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Brainstem control of spinal sensory processing in normal and pathophysiological states

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Plasticity in the nervous system underlies the adaptive, non-dependable relationship between the intensity of a noxious stimulus and the perception of pain. Sensitisation of the nervous system can contribute to the development and maintenance of some long-term pain states and may occur at peripheral, spinal and supraspinal levels. With respect to the latter, complex networks of pathways integrate and project from various brain structures to influence the spinal processing of nociceptive input in a top-down fashion. The rostroventromedial medulla (RVM) is a major endogenous modulatory system implicated in the supraspinal control of spinal sensory information. We determined the extent to which facilitatory and inhibitory neurones in the RVM shape the responses of spinal cord neurones to primary afferent input. Single unit extracellular recordings were made *in vivo* from L4–L5 deep dorsal horn neurones in anaesthetised rats (0.7% halothane in 66% N_2O and 33% O_2) both before and after the stereotaxic injection of 0.8µl lidocaine into the RVM. The effects of this local anaesthetic on electrical and natural (brush, von Frey filaments and heat) evoked responses in each animal were measured against pre-drug control responses. Experiments were performed on normal rats (n=16) and rats that had undergone spinal-nerve-ligation surgery under 1% halothane anaesthesia 2 weeks previously and who accordingly displayed behavioural signs of mechanical and cold hypersensitivity (n=17). Results from these studies show that in normal and neuropathic rats alike, intra-RVM lidocaine differentially (and significantly) inhibited or facilitated the responses of dorsal horn neurones to the range of stimuli tested (comparison of pre- and post-drug responses using a Student's paired t test with significance set at $p<0.05$).

In both sets of animals, descending facilitatory influences predominated over inhibitory influences, evidenced by the fact that in the majority of experiments, neuronal responses decreased following the histologically verified injection of lidocaine into the RVM. This inhibition was particularly pronounced for input, C-fibre responses and post-discharge, whilst the reduction in dorsal horn responses to higher threshold mechanical and thermal stimuli (vF 30g, 75g & 45°C, 48°, 50°C, respectively) was significantly greater than the reduction in responses to lower threshold stimuli (vF 5g, 9g, 15g & 35°C, 40°C). Moreover, the proportion of neurones that showed an overall inhibitory action of lidocaine increased in the neuropathic condition; in the normal state, 65% of recorded cells had reduced responses post-lidocaine injection whereas in the neuropathic state this increased to 88%. Thus, results from this study have shown that descending facilitations on dorsal horn neurones dominate in normal rats and support previous data suggesting their increase after nerve injury.

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Modulation of A- and C-nociceptor-evoked withdrawal reflexes by cyclooxygenase isoforms in the midbrain periaqueductal grey

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Descending control of spinal nociception that originates from the midbrain periaqueductal grey (PAG) is an important determinant of the pain experience. The current study investigated the role of different cyclooxygenase (COX) isoforms in regulating, at the level of the PAG, descending control of A- versus C-nociceptor evoked spinal reflexes.

At 8 min intervals, either fast (7.5°C s^{-1}, 30-59°C) or slow (2.5°C s^{-1}, 30-57°C) rates of heating were applied to the hind paw dorsum to preferentially activate Aδ- or C-heat nociceptors, respectively (Yeomans *et al.*, 1996a, b; McMullan *et al.*, 2004) in propofol-anaesthetised (~30mg kg^{-1} h^{-1}, i.v.) male Wistar rats (280-300g). Withdrawal EMG thresholds to fast or slow heat ramps were recorded from biceps femoris, before and after microinjection of substances into the PAG.

Ketoprofen (100µg in 300nl), a non-selective COX inhibitor, had no significant effect on withdrawal thresholds to fast (ANOVA, $p=0.6731$; n=4) or slow ($p=0.0973$; n=4) heat ramps compared to vehicle when injected into dorsolateral/lateral regions of the PAG. However, it significantly increased ($p<0.0001$) withdrawal thresholds to fast (54.33±0.25 to 57.95±0.57°C, mean±s.e.m.; n=4) and slow (52.3±0.22 to 56.63±0.38°C; n=4) heat ramps when injected into the ventrolateral-PAG. The duration of this antinociceptive effect was longer for C-nociceptor ($p<0.05$ for all heat ramp trials between 1-41 and 57-65min post-injection, Bonferroni post-test) than A-nociceptor evoked responses ($p<0.05$ for trials between 17-25min post-injection). The COX-1 inhibitor SC-560 (50nM) produced similar effects to ketoprofen on both A-nociceptor ($p<0.05$ for trials between 17-25min post-injection; n=3) and C-nociceptor evoked responses ($p<0.05$ for trials between 1-65 and 81min post-injection; n=4), again with greater influence over C-nociceptor evoked responses. The COX-2 inhibitor NS-398 (5µM) had no significant effect on withdrawal thresholds to fast ($p=0.4991$; n=3) or slow ($p=0.1918$; n=4) heat ramps. Vehicle (70% DMSO, 30% physiological saline) had no significant effect on thresholds to fast or slow heat ramps in any PAG region.

The data suggest that COX-1-derived products are tonically active in ventrolateral-PAG and play a role in setting the gain during acute nociceptive processing, with a greater influence over C- than A-nociceptor-evoked responses.

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Loss of superficial NK1-expressing dorsal horn neurones attenuates inhibitory neurotransmission mediated by spinal $GABA_A$ receptors

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Superficial neurokinin 1 (NK1)-expressing neurones drive a descending serotonergic pathway from the brainstem back onto spinal dorsal horn neurones to enhance nociceptive transmission via a facilitatory action at spinal 5HT3 receptors. Ablation of these cells attenuates allodynia and hyperalgesia which is mirrored by marked deficits in the evoked responses of deep (lamina V-VI) wide dynamic range (WDR) cells to noxious mechanical, thermal and chemical stimuli.

To assess whether removing the origin of this facilatatory spino-bulbo-spinal loop results in any potential alterations in GABAergic spinal inhibitory systems we investigated the effects of spinal bicuculline, a selective $GABA_A$ receptor antagonist, on the evoked activity of deep dorsal horn WDR neurones following selective ablation of superficial NK1-expressing dorsal horn neurones.

Ketamine (1.5 mg/kg I.P.)-anaesthetised Sprague Dawley rats (120-130g) were given an intrathecal injection of substance P (SP) conjugated to a cytotoxin, saporin (SAP) delivered to the L4-5 region to ablate NK-1 neurons. SAP alone was used as control. Animals were allowed to recover. Twenty-eight days later in vivo electrophysiology experiments under halothane (1%) anaesthesia were performed. The effects of cumulative doses of bicuculline (5, 50, 250 μg/50μl) on the evoked responses of lamina V WDR neurons to electrical (Aβ- Aδ- and C-fibre) and innocuous and noxious mechanical (brush, prod and von Frey (vF) 8 and 26g) stimuli were measured. Statistical analysis was performed using repeated measures ANOVA.

In the SAP control group (n=9), bicuculline significantly facilitated the Aδ- and C-fibre-evoked responses as well as the post-discharge and non-potentiated response of the neurones in a dose-dependent manner (257±45, 162±18, 259±64 and 264± 67% of control, respectively; $P<0.05$). The evoked neuronal responses to prod and mechanical punctate stimuli (vf8 and 26g) were also significantly increased (247±57, 550±215 and 305±130% of control, respectively; $P<0.05$). Remarkably, the marked excitatory effect on lamina V neuronal responses by blocking spinal $GABA_A$ receptors was lost in the SP-SAP-treated group (n=9) on all neuronal responses measured. The effect of bicuculline on the electrically and naturally evoked responses in these animals ranged from 96±37 to 148±55% of control.

These data suggest an intact facilitatory spino-bulbo-spinal loop is essential for GABA-mediated inhibitory controls mediated via spinal $GABA_A$ receptors since ablation of superficial NK1 neurons blocked any GABA-mediated enhancement of neuronal responses. Moreover these findings provide further evidence to support that activation of NK1-expressing cells predominantly drives a facilitation to enhance deep dorsal horn neuronal responses since the overall excitability of the spinal cord is reduced after ablation of NK1 neurones, yet intrinsic GABA inhibitory controls do not increase.

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C29

Postnatal spatial tuning of inhibitory cutaneous receptive fields in the rat dorsal horn

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There is evidence to suggest a lack of inhibitory control in developing spinal sensory pathways: excitatory cutaneous receptive fields are large and mechanical thresholds low in the first weeks of life (Fitzgerald, 2005). Since robust $GABA_A$ receptor-mediated inhibition of receptive fields is evident by the third postnatal day (P3) (Bremner et al. 2005), we hypothesized that developmental differences in dorsal horn inhibition may occur at the network level. To test this, we studied the spatial organisation of inhibitory receptive fields in adult and neonatal spinal cord.

Anaesthesia was established in adult Sprague Dawley rats (~180g) with Hypnorm (0.6 mg/kg; i.p.) and Diazepam (2.5 mg/kg; i.p.) and in neonates (P3) by cooling on ice. The trachea was cannulated and a midcollicular decerebration performed; anaesthesia was then withdrawn. Neuromuscular blockade was induced with pancuronium bromide (2 mg/kg) and animals ventilated with O_2 and spinalised. ECG was monitored throughout.

Extracellular spikes were recorded from single dorsal horn cells of the lumbar spinal cord. Excitatory receptive fields (RFs) were mapped with brush and pinch of the ipsilateral plantar hindpaw and inhibitory fields were mapped on the contralateral paw. Pinch of the contralateral plantar hindpaw inhibited spontaneous or evoked activity in 23/29 adult cells and 20/29 P3 cells. Strength of inhibition was quantified as the number of spikes inhibited relative to the baseline rate of firing. For each cell, the contralateral paw area that produced the maximal inhibition was established and the remaining hindpaw areas were mapped according to whether they produced <20%, 20-39%, 40-59%, 60-79% or ≥80% of the maximal inhibition. Data are presented as mean ± SEM.

The spatial organisation of the inhibitory RFs differed significantly at the two ages. In the adult, the strongest (≥80%) inhibition covered 27.7 ± 5.3% of the plantar surface and was restricted to the toes and pads in 17/21 cells. In contrast, at P3, inhibition was more evenly distributed, with pinch to large

regions of the paw often inhibiting firing equally strongly: the strongest inhibition covered 69.1 ± 6.5% of the plantar surface and was restricted to the toes and pads in only 2/17 cells. Plotting the strength of inhibition against cumulative plantar coverage and measuring the area under the curve (AUC) for each cell revealed a highly significant difference between the two ages (adult: 5388 ± 318; P3: 7223 ± 298 (Mann Witney $p < 0.001$), where 8000 represents equal inhibition across the whole plantar area).

These results demonstrate substantial postnatal changes in the spatial organisation of inhibition in the developing dorsal horn. Contralateral inhibition is present at P3 but the area of maximal inhibiton is less spatially restricted than in the adult. This lack of inhibitory tuning may contribute to the observed excitability of neonatal cutaneous sensory processing.

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L.B. is a Wellcome Trust PhD student.

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SA17

Positive control; regulation of spinal afferent input

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It has recently become necessary to extend our concept of how information flow in the spinal cord is regulated presynaptically. Classical presynaptic inhibition occurs at terminals of primary afferent axons and is mediated through interneurons that release GABA at axo-axonic synapses. The release of GABA is associated with a depolarisation of the primary afferent terminal (PAD). Although PAD-like phenomena have been recorded from terminals of some classes of interneuron, there is no evidence that axo-axonic synapses are present on interneuron terminals. Nevertheless the discovery that receptors are present not only on primary afferent axon terminals but also on terminals of interneurons, along with the acceptance of volume transmission as a mode of signalling in the CNS, suggests that some form of presynaptic regulation is likely to occur at terminals of interneurons. Recently, we examined the organisation of two classes of receptor that are activated by descending monoamine systems: the α2c adrenergic receptor (α2c –AR) and the 5-HT3 serotonin receptor. The α2c–AR, (unlike the α2A–AR which is present on primary afferents) is found principally on terminals of spinal interneurons. The majority (84%) of these interneurons are excitatory but a small group (11%) is inhibitory. A proportion of α2C-AR-immunoreactive terminals also contain peptides such as enkephalin, somatostatin, neurotensin, neuropeptide Y but they are not present on noradrenergic terminals and thus do not function as autoreceptors. Excitatory axons that possess the α2c–AR target projection cells in lamina I that are immunoreactive for the neurokinin 1 (NK-1) receptor. Therefore noradrenaline appears to modulate excitatory synaptic transmission from spinal interneurons to projection cells by acting at α2C-ARs; this could be one of the mechanisms that underlie its antinociceptive action. Serotonin 5-HT3 receptors are present on primary afferent terminals and terminals of interneurons. This receptor is found on excitatory terminals. Like the α2c–AR axons, axons possessing the 5-HT3 receptor also form synaptic relationships with lamina 1 projection cells. However, serotonin probably facilitates excitatory transmission at such synapses and has a pronociceptive action when it acts through 5-HT3 receptors. Thus sensory transmission is not only regulated presynaptically at primary afferent synapses in the spinal cord but is also regulated presynaptically at synapses formed by interneurons which are components of polysynaptic pathways.

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SA18

Descending controls that modulate spinal sensory processing and therapy

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Pain from peripheral nerve injury, inflammation and cancer, characterised by ongoing pain, hyperalgesia and allodynia arise from peripheral and central processes. There is clear evidence from both preclinical and clinical studies that both peripheral and central hyperexcitability play important roles in determining the level of pain perceived. Rightly, much emphasis has been put on spinal cord mechanisms in central excitability such as wind-up and long term potentiation but it also clear that spinal excitability can be regulated by descending pathways from the brain. These originate from predominantly monoamine systems which are additionally implicated in control of emotions, fear, anxiety, thermoregulation and the sleep cycle and so may mediate these pain induced co-morbidities. Furthermore, they are likely targets for antidepressants used in pain states.

Much of the early work in this area concentrated on descending inhibitions, now known to be predominantly noradrenergic, and failure of descending inhibitions has been reported in patients. However, pain could equally be increased by enhanced descending facilitations and data is accumulating on exactly this. Thus, increases in pain sensitivity that follow injury can be regulated by superficially located projection neurons of the dorsal horn of the spinal cord that express the NK1 receptor. These neurones project through the parabrachial area, an area activated in humans in hyperalgesic states, to the amyglada and hypothalamus where they engage systems related to the affective, autonomic and aversive aspects of pain. Following selective ablation of these neurons we have identified changes in receptive field size, mechanical and thermal coding and central sensitisation of deeper lying dorsal horn neurons in the rat, important for both pain sensations and reflexes. The pathways relay on 5HT positive and other neurones in the brainstem rostroventromedial medulla (RVM).

The final arm is a descending serotinergic facilitatory pathway terminating on excitatory 5HT3 receptors in the spinal cord that has a major influence on mechanical and chemical evoked responses of deep dorsal horn neurones and also enhances their thermal responses. 5HT3 receptors therefore play a pronociceptive role in the spinal cord as supported by previous studies. Interestingly, these 5HT facilitations are not required for either windup or long term potentiation, reinforcing the idea that these are intrinsic spinal events. Further we have demonstrated that in animal models of neuropathic and bone cancer pain states there are enhanced descending facilitatory controls of mechanical responses of spinal neurones, mediated through the activation of these spinal 5HT3 receptors. Depletion of spinal 5HT reduces behavioural hypersensitivity after peripheral nerve injury. After inflammation, the changes seen are minor, suggesting that these facilitations impact more upon pathophysiological processes.

Finally, based on the changes in these controls after nerve injury and the ability of gabapentin to modulate neuropathic pain, we have shown that this 5HT3 mediated descending pathway plays a major role in the state-dependent actions of this drug.Since gabapentin binds to a subunit of calcium channels and some 5HT3 receptors are on afferent terminals, this interaction may occur on the terminals of afferent fibres as they enter the spinal cord.

These excitatory influences are likely to contribute to the development and maintenance of central excitability in the spinal cord, and furthermore, to the behavioural manifestation of tactile allodynia. These pathways, therefore form a link between emotional states and levels of pain and when dysfunctional, could contribute to pains where there are mood and sleep disturbances such as fibromyalgia.

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SA19

The imbalance of descending inhibition and facilitation in defining the perception/response characteristics of a nociceptive event

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Pain perception is modulated by a complex network of neuronal circuits that ultimately influence nociceptive transmission at the spinal cord. Although a lot of emphasis has been given to the pain inhibitory components, the occurrence of pain facilitation gained increasing relevance during the last years. Its weight on defining ascending nociceptive signalling is, however, far from understood. The role of descending facilitation in pain control was first committed to anti-analgesic actions and the establishment of sensitisation during chronic pain (Lima and Almeida, 2002). The possibility that facilitation is important to tune pain perception and reactions in everyday physiological conditions was brought into consideration after the discover of supraspinal centres that primarily induce increased nocifensive behaviour and enhanced responsiveness of nociceptive neurons. This is the case of the medullary dorsal reticular nucleus (DRt) (Lima and Almeida, 2002) . The DRt was shown to establish closed reciprocal loops with the spinal cord lamina I, as were some inhibitory centres such as the caudal ventrolateral reticular formation (VLM) and the nucleus tractus solitarii (NTS). In lamina I, but not in the deep dorsal horn, axons projecting down from the DRt or the VLM establish synaptic contacts with neurons that in turn synapse upon DRt and VLM lamina I-projecting neurons. While in the first case synapses are excitatory at both spinal and medullary levels (Almeida et al., 1993, 2000), in the latter the spinal link is either excitatory or inhibitory, the inhibitory circuit being dominant (Tavares and Lima, 2002). These reciprocal circuits are well fitted to subserve a direct control of activation of projection neurons from the respective target, and may explain the observed differences in the relative amount of projecting lamina I neurons that are activated in different pathways. Not so easy to explain is the fact that, for the same pathway, different lamina I cell groups are activated in different amounts. Nevertheless, the data imply that for fixed stimulation conditions there is a pattern of supraspinal distribution of nociceptive input determined by the amount of lamina I neurons of different types that contribute to different pathways (Lima, 1998). The activation pattern varies by varying stimulus properties, such as the nature of the stimulus, and desappears in conditions in which discrimination is impaired, such as secondary hyperalgesia. These findings indicate that the structural diversity of lamina I neurons may contribute to adapt ascending nociceptive signalling to stimulation conditions through the differential activation of the various cell groups contributing to the various pathways.

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