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A12–NOICEPTION IN VERTEBRATES: ANATOMY, NEUROPHYSIOLOGY, GENOMICS AND BEHAVIOUR

Organised by L.U. Sneddon for the Neurobiology Group

A12.1 Sensory properties of articular afferents following arthritis in the chicken

M.J. Gentle, Roslin Institute, UK

Birds, like humans, are bipeds and not only suffer the attendant mechanical problems imposed by their gait but can also develop articular gout. Arthritis especially in heavy breeds of poultry is common and as such they are good models in which to study the relationship between peripheral nociceptors and arthritic pain. The chicken has large numbers of group IV and group III afferent fibres in the joint with nociceptive functions physiologically similar to afferents identified in the mammalian joint capsule. In the experiments to be described the ankle joint was subjected to 3 different arthritic conditions using intra-articular injection to mimic either acute gout (sodium urate crystals), bacterial infection (Freund's complete adjuvant) or *Mycoplasma* arthritis (live *Mycoplasma* culture). Electrophysiological recordings from single afferent group IV and group III fibres showed that all 3 diseases resulted in nociceptor sensitisation but the extent of this sensitisation as well as the fibres affected differed in each arthritic condition. Following *Mycoplasma* arthritis the absence of any clear relationship between synovial pathology and nociceptor sensitisation in the acute stage of the disease, and the normal properties of these nociceptors during chronic prolonged synovitis, illustrates the difficulties of predicting nociceptor sensitisation on the basis of histopathological findings. This demonstrates the complex relationship between the responses of peripheral nociceptors and the disease process and the different patterns of nociceptor sensitisation suggest a peripheral neural basis for both qualitative and quantitative differences in the pain experienced.

A12.2 Physiology and pathophysiology of nociceptors in mammalian skin

B. Lynn, University College London, UK

This presentation will review selected aspects of nociceptor behaviour in mammalian skin. Cutaneous nociceptors are not all alike. Many C-fibre nociceptors are polymodal-responding to heat, mechanical and chemical stimuli. Many A-fibre nociceptors are mechanical nociceptors, only responsive to strong mechanical pressure. But several other types of nociceptor are found. Some C-fibre nociceptors show responses only to pressure, while others (heat nociceptors) only respond to heat and chemical irritants. A significant group of C-fibres only respond to chemical stimuli (the silent or sleeping nociceptors). Nociceptor properties change with animal size, with for example pressure thresholds increasing as body size increases. Conduction velocity can also vary widely, particularly in the A-fibres where some nociceptive neurones conduct in the A-beta range. In the presence of tissue inflammation nociceptors sensitize and also tend to become polymodal (if not polymodal already). For example C-heat nociceptors become sensitive to pressure. Injury to nerve trunks produces a different set of changes in properties and mechano-transduction, normally seen only at nerve endings in the tissue, is now seen at the lesion site. The underlying mechanisms causing changes in nociceptor properties after inflammation or trauma will be discussed.

A12.3 Evolution of nociception: evidence from lower vertebrates

L.U. Sneddon, University of Liverpool, UK

Comparative analysis of nociception may provide insights into the evolution of nociceptive systems. By examining results from lower vertebrates, we can compare and contrast properties of nociceptors with higher vertebrates including humans. Research investigating nociception in the lamprey, elasmobranch (cartilaginous) fish and teleost (bony) fish shall be reviewed and

compared with anatomical and physiological properties of nociceptors in avian and mammalian models. The primitive lamprey has no myelination and receptors were identified that respond to damaging stimuli. Anatomical and electrophysiological investigations on several species elasmobranch fish have failed to find any evidence of nociception yet polymodal and mechanothermal nociceptors have been identified in a bony fish. This may represent an evolutionary difference between these lineages. Many of the electrophysiological properties of the teleost nociceptors, such as conduction velocity, thermal threshold, action potential shape and amplitude were similar to characteristics of mammalian nociceptors. Teleost nociceptors were slower in duration but this may be due to the temperature difference between these cold-blooded animals and mammals. Anatomically, the teleost fish possessed C fibres and A-delta fibres of the same diameter to those found in higher vertebrates although C fibres only comprised 4% fibre type whereas higher vertebrates have 50–65%. Hypotheses shall be presented to discuss the possible causes of the different properties between lower and higher vertebrates. Future studies will examine gene expression changes associated with nociception in fish to examine evolutionary conserved mechanisms at the molecular level.

A12.4 Opioid research in amphibians: an alternative pain model yielding insights on the evolution of opioid receptors

C.W. Stevens, OSU-Center for Health Sciences, Tulsa, Oklahoma, USA

Over the last decade, we established an alternative, amphibian model for the investigation of opioid analgesics. Using the most common frog in North America, *Rana pipiens*, an antinociceptive assay based on a weak acid stimulus to evoke a wiping response produced novel data on opioid analgesic effects comparable to that observed in mammals. In humans and other mammals, opioid analgesia is mediated by three types of GPCRs; *mu*, *delta*, and *kappa* opioid receptors. There is little known about opioid-like receptors mediating analgesia in earlier-evolved vertebrate species. Behavioral studies from our laboratory using amphibians demonstrated that opioid analgesia is mediated by type-selective opioid agonists and data from radioligand binding sites suggested the expression of the three types of opioid receptors. We recently cloned and sequenced three types of opioid-like receptors in *Rana pipiens*, *rpMOR*, *rpDOR*, and *rpKOR*. These receptors are 78–84% identical to mammalian receptors and phylogenetic analysis places them between fish opioid-like receptors and mammalian orthologues. Within species, *mu*, *delta* and *kappa* opioid-like receptors are significantly more divergent in mammals than non-mammals. This supports the hypoth-

esis that opioid receptors in earlier-evolved vertebrates are less selective. Novel comparative analysis of all vertebrate opioid receptor sequences suggest conservation of specific receptor domains correlated to type of opioid receptor. These findings highlight the importance of alternative models in biomedical research and the advances made in understanding nociception in non-mammalian species. Supported in part by the National Institutes of Health, USA, and the Oklahoma Center for Advancement of Science and Technology.

A12.5 Properties of mammalian nociceptive primary afferent neurons

S.N. Lawson, X. Fang & L. Djouhri, University of Bristol, UK

Nociceptive primary afferent neurones in dorsal root ganglia (DRGs) differ from non-nociceptive neurones in many ways. It is important to find ways of identifying neurones that are nociceptive even, or perhaps especially, when it is not possible to test their sensory properties. Unfortunately a number of these properties change when neurones are isolated *in vitro*. We have therefore studied the properties of nociceptive neurones *in vivo* in deeply anaesthetised guinea pig and rat primary afferent neurones in DRGs. Intracellular recordings in DRG neurones were made with dye filled microelectrodes. Conduction velocity of the dorsal root was measured and the properties of the dorsal root evoked intracellular action potential was analysed later, offline. After sensory receptive properties of the neurone were established, dye was injected into the neurones enabling subsequent immunocytochemistry on the same neurones. The properties found to be different in nociceptors compared with low threshold mechanoreceptors include: slower conduction velocity, long action potential duration, large action potential overshoot, long afterhyperpolarisation, expression of substance P, CGRP, trkA (the high affinity NGF receptor), binding of the lectin IB4, and certain Na channel subunits. During inflammation, some of the properties of nociceptive neurones *in vivo* are altered, with lowered thresholds, increased conduction velocity, shorter duration action potentials and afterhyperpolarisations, increased rate at which fibres can fire action potentials, and expression of certain Na channel subunits. Thus both the kinetics of active membrane properties and the excitability of the nociceptive neurones are increased. NGF plays an important role in many of these changes.

A12.6 The organisation of motor responses to noxious stimuli

R.W. Clarke & J. Harris, School of Biosciences, University of Nottingham, UK

Limb withdrawal reflexes were first characterised by Sherrington at the beginning of the 20th century (*J.Physiol.* **40**, 28–121, 1910). His work suggested that noxious stimuli evoke a reflex that is more or less the same irrespective of the location of the stimulus, *i.e.* excitation of all flexor muscles in the stimulated limb, with concomitant inhibition of all extensors. More recent studies by Schouenborg (*e.g. News Physiol.Sci.* **9**, 261–265, 1994) indicate that each hind limb muscle has an excitatory receptive field overlying the area withdrawn by contraction of that muscle, leading to the suggestion of a ‘modular’ organisation of withdrawal reflexes. In this view the total movement generated by a noxious stimulus is the result of collective activation of reflexes to those muscles that have excitatory receptive fields overlapping the point of stimulation. A corollary of this idea is that there may be differential control of reflexes to individual muscles. Recent results from our laboratory provide several examples of fractionated control of withdrawal reflex pathways. In studies on reflexes evoked by stimuli applied at the toes *versus* responses elicited from the heel in the rabbit, we have found differences in:– (i) the body regions from which reflexes can be sensitized by acute and chronic noxious stimuli; (ii) tonic descending control of reflexes and their sensitivity to anaesthesia; and (iii) the sensitivity of reflexes to cannabinoids. We are now beginning to assess what this may mean in terms of the movements generated by noxious stimuli using motion analysis.

A12.7 Pathophysiology of Hyperalgesias

H.O. Handwerker, University of Erlangen/Nuernberg, Germany

Hyperalgesias cannot be explained by one single peripheral or central mechanism. In particular, the simple assumption that hyperalgesia is a linear consequence of the sensitization of a uniform group of primary afferent nociceptors is untenable in the light of the diversity of the phenomenon. Different forms of hyperalgesia are mediated by quite different peripheral and central nervous mechanisms. For discussing hypotheses on pathophysiology it is important to distinguish between hyperalgesias according to: 1. neuropathic or inflammatory origin; 2. extension only to the primary zone of a trauma or also into a secondary zone; 3. stimulus modality and quality, *e.g.* thermal or mechanical; and 4. stimulus form and dynamics. In experimental models applied to human volunteers it has been shown that different forms of trauma induce different patterns of

hyperalgesia. Models will be discussed employing topical application of capsaicin to small skin patches, burn lesions, and lesions induced by freezing a skin site. Microneurography, the percutaneous recording of single nociceptor units from skin nerves of awake, cooperating human volunteers contributed another dimension to this analysis by allowing correlation of the input from primary afferents directly to experiences of hyperalgesia. On this basis, hypotheses on the neuronal mechanisms underlying hyperalgesias have been developed which take into account that these sensory phenomena depend on a complex interplay of peripheral and central pathophysiological mechanisms.

A12.8 Behaviour based pain assessment in laboratory animals

J.V. Roughan & P.A. Flecknell, University of Newcastle upon Tyne, UK

Implementing methods for assessing animal pain is not only desirable but an ethical and legal requirement for researchers conducting experiments liable to cause pain. Guidelines on assessment exist, but the techniques currently available are highly subjective and most have not been validated, so they probably fail to accurately quantify animal pain. Without validated methods we cannot reliably assess pain or judge analgesic requirements. This has contributed to the current lack of analgesic dosing information for many laboratory species. Assuming pain will cause changes in the normal behaviour pattern, and providing any non-specific effects of the analgesics upon behaviour are eliminated, the magnitude of these changes and the extent of their attenuation by analgesic therapy can be used to estimate both pain intensity and the efficacy of treatment. Working on this assumption, and utilising animals undergoing surgery as part of other projects, our work has identified some key activities that can be used to assess pain following different surgical procedures (midline laparotomy, bladder surgery, adrenalectomy). These are back-arching, fall/stagger, writhe and twitch. Since the occurrence of these activities is not age or strain specific, and assessments have been successfully implemented by inexperienced staff, we believe we have made significant progress in developing a practicable technique for assessing post-operative pain in rats. Validation of this type has rarely been conducted successfully before. We are currently determining whether a similar approach can be used to assess post-operative pain in mice and rabbits and hope to present some preliminary data at the meeting.

A12.9 A translational approach to pain research in animals

A.M. Nolan & S. Dolan, Institute of Comparative Medicine, University of Glasgow, UK

Experimental animal models of inflammatory and neuropathic pain have helped provide a basis for better understanding of pain, however, these studies are often limited in addressing the diverse nature of clinical pain. Recently, there has been a move to develop animal models more representative of clinical pathological conditions. In an attempt to address this issue, we have characterised two clinical models of inflammation in sheep: a naturally-occurring inflammatory disease model, 'footrot', and a model of post-surgical pain and inflammation, and compared these to an experimental model of inflammation (intradermal carrageenan). Significant, long-lasting mechanical hyperalgesia was observed in sheep with 'footrot', a bacterial infection of the digital tissues. In contrast, carrageenan treatment induced only short-lasting hyperalgesia (hours). Cellular mechanisms of inflammatory pain and hyperalgesia are intimately linked with neuronal plasticity in spinal cord, and consequently with altered gene expression. Preliminary work on spinal cord metabotropic glutamate receptors (mGluRs) has demonstrated that clinical inflammation is associated with a markedly different qualitative pattern of gene expression in spinal cord than that induced by experimentally-induced inflammation. Furthermore, each clinical model displayed a distinct temporal alteration in the pattern of mGluRs expression. Thus, we have evidence to support the assertion that differences exist between clinical and experimental inflammatory pain, both in behavioural consequences and in the molecular mechanisms underlying these conditions, supporting the need to integrate basic and clinical pain research and to study animal models more representative of clinical pathological conditions.

A12.10 Avian mucosal nociceptors: responses to gaseous chemical stimulation

D.E.F. McKeegan, Roslin Institute, UK

The physiological characteristics of trigeminal receptors responding to gaseous chemical stimulation have not been well described. We have recently demonstrated the presence of polymodal nociceptors responding to gaseous chemical stimulation in the nasal mucosa of the hen (*Gallus domesticus*), providing the first chemical thresholds and stimulus response curves for this type of receptor. This study compared the properties of chemically sensitive nociceptors in the nasal and buccal epithelia recorded from either microelectrode stabs of the trigeminal ganglion (nasal mucosa) or microdissected twigs of

the nasopalatine nerve (palatine mucosa) in urethane anaesthetized hens. Slowly and rapidly adapting nasal and palate mechanoreceptors exhibiting a range of von Frey thresholds were identified using a mechanical search stimulus (gentle probing with a glass rod). A subset of these (classified as polymodal nociceptors) exhibited chemical sensitivity when exposed to noxious levels of ammonia gas, acetic acid vapour or carbon dioxide applied via either a cannula attached to a gas tight syringe or a purpose built gas delivery system. The magnitude and specificity of responses varied between epithelial areas and between individual receptors – some responded to only one of the gases applied while others responded to all three. In general, response thresholds were higher in buccal nociceptors compared to nasal receptors. These results demonstrate buccal afferent nociceptive responses to gaseous chemical stimulation and highlight different sensitivities to potentially painful chemical stimulation between mucosal regions.

A12.11 Glutamate receptor interactions with adapter proteins in chronic pain states

E.M. Garry & S.M. Fleetwood-Walker, Royal (Dick) School of Veterinary Studies, University of Edinburgh, UK

Chronic pain resulting from various forms of injury is sustained by central neuronal sensitisation. The pain arising from nerve injury (neuropathic pain) is however, resistant to centrally acting analgesics, while inflammatory pain responds well. New research suggests molecular differences in how spinal glutamate receptors are organised in complexes with partner proteins and this may explain the distinction in central nervous system (CNS) processing when faced with either type of pain. Both neuropathic and inflammatory pain mechanisms involve NMDA and AMPA types of glutamate receptor. The NMDA receptor forms a complex of proteins with signalling and structural functions, and is crucial in various forms of neuronal plasticity both in spinal cord and in other parts of the CNS. Membrane associated guanylate kinase MAGUK proteins (PSD-95/SAP90, Chapsyn-110/PSD-93, SAP102 and hDlg/SAP97), link the NMDA receptor to an array of signalling/scaffolding proteins, as well as cytoskeletal elements and regulatory kinases and phosphatases. Recently, spinal PSD-95 and PSD-93 have both been shown to play important roles in the plasticity that underlies chronic pain, but show some striking differences in their mode of action. AMPA-type glutamate receptors are translocated to synaptic sites following sensitising stimuli and have their own array of adapter proteins and secondary partners. These interactions also play an essential part in the mechanisms of spinal cord sensitisation in chronic pain.

Elucidation of the distinct roles of different NMDA and AMPA receptor-interacting proteins in various chronic pain states may provide new molecular models of pain states and potentially generate new targets for therapeutic intervention.

A12.12 Stimulus transduction and encoding by mammalian primary sensory neurones

C. Belmonte, E. de la Peña & F. Viana, Instituto de Neurociencias de Alicante, Spain

The mechanisms for transduction by sensory nerve endings of low and high intensity mechanical forces and cold are not fully understood. We recorded the electrical activity of single nerve endings in the cornea of the eye and performed calcium imaging combined with intracellular recording in trigeminal ganglion (TG) neurones in culture, and in the ganglion 'in vitro' and 'in situ'. Cell volume increases elicited by hypo-osmotic solutions evoked $[Ca^{2+}]_i$ rises in 80% of TG neurones. $[Ca^{2+}]_i$ rises were rapid and prominent in neurones with fast, short-lasting action potentials (AP) while they were slow and less pronounced in neurones with slow-rising, long duration APs. *In vivo*, fast AP neurones responded to low intensity mechanical stimulation whereas slow AP neurones were activated by high threshold mechanical stimulation of the skin. 10% of cultured TG neurones responded with a $[Ca^{2+}]_i$ rise to cold solutions and menthol. Perfusion with 4-AP induced cold sensitivity in previously unresponsive neurones, suggesting that the specific temperature sensitivity of cold neurones depends in part on the low expression of a transient K⁺ current (IK_D) that normally acts as an excitability brake against the depolarizing effect of temperature decreases. Single corneal cold nerve endings exhibited spontaneous activity at 33°C that increased with cooling. In contrast, neurones of the intact ganglion 'in vitro' remained silent even when perfused with cold solutions. However, after exposure to 4-AP, a fraction of the fast AP neurones developed cold-sensitivity.

A12.13 Putative nociceptor responses in avian skeletal muscle

D.A. Sandercock & M.J. Gentle, Roslin Institute, UK

While there has been extensive characterisation of nociceptors in mammalian skeletal muscle there have been no equivalent studies in birds. This study is the first to demonstrate responses of avian (*Gallus domesticus*) skeletal muscle primary sensory afferents to noxious mechanical and chemical stimulation. Single unit record-

ings were made from small nerve filaments dissected from the lateral tibial and fibular nerves, which innervate the gastrocnemius (lateral head) and fibularis longus muscles of the lower leg. A total of 17 "nociceptive" units were identified. Units did not exhibit spontaneous activity prior to mechanical stimulation. All units responded in an intensity dependent manner to moderate (50–125 N/m²) and noxious (>200 N/m²) local mechanical pressure in the area of the receptive field. Responses to mechanical pressure lasted the duration of the stimulus and units exhibited slowly adapting, irregular discharges and did not demonstrate any of the characteristics of proprioceptors. Injection of 0.2 ml acetic acid (1% v/v) in isotonic saline (pH 2.8) into the receptive field stimulated discharge activity in 11/17 units (latency range 5–45 s). Firing activity increased over time, with peak firing frequencies observed 200–300 s post-injection. Median and peak firing frequencies ranged from 4–8 and 6–15 impulses/s respectively. The pattern in the responses showed irregular single or clustered discharges, which continued beyond the 15 minute recording period. In units where conduction velocities were measured (n=11), CV's ranged from 2.8 to 11.3 m/s. Responses to mechanical and chemical stimulation were comparable with avian cutaneous nociceptors and consistent with mammalian group III muscle nociceptors.

A12.14 Spinal dorsal horn neurone targets for unmyelinated primary afferents: do single neurone morphological characteristics suggest how nociceptive information is processed at the spinal level

R. Morris¹, O. Cheunsuang¹, A. Stewart¹ & D. Maxwell², ¹Department of Veterinary Preclinical Sciences, University of Liverpool, ²Institute of Biomedical and Life Sciences, University of Glasgow, UK

Unraveling the complexity of the functional anatomy of the spinal cord dorsal horn is an immense challenge due to the diversity of afferent inputs and the complexity of the efferent processes they activate ranging from the monosynaptic reflex through to the perception of pleasure and pain. New insights into this organization have been facilitated by an increasing array of selective markers of ion-channels, enzymes, neurotransmitters and receptors. The distribution of the neurokinin 1 (NK₁) receptor gave a completely new insight into the principal target neurons for substance P. In the rat, one target group consists of large LI neurones with dendrites that radiate from the soma parallel to the dorsal surface.

Their dendritic territory would fit into a disc approximately 0.5mm diameter, and the mean intersoma distance is 0.2 mm. Thus any point in LI could fall into the dendritic spread of 20 of these neurons. These large neurons are known to be projection cells and probably have relatively fast conducting axons (25m/s). They receive A-delta and C-fibre afferent input and are either nociceptive specific or wide-dynamic range neurons. From the mapping of dermatomes onto the spinal cord (Takahashi *et al.*), these cells potentially receive input from receptive fields 20mm across. These neurons would be suited to analyzing the extent of injury as opposed to the precise location of a noxious stimulus. This review will explore whether the emergent morphology of LI and LII neurons can provide clues to function. Reference: Takahashi *et al.* J Comp Neurol, 462: 29–41.

A12.15 Nociception and the Feeling of Pain

S. Hunt, University College London, UK

In the last decade remarkable progress has been made in unraveling two areas of pain research; the molecular biology of the nociceptor and utilization of fMRI imaging to localize areas of the forebrain that are involved in processing pain. Thus, for the first time we are beginning to identify the specific receptors and channels in nociceptors that transduce particular types of noxious stimulation associated with tissue damage. Similarly, using imaging tools such as fMRI in humans and experimental animals we have begun to understand what specific brain regions are activated in acute and the more persistent chronic pain state. However, the relationship between pain and injury, contingent as it is on the environmental context, and the disturbance of this relationship in chronic pain states, has largely eluded molecular or cell biological explanation. However, there is now an emerging focus on a small subset of spinal cord neurons. These neurons were identified from a confluence of studies using different techniques such as electrophysiology, morphology, the targeting and ablating specific populations of neurons, cell and molecular biology and behavioral analysis. Understanding function and plasticity of these lamina I cells in acute and chronic pain states seems to be an important key in our understanding of how pain information is processed in the CNS. Understanding how peripheral nociceptive events are ultimately appreciated as pain should not only shed light on how somatosensory information is modulated at the cellular and molecular level but may also reveal novel targets for the treatment of chronic pain.

A12.16 Nociception and Naturally-Occurring Bovine Mastitis

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Mastitis is a major disease problem for the dairy industry, causing significant economic losses and adverse welfare. The use of NSAIDs is generally limited to cows with severe forms of mastitis and the benefits of these drugs in terms of their analgesic effect have not been quantified. Field studies evaluating the use of NSAIDs were conducted on commercial dairy farms with 'mild' or 'moderate' clinical mastitis. Measurements included heart and respiratory rates, rectal and mammary gland temperature, and milk samples were collected for bacteriological diagnosis and measurements of the acute phase proteins (haptoglobin and milk amyloid A). The distance between the hocks was measured as a proxy indicator of altered cow stance. Mechanical nociceptive response thresholds were measured on each hindlimb. Our data indicated that the acute phase proteins were useful diagnostic indicators of disease severity, as was hock-to-hock distance, a potential indirect measure of pain, and rectal temperature. Cows with both mild and moderate mastitis showed evidence of mechanical hyperalgesia when threshold measurements were made on the ipsi-lateral hindleg. The duration of hyperalgesia was shown to persist for less than 7 days for mild cases, but for weeks in moderate cases of mastitis. The use of NSAIDs reduced mechanical hyperalgesia and occurred only when the drugs were given systemically rather than locally. Thus, results indicate that mechanical hyperalgesia may be used as an indicator of altered nociceptive processing in dairy cows, that hyperalgesia is centrally mediated, and that NSAIDs may be used clinically to alleviate pain and improve welfare.

A12.17 Chronic allodynia in rats infected with varicella zoster virus: A small animal model for post herpetic neuralgia?

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Varicella-zoster virus (VZV) is the causative agent of two human diseases: chicken pox following primary infection and herpes zoster or 'shingles' following reactivation from a latent infection. A common complication of herpes zoster is post-herpetic neuralgia, pain that persists after the rash has disappeared and which is char-

acterised by the development of chronic hyperalgesia and allodynia. Work on understanding the molecular basis of PHN has up to now been hampered by the lack of an appropriate animal model. We have previously described a rat model of VZV latency and have demonstrated striking changes in behavioural reflex responses indicative of mechanical allodynia and hyperalgesia which were undiminished up to five weeks post-infection, by which time all evidence of local injection trauma has disappeared. We have now shown that the chronic allodynia persists for up to 75 days post infection but resolves by day 100-post infection, this chronic/resolving course is similar to that seen in PHN patients. We have further characterised this model by assessing the efficacy of various compounds either known to be at least partially effective in the treatment of PHN, to play a role in immune modulation or to have antiviral activity. We have also carried out immuno-histochemical studies investigating potential changes in neuronal and glial cell phenotypes.

A12.18 Cerebral correlates of human experimental and clinical pain-changing views

T.J. Nurmikko, Department of Neurological Science, University of Liverpool, UK

Not long ago research on human pain was limited to reports of healthy volunteers or patients on their subjective experience, and evaluation of concomitant behavioural changes. Much of the understanding of how the human brain processes painful signals was based on results from cortical stimulation during neurosurgical operations in awake patients and interpretation of reports on pain by patients with brain injury. While neurobiology of nociception made steady progress in line with more sophisticated animal models, human conscious experience of pain and generation of associated emotional and behavioural dimensions remained poorly understood until the adoption of new techniques in the last 10 years. Of these methods, MEG (magnetoencephalography), PET (positron emission tomography), fMRI (functional magnetic resonance imaging), hold most promise and are now being used as research tools to study regional brain activation in experimental and clinical pain conditions. Despite inherent differences in what they measure and the pain states they have been used in, they all support the concept of “pain matrix” – a network of cortical and subcortical connections activated during perception of a painful stimulus. The details, however, of how intensity and localisation of pain are coded, and what processes are involved in determining quality and unpleasantness of pain are only partially understood. More complex cognitive processes as well as motivational and emotional consequences of

pain have only recently been subjected to functional brain imaging. Clinical applications of these methods in patients with chronic pain remain few, but show considerable potential in depicting cortical reorganisation in various conditions.

A12.19 The SNSR/Mrg family – where’s the pain?

S.A. Trim, P.J. Cox, B.I. Williams-Jones, M.J. Wyatt, H. Melrose, M. Ackley, R. Monhemius & R.A. Kinloch, Pfizer

A family of G-protein coupled receptors (GPCR) termed the sensory neuron specific GPCR (SNSR) or Mas Related Gene (Mrg) family was recently discovered. Some members (MrgX1) are uniquely expressed in small diameter sensory neurons and may play an important role in nociception. To date, rodent orthologues of MrgX have not been reported. Degenerate PCR and a bioinformatics approach were used to identify novel Mrg gene family members from rodent species. Although rodent MrgX orthologues were not identified we did identify guinea pig and hamster Mrgs. Analysis of the expression of select rat Mrg family members revealed that MrgD is dorsal root ganglion (DRG) specific and rat MrgE is found in the DRG, spinal cord, sciatic nerve and brain, whereas rat MrgG is expressed in heart, lung and thymus as well as nervous tissue. The Human MrgX1 receptor is agonised by proenkephalin A peptide fragments e.g. Bam 8–22 (Bovine Adrenal Medulla peptide) and shows weak activation by Opioid ligands. Here, we show that Bam 8–22 increases excitatory post-synaptic discharges in isolated rat spinal cord preparations and modulates firing of wide dynamic range neurones in response to noxious stimuli. The activity of Bam8–22 against rat Mrgs expressed in mammalian cells is being investigated to determine if any of the aforementioned effects are potentially mediated by the activity of this peptide against a specific rat Mrg encoded GPCR.

A12.20 Stretch responses of axons in regions of local inflammation in rat peripheral nerves

A. Dillely & B. Lynn, University College London

Increased axonal excitability and mechanosensitivity at sites of nerve injury are contributing factors in causing neuropathic pain. Normal limb movements resulting in nerve stretch can be painful in these patients. This study examines the stretch sensitivity of nerve fibres in regions of local inflammation. Inflammation was induced by applying Freund's adjuvant locally to the sciatic nerve of SD rats. Recordings were performed in vivo 2–8 days

post surgery from filaments dissected proximal to the lesion site. Mechanical sensitivity was assessed at the lesion site using a fine glass probe and by stretching using watchmakers forceps positioned above and below it. Post surgery approximately 13% of C-fibres from sural or peroneal nerves fired spontaneously. Responses to pressure at the lesion site were seen in 9/138 (6%) of C-fibres and 2/67 (3%) were stretch sensitive. Further examples of pressure or stretch sensitive C-fibres were seen in larger multi-fibre filaments. Some stretch sensitive A-fibres were also seen in multi-fibre preparations. The most sensitive C-fibre fired to less than 4% stretch. Following testing at the lesion site, a number of mechanically sensitive units began firing spontaneously. No receptive fields were found for any of the mechanical or stretch sensitive units. In summary, nerve inflammation can cause small numbers of A and C fibres to become stretch sensitive at the inflamed site. The most sensitive units respond to stretches within the range seen during normal limb movement suggesting that their responses could play a part in generating pain to limb movement in neuropathic conditions.

A12.21 Nociception and Neuronal Gene Expression in the Brain of Fish

S.C. Reilly, A.R. Cossins, J.P. Quinn & L.U. Sneddon, University of Liverpool, UK

There is substantial evidence that fish experience nociception, the detection of damaging stimuli. The nervous system of fish contains the cellular components required for nociception to occur. A recent study found Ad and C fibres in the head of a rainbow trout which responded to potentially harmful stimuli. In addition to this, a noxious event causes adverse behavioural reactions. The model of nociception in fish is relevant to animal welfare, the evolution of the nervous system and biomedical research. Many genes alter their levels of expression after a noxious experience and a global overview of this event can be obtained with microarray technology. A microarray study of nociception in fish may give insights into the molecular mechanisms underlying the changes in behaviour and physiology caused by a noxious event. The present study aims to examine the changes in mRNA production in the brain of fish after a noxious stimulation. Acetic acid was injected to the lip of common carp (*Cyprinus carpio*) and the animals killed after 6 hours. Regulation of gene expression was examined by comparing levels of mRNA produced in the experimental group to a saline injected control using a cDNA microarray. Genes regulated specifically by the noxious event were identified. The results will be discussed with particular reference to the evolution of the nociceptive system.

A12.22 A model of organotypic rat spinal slice culture and biolistic transfection to elucidate factors that drive the preprotachykinin-A promoter

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A combination of a neuronal culture system and a high-throughput transfection system can provide a way to study promoter function in the CNS. The preprotachykinin-A (PPT-A) gene encodes for neurokinins that are upregulated in sensory and spinal neurons after injury but there is limited knowledge of the intrinsic factors driving the promoter. An interface organotypic culture system was set up of 8-day old Wistar rat spinal slices. Morphology and viability were determined by immunostaining for neuron specific-nuclear protein (NeuN), β III-tubulin (neuron specific), neurokinin 1 receptor (NK1 R) and substance P. Biolistic transfection was undertaken and confirmed with an enhanced green fluorescent protein cDNA construct with a cytomegalovirus promoter (CMV-EGFP). The resultant cell populations were assessed with glial fibrillary acidic protein (GFAP), NeuN and β III-tubulin. Slices were transfected with a PPT-A promoter construct driving EGFP (kindly provided by J. Quinn, University of Liverpool) and a CMV-DsRed construct, as a transfection efficiency control. EGFP fluorescence was significantly induced by forskolin (10 μ M)/high K^+ (10 mM) application. EGFP fluorescence was also significantly induced by [Sar⁹,Met (O₂)¹¹]-substance P (10 μ M). These data show that this model offers a means to study the role of receptor specific ligands relevant to nociceptive processing in driving promoter activity. In addition, a combination of slice culture and biolistic transfection could be utilised for promoter studies in other CNS areas.

A12.23 Responses of neurones, in the spinal dorsal horn (LI-LIII) of the rat, to application of histamine and capsaicin to the DRG and spinal cord, *in vitro*

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Some dorsal root ganglion (DRG) neurones contain mRNA for the H1 histamine receptor and the present study has tested whether this can be exploited to identify spinal neurones processing itch information. Parasagittal or transverse slices (400–600 μ m thick) were cut from the lumbar spinal cords, of juvenile rats (15–25 days old). They were cut with attached dorsal roots, dorsal root ganglia and peripheral nerves, which were

arranged in individual chambers separated by grease seals. Intracellular recordings were made from neurones in LI-III and their responses to the application of histamine (1–100 μM) and capsaicin (1–10 μM) were tested. DRG application of histamine produced very small increases in EPSP activity in only 3 out of 19 neurones tested. In contrast 6 out of 12 neurones responded with marked increases in EPSP activity to DRG application of capsaicin with no consistent changes in resting membrane potential. A further neurone responded with an increase in IPSP activity. Direct application of histamine to the slice (1–100 μM) also produced no effect in 11 out of 12 neurones tested, with only one neurone showing a small increase in EPSPs. In contrast application of capsaicin to the slice evoked EPSPs in 12 out of 44 neurones tested. These data suggest that functional H1 receptors may not be expressed on the DRG neurone soma or the central terminals of primary afferents in intact animals. The action of histamine on cultured DRG neurones probably reflects the incorporation of functional receptors into newly formed neuritis.

A12.24 An integrative approach to assessing nociception and pain in the rainbow trout

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Nociception acts as a warning system to alert an animal to a potentially damaging stimulus and is an important

somatosensory system. The criteria for animal pain which may result from a tissue damaging event is 1. detection of a harmful stimulus; 2. reacting to the stimulus; 3. suspension of normal behaviour such as feeding and behaving socially; 4. physiological reactions such as increased respiration rate; and finally 5. amelioration of these responses by the use of analgesics. A review of recent research on a teleost fish shall be presented. To prove the rainbow trout was capable of detecting potentially painful stimuli, anatomical techniques were employed to look for the presence of A-delta and C fibres that act as nociceptive nerves in higher vertebrates in the trigeminal nerve of the fish. Electrophysiological recordings were made from afferent cell bodies in the trigeminal ganglion and receptors were found on the head of the fish which were preferentially sensitive to damaging stimuli. These receptors responded physiologically in a similar way to polymodal and mechanothermal nociceptors in mammalian models. A pain-causing agent was administered to the fish which showed suspension of feeding behaviour, increased respiration rate and no response to external stimuli. All of these responses were ameliorated by morphine, a painkiller. These studies have shown that a teleost fish is capable of nociception and there is the potential for pain perception in this lower vertebrate.