of physiological insults. Exploratory data analysis found a peak in the distribution of the percentage of monitoring time spent at given insult levels occurred at 20% of monitoring time. We chose to dichotomise each secondary insult parameter into "few" versus "many" insults based on this 20% cut-off.

Results: A total of 149 patients had monitoring data for arterial pressure, CPP, ICP and SaO2. The mean and median duration as a percentage of monitoring time for hypotensive insults was (31% and 25%), for CPP was (17% and 15%), for ICP was (25% and 11%) and for SaO2 was (10% and 6%) respectively. Patients with "Many" CPP and ICP insults were twice as more likely to die compared to "Few" insults group, $\chi^2 = 7.35$, 13.13, d.f. 2 ($p =< 0.05, < 0.01$, respectively). Formal analysis of patients monitored in the first 48 hours after admission failed to show a significant positive association between APOE genotype and "Many" insult burdens for either ICP or BP (χ^2 d.f.1 $= 0.111$; $p = 0.739$ for ICP and χ^2 d.f.1 $= 1.764$; $p = 0.184$ for BP).

Conclusion: Using this dichotomy approach the APOE genotype does not influence the secondary insults after TBI. Further studies using different methodologies to quantify secondary insult burden are required to validate the findings of this study.

0 - 45

REBUILDING THE BRAIN AFTER TRAUMATIC BRAIN INJURY: A JOURNEY FROM CELL DEATH TO PLASTICITY AND REGENERATION

TK McIntosh, N Royo, N Marklund, V Conte, C Fulp, H Thompson, S Shimizu, J Schouten, A Bakshi, L Longhi, H Laurer, P Lenzlinger, K Saatman, R Raghupathi, MS Grady, J Eberwine, JQ Trojanowski, DI Graham (*Philadelphia, USA; Glasgow, UK*)

To date, research studies designed to develop an effective treatment for traumatic brain injury (TBI) have not translated well into successful clinical trials. There is accumulating evidence for the involvement of both passive and active cell death processes in both experimental models of TBI as well as in the clinical setting. The pioneering work of Dr. David I. Graham and colleagues has set the stage for much that is currently known concerning mechanisms of cell death and dysfunction following TBI. It is these seminal studies that have laid the groundwork for novel strategies that protect against both acute and chronic cell death. In addition, the development of cell replacement therapies offer an alternative or complementary therapeutic modality, and recent experimental studies have identified a number of candidate cell lines for engraftment into the injured brain. In addition, the characterization of the neurogenic potential of specific regions of the adult brain and the elucidation of the molecular controls underlying regeneration or repopulation may allow for the development of neuronal replacement therapies that does not rely on transplantation of exogenous cells or infusion of neuroprotective pharmacotherapy. Dr. Graham's lifelong work and philosophy remains an inspiration to contemporary and future researchers dedicated to an understanding of the pathobiology and treatment of human TBI.

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