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Evidence that spinal lamina II stalked cells receive input from nociceptive epidermal primary afferents, and the parallel processing of pain

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By combining two parallel sets of studies, we present evidence on the functional assembly of neuronal circuitry underlying the processing of nociceptive sensations in the dorsal horn of the spinal cord. One set of observations was made using a transgenic mouse line expressing a membrane-targeted version of green fluorescent protein (GFP) selectively in epidermal primary afferents. The second set were made using whole cell patch recordings from spinal cord slices isolated from juvenile rats.

All animals used were deeply anaethestised (pentobarbitone 80mg/kg, for adult mice or ether for juvenile rats) prior to either vascular perfusion (adult mice) for histology or decapitation (juvenile rats) and spinal cord slice preparation for whole-cell patch studies. Standard immunochemical and intracellular labelling methods were employed.

In transgenic mice in which the Thy1.2 promoter was used to drive expression of GFP, exclusively small diameter dorsal root ganglion (DRG) neurones expressed GFP and these also bound IB4 lectin. GFP expression was detected in their peripheral axons selectively terminating in the epidermis whereas their central terminals were restricted to LII. No colocalisation with substance P was found. Many IB4 binding primary afferents terminate in glomerular synapses forming contacts with spines on stalked cells. Whole cell patch recordings from LII neurones (n=55) in rat lumbar spinal cord slices revealed that many had the morphology of stalked cells (n=10) and were excited by capsaicin. This excitation persisted in the presence of TTX and could be blocked by AMPA receptor antagonists (NBQX, 10μM). In the absence of magnesium the post-synaptic responses were prolonged and this could be reversed by an NMDA antagonist. Intracellularly labelled stalked cells were shown to express the glutamate vesicular transporter and hence, are excitatory interneurones.

These observations provide strong evidence for the existence of a system in which epidermal primary afferents are activated by noxious heat, and in which centrally projecting afferents synapse on LII excitatory interneurones. A parallel system involving substance P containing primary afferents innervating LI spinothalamic neurones is also nociceptive. We hypothesize that epidermal neuronal circuitry may be involved in detection of superficial burning sensations such as would occur in sun-burn. In contrast, substance P containing circuitry is more concerned with tissue injury and inflammation. These findings will have important implications for the treatment of pain.

Where applicable, the experiments described here conform with Physiological Society ethical requirements.

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Localization of touch versus heat pain in the human hand: a dissociative effect of temporal parameters on discriminative capacity and decision strategy

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We studied the influence of temporal parameters on localization of touch versus pain in the palmar skin of the human hand. A monofilament was used for tactile stimulation (n=14) and a thulium laser for inducing C fibre-mediated pain (n=8). Stimuli were applied sequentially to determine discrimination between successive stimulus sites. The interstimulus interval (ISI) varied from 1 to 9 s. Localization threshold was about two times higher for heat pain than touch (13.0±0.8 mm vs. 6.9±0.4 mm; mean±S.E.M., respectively). The localization threshold for pain, but not touch, decreased with prolongation of the ISI from 1 to 7-9 s ($F_{3,80}$=6.36, p=0.0006), and it remained higher for pain even at the ISI of 9 s. The response time was longer for pain than touch (1857±100 ms vs. 1165±96 ms, respectively), and it increased with an increase in the ISI ($F_{3,80}$=3.37, p<0.04), independent of the modality ($F_{3,80}$=0.38). Discriminative capacity, as assessed by the receiver operating characteristics curve, was markedly better for touch than pain (e.g. at the interstimulus distance of 10 mm, the 95% confidence limits of the areas under the receiver operating characteristics curves for touch and pain were not overlapping at any of the ISIs). The discriminative capacity decreased with an increase of the ISI, but only for touch ($F_{3,364}$= 7.34, p<0.0001).

The results indicate that localization is more accurate for touch than pain. Temporal summation of C fibre-evoked pain contributes to the reduced accuracy of pain localization if the ISI is ≤ 3 s. Additionally, temporal factors dissociatively influence the response strategy in the tactile versus pain localization task as indicated by changes in the response time, false alarm rate and discriminative capacity with the prolongation of the ISI from 1 to 9 s. Due to this strategy change, localization threshold for touch remains constant at prolonged ISIs, in spite of a decrease in discriminative capacity.

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Where applicable, the experiments described here conform with Physiological Society ethical requirements.

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A pharmacological comparison of two different sensory readouts in a rat model of chronic inflammatory joint pain

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The aim of this study was to compare two different methods of evaluating the efficacy of known analgesics, currently available

in the clinic, for the treatment of the chronic pain associated with rheumatoid arthritis. The paw pressure withdrawal threshold assay (Randall & Selitto, 1957) has long been used as a mechanical test of assessing hypersensitivity in models of acute inflammatory pain (Anseloni et al. 2003). The recent development of the weight bearing assay has seen the introduction of an alternative readout providing an objective assessment of incident hypersensitivity (Clayton et al. 1997). Using a rat model of chronic inflammatory joint pain (Donaldson et al. 1993), a monoarthritis was induced by injecting 150µl (1mg/ml) of Freund's complete adjuvant (FCA) into the intra-articular space of the knee joint. Injections were done under brief anaesthesia (3% isofluorane with O2 at 1.5l.min-1). A series of six, blind and randomised experiments (n=40, for each experiment) were conducted assessing the analgesic potency of Rofecoxib (1, 3 and 10mg.kg-1), Etoricoxib (1, 3 and 10mg.kg-1) and Ibuprofen (3, 10 and 30mg.kg-1), using both the paw pressure withdrawal threshold and the weight bearing readouts. Drug or vehicle (DMSO 1%, distilled water 33% and PEG400 66%) were chronically dosed p.o. on days 13-17 post FCA injection and the above readouts were used to assess the ability of the test drug to reverse the induced hypersensitivity. The contralateral paw pressure withdrawal threshold readout can be taken to negate any compound-induced motor deficits being falsely concluded as analgesia. All data were transformed into an area under the curve (AUC) value, for days 13-17 for each dosing group. Using Statistica V.6 the AUC data were statistically analysed by an ANOVA followed by a Duncan's post-hoc test. A difference in groups was only considered significant when $P<0.05$. The relationship between the AUC group means were then assessed using Pearson's linear regression correlation analysis in order to produce a correlation coefficient (where 1 = positive correlation, 0 = no correlation and -1 = negative correlation) and an associated P value stating the statistical significance of the test. Animals were humanely killed at the end of the experiment.

Results revealed strong correlations between the two readouts (Rofecoxib r = -0.9949, Etoricoxib r = -0.9834 and Ibuprofen r = -0.9514) with both assays producing similar 50% effective dose (ED50 values) for each drug. These results provide evidence that the weight bearing assay can be used alongside the paw pressure withdrawal threshold assay to increase confidence in the preclinical potency of novel analgesics and hopefully lead to improved clinical efficacy.

Clayton NM, Oakley I, Thompson S, Wheeldon A, Sargent B & Bountra C (1997). BJP 120, 219P.

Donaldson LF, Seckl JR & McQueen DS (1993). JNM 49, 5-10.

Randall LO & Selitto JJ (1957). AIPT 111, 409-419.

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Where applicable, the experiments described here conform with Physiological Society ethical requirements.

SA17

Learning in sensorimotor circuits

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The study of plasticity in CNS is a major and very dynamic neuroscience research field with enormous clinical potentials. It now appears that most circuits in the brain and spinal cord show plasticity and that they can be modified by experience. Understanding the mechanisms of plasticity in the nervous system is therefore essential for the understanding of how the nervous system is wired during development and how it adapts in response to changes in the body and environment. This lecture focuses on self-organizing adaptive plasticity in spinal sensorimotor circuits.

To be useful in motor control, somatosensory information must be encoded (weighted) with respect to body anatomy and movement patterns produced by the sensorimotor circuits. This is a difficult task since the multisensory information (nociception, pressure, temperature, joint angles, muscle force and length) arises from a complex body constitution. The information processing that needs to be performed is therefore staggering. Understanding how the basic sensorimotor system functions are adapted to the body anatomy and biomechanics is therefore a major task. To understand the cellular mechanisms underlying functional adaptation in the spinal cord, knowledge on the functional organization of the neural circuits is essential.

During the last 10 years the concept of a modular organization of the spinal cord has grown stronger. A modular type of reflex organisation in the mammalian spinal cord was first demonstrated for the nociceptive withdrawal reflex (NWR) system. In this system, each excitatory module preferentially acts on a single muscle and performs a detailed sensorimotor transformation resulting in a graded withdrawal of the limb (or part of the limb) from its receptive field. For each excitatory NWR module, the input strength has a characteristic pattern on the skin that mimics the pattern of withdrawal efficacy when the output muscle of the module contracts. In a sense, the pattern of withdrawal efficacy is imprinted on the receptive field of the module. A corresponding set of inhibitory reflex modules also exists. In this case, the receptive fields correspond to the graded movement of the skin area towards external stimulation (i.e. increase in load) on contraction of the muscle in the module. As a result of this organisation, the excitatory and inhibitory modules are engaged to a degree that is proportional to their respective withdrawal/loading efficacy on skin stimulation. Given that the adult sensorimotor transformations performed by the spinal cord reflect precisely weighted connections in modules - how can this weighting be achieved during development? Previous studies in this laboratory have shown that an experience dependent mechanism termed somatosensory imprinting underlies the functional adaptation of this system. Recently, we found that tactile feedback ensuing on spontaneous motility in spinal sensorimotor circuits is used to tune the connection strengths in nociceptive withdrawal reflex modules during postnatal development in the rat. Thus, tactile inputs from the skin area normally withdrawn and arriving in conjunction with the spontaneous movements had an adaptive effect on the reflex modules. This learning took place over postnatal days 12-17. Uncorrelated input (given at random time points) did not cause a learning effect. Since this process results in an imprint of the withdrawal efficacy on the reflex modules, it was termed motor directed sensorimotor imprinting (MDSI). Notably this novel form of unsupervised learning occurs during active sleep, characterized by atonia in the musculature. This state may be particularly advantageous for learning since the sensory feedback on muscle contraction stands out from a more or less silent background.

Spontaneous movements are a ubiquitous phenomenon during embryonic development in all vertebrates and mammals. Their role in sensorimotor learning has, however, not been known. The activity appears to be caused by spontaneous endogenous activity in neuronal circuits the spinal cord and brain stem. Although present classifications tend to lump the spontaneous motility broadly into a few categories, detailed studies in humans have distinguished up to 16 different types. The prevalence and complexity of these movements lead us to suggest that all major spinal motor systems contribute to the spontaneous movements during development. Furthermore, since this adaptive learning is highly effective, it may well be that all major groups of spinal motor systems learn relevant aspects of the body anatomy and biomechanics during development by probing the sensory feedback after spontaneous endogenous activation.

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Plastic Changes in Dorsal Horn Nociceptive Neurons Can Help Account for Allodynia

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Allodynia is pain that results from a normally innocuous stimulus. Allodynia in human patients is a distressing condition that commonly occurs in neuropathic pain states and in inflammation. Several proposals have been made concerning mechanisms that underlie allodynia. We hypothesize that a plastic change in nociceptive dorsal horn neurons called central sensitization is responsible for the development of mechanical allodynia.

Our laboratory has examined the possible role of central sensitization of dorsal horn nociceptive neurones, including spinothalamic tract cells, in mechanical allodynia. Our experimental models were peripheral neuropathy following tight ligation of one or more spinal nerves (Chung model) and intradermal injection of capsaicin. Spinal nerve ligations were done under sodium pentobarbital anaesthesia. Capsaicin injections were made under halothane or pentobarbital anaesthesia. Most experiments have been done in rats, but some, particularly those in which electrophysiological recordings were made from spinothalamic tract neurones, were done in monkeys (Macaca fascicularis). These animals were anaesthetized initially with halothane and nitrous oxide, and then anaesthesia was maintained with a mixture of α-chloralose and sodium pentobarbital. At the end of the experiments, the animals were humanely killed.

Wide dynamic range dorsal horn neurones normally respond weakly to tactile stimuli and maximally to noxious stimuli. After peripheral nerve injury or acute inflammation, such as following an intradermal injection of capsaicin, these neurones could respond as strongly to tactile stimuli as they previously had to noxious mechanical stimuli. If thalamo-cortical neurones reflect these changed responses, the resulting sensation may be interpreted as pain rather than as touch. This could account for mechanical allodynia.

We attribute the enhanced tactile responses of nociceptive dorsal horn neurones to central sensitization. We have evidence from pharmacological experiments that this process is initiated by synaptic release of glutamate and peptides, including substance P and calcitonin gene-related peptide, from the terminals of nociceptors in the dorsal horn. Actions at NMDA, NK1, CGRP1 and other receptors result in the activation of several signal transduction pathways (including the CaMKII, PKC, PKA, and NO/PKG cascades), which in turn cause the phosphorylation of proteins, such as the NR1 subunits of NMDA receptors and GluR1 subunits of AMPA receptors. Based on the work of others, we presume that phosphorylated glutamate receptors become more responsive to glutamate and more receptors may be inserted into the surface membrane. These changes then increase the responses of wide dynamic range nociceptive neurones to glutamate released from tactile afferents. Simultaneously, the responses of inhibitory amino acid receptors to GABA and glycine are reduced. We speculate that it is possible that phosphorylated inhibitory amino acid receptors become desensitized, reducing the amount of inhibition of dorsal horn neurones. We have found that the duration of these changes is regulated by protein phosphatase activity.

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