Review

The Neurobiology of Acute Pain

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Abstract

The mechanisms by which noxious stimuli produce the sensation of pain in animals are complex. Noxious stimuli are transduced at the periphery and transmitted to the CNS, where this information is subject to considerable modulation. Finally, the information is projected to the brain where it is perceived as pain. Additionally, plasticity can develop in the pain pathway and hyperalgesia and allodynia may develop through sensitisation both peripherally and centrally. A large number of different ion channels, receptors, and cell types are involved in pain perception, and it is hoped that through a better understanding of these, new and refined treatments for pain will result.

Keywords: Pain; Neurobiology; Nociception; Analgesia; Spinal cord

Introduction

Pain is the subjective experience of harm in a part of one's body, and is currently more strictly defined as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage" (Williams and Craig, 2016). By this definition it is an experience, which therefore requires activity of structures in the brain to be perceived. This is in contrast to nociception which is defined as the encoding and processing of noxious stimuli in the nervous system. Pain has a fundamentally important protective role, alerting us to threats and providing an impetus for the preservation of the integrity of the body. However, in the context of veterinary treatment of illness or injury, acute pain may become an unwanted consequence that compromises the welfare of animals in our care. The implications of chronic pain, which is generally defined as pain that extends beyond the normal duration of healing, can be even more detrimental to wellbeing and is covered in another article within this issue.

A wide variety of thermal, chemical, mechanical and inflammatory stimuli can trigger pain and the experience of clinical pain is likely a complex amalgam of these stimuli perceived after significant modulation in the central nervous system. Pain may also result from a lesion or disease of the nervous system itself. However, the complex mechanisms of this so-called neuropathic pain, which have received significant attention from pain researchers, are not covered in detail in this review.

The nociceptive pathway that carries signals from the periphery to the brain where pain is perceived can be broken into components: *Transduction* of noxious stimuli at the periphery, *transmission* of those stimuli to the central nervous system (CNS), *central integration and modulation* of the signals at the CNS level, and finally *projection* to the brain followed by

perception. This article aims to detail the important cellular and molecular mechanisms of each of these stages.

Transducing the stimulus at the periphery; the role of the nociceptor.

Nociceptors are primary afferent neurons which project from tissues including skin, muscle, joints and viscera to the spinal cord or its trigeminal equivalent in the brainstem. Unlike other classes of primary afferents, e.g. those that convey touch, nociceptors preferentially transduce stimuli with intensities in the noxious range allowing them to respond to injurious stimuli (Basbaum et al., 2009). Anatomically, the cell bodies of nociceptors are located in the dorsal root ganglia (DRG) adjacent to the spinal cord or in the trigeminal ganglia in the case of sensory information arising from the face. Their axons arise from these cell bodies and have both a peripheral branch that innervates the tissues where stimuli are transduced, and central branch innervating the spinal cord. While other classes of sensory primary afferents may have complex peripheral apparatus for the detection of stimuli, e.g. Meissner's corpuscles for low threshold touch, nociceptors are present at the periphery as simple branched free nerve endings (Lumpkin and Caterina, 2007). Despite this apparently simple anatomical arrangement, nociceptors have a complex array of cellular and molecular machinery that enables stimulus transduction, as detailed below.

Classifying Nociceptors

Primary afferent neurons are classically characterised by their diameter and degree of myelination which both determine their conduction velocity (see Table 1). Most large myelinated afferents (termed Aβ fibres) are low frequency mechanoreceptors which respond to touch or hair movement (Abraira and Ginty, 2013). Two major classes of nociceptor exist. The first are medium diameter, myelinated afferents (Aδ) and these are responsible for the transmission of well-localised 'fast' or 'first' pain (Ringkamp et al., 2013). The second are

unmyelinated, small C-fibre nociceptors which convey poorly localised ‘slow’ pain. The Aδ nociceptors can be further subdivided into two functional groups (Treede et al., 1998). Type I Aδ nociceptors respond to mechanical and chemical stimuli but have high heat thresholds but will sensitise in the context of tissue injury. These fibres are probably responsible for ‘first’ pain from mechanical stimuli such as a pin prick. Type II fibres have a much lower heat threshold and a high mechanical threshold and are involved in ‘fast’ pain responses to heat.

Mammalian C-fibre nociceptors can be further classified, not by their functional or conduction properties but on a molecular basis based on receptors and neurochemicals that they express. A wide range of markers has been studied with the aim of defining neuronal subpopulations and correlating these with the response properties of the nociceptors. It is common practice to divide the nociceptive C-fibres into two groups; the peptidergic fibres marked by the expression of calcitonin gene-related peptide (CGRP) and substance P, and the non-peptidergic group identified by their binding of isolectin B4 (IB4) (Snider and McMahon, 1998). A plethora of other single markers, such as the transient receptor potential channels (TRP channels) and the Mrg family of G-protein linked receptors, have been suggested to define functional populations (Zhang et al., 2013) but recent unbiased molecular strategies aiming to define classes within all sensory primary afferents indicate the situation is clearly more complex (Li et al., 2016; Usoskin et al., 2014). These studies have sought to define primary afferent heterogeneity using modern molecular techniques to analyse mRNA transcripts in the cell bodies of DRG neurons. Individual cells can then be classified, not by a single marker, but by the constellation of genes that they express. The result is that primary afferents are grouped on a molecular basis into ten or eleven subgroups and in one study the functional significance of these groups has been interrogated (Li et al., 2016).

It is of interest that not all sensory C-fibres are nociceptive. Small subgroups of C fibres appear to be specifically responsible for itch transduction and are termed pruritoceptors (Han

et al., 2013; Mishra and Hoon, 2013). Additionally, two small classes of C-fibres are low threshold afferents (termed C low threshold mechanoreceptors, (C-LTMR)) that are involved in the transduction of pleasant or gentle touch sensations (Seal et al., 2009; Vrontou et al., 2013).

Much of the knowledge about nociceptors is derived from those that innervate the skin, so called cutaneous nociceptors, rather than those that innervate the viscera and convey the impulses that can lead to the sensation of visceral pain. As such, much of what is presented in this section is relevant only to cutaneous nociceptors. In some respects, visceral sensory neurons with the capacity to convey nociceptive information are similar to cutaneous nociceptors; their cell bodies are present in the DRG (or nodose ganglia in the case of vagal visceral afferents), and they generally possess thinly myelinated or unmyelinated axons and small to medium sized cell bodies (i.e. Aδ & C fibres). Some visceral afferents, however, traverse pre- and paravertebral ganglia en route to the spinal cord (Gebhart and Bielefeldt, 2011). Importantly, the viscera are sparsely innervated compared to the non-visceral tissues, and visceral nociceptors have markedly different response properties. Specifically, visceral nociception and hence pain does not arise from cutting or burning of organs, rather it arises from distension, traction, ischaemia and through release of chemical mediators of inflammation. While our knowledge of the biochemical differences underlying these functional differences is incomplete, it is safe to say that the make-up of receptors and ion channels present on visceral nociceptors is unique, and several notable differences have been reported (Robinson and Gebhart, 2008).

Mechanisms of stimulus transduction

Acute noxious stimuli may be thermal, mechanical or chemical and specific ion channels and G-protein linked receptors are involved in conversion of the stimulus into electrical signals in

the primary afferents. These channels generate an electrical current through either opening, hence allowing the influx of Na^+ or Ca^{2+}, or closing if the channel is responsible for a hyperpolarising current (e.g. a K^+ channel) (Gold, 2013). Many chemical stimuli act via G-protein linked receptors and in these cases intracellular signalling pathways indirectly modify ion channel activity.

The specific channels and receptors that transduce stimuli have been studied extensively with the transient receptor potential channels (TRP channels) being of importance. In the case of heat sensation the TRP Vanilloid 1 (TRPV1) channel would appear to play a prominent role (Cavanaugh et al., 2009). This is of particular interest as TRPV1 is the receptor for capsaicin, the active ingredient in chilli peppers, and there has been significant attention paid to developing drugs acting here (Brown, 2016). TRPV1 is one of some 30 or so members of the transient receptor potential family with other channels also important for stimulus transduction. TRP Melastatin 8 (TRPM8), the receptor for menthol, is proposed to have major roles in the transduction of cold stimuli (Bautista et al., 2007). Other thermotransducers also contribute to temperature sensation and these include two-pore potassium channels (K2P) (Noël et al., 2009) and voltage gated sodium channels (Zimmermann et al., 2007). A number of candidate proteins have also emerged as important contributors to noxious mechanosensation. These include the acid-sensitive ion channels (ASICs) (Omerbašić et al., 2015), Piezo channels (Coste et al., 2010) , TRP Ankyrin 1 (TRPA1) (Corey et al., 2004), and K2P channels , although the molecular basis for mechanotransduction requires further clarification (Basbaum et al., 2009).

The ability to detect chemical signals is an important requirement for an organism for avoiding both environmental noxious chemicals and also to detect endogenous irritants that may be produced as a result of injury and inflammation. The TRP channels are particularly important here acting as the receptors for capsaicin (TRPV1), mustards and garlic (TRPA1),

and a wide array of other chemical irritants (e.g. TRPA1 transduces the aversive smell of isoflurane). A number of ion channels (e.g. ASICS) and a wide variety of G-protein linked receptors are present on peripheral nociceptor terminals (Yaksh et al., 2015) which can sense the substances produced by the process of inflammation and sensitise the nociceptor giving rise to lower thresholds. These mechanisms are discussed in more details below. However, it is also worth stating that a number of mechanisms exist whereby nociceptor activity can be modulated peripherally (Pan et al., 2008). Antinociceptive G-protein receptors involved include opioid, cannabinoid, somatostatin, muscarinic acetylcholine, $GABA_B$, and α2-adrenergic receptors and most appear to primarily act via modulation of Ca^{2+} channels thus reducing Ca^{2+} entry. This appears to be highly relevant following clinical administration of exogenous opioid agonists at peripheral sites where they can produce significant analgesic and anti-inflammatory effects (Iwaszkiewicz et al., 2013; van Loon et al., 2010).

Transducing the stimulus in the context of inflammation

Disease and injury often results in pain that is not explained simply by the transduction of noxious stimuli as explained above. This so called inflammatory pain is the result of endogenously generated factors which can activate nociceptor terminals (Dawes et al., 2013). In addition, these substances can also sensitise nociceptors, which is to lower their response threshold and increase their response to a given stimulus. This process is termed peripheral sensitisation and the result for the animal are the phenomena of hyperalgesia, where painful stimuli are perceived as more painful, and allodynia, where non-noxious stimuli are perceived as painful.

Inflammatory mediators may be released by a multitude of non-neuronal cells including fibroblasts, keratinocytes, platelets and immune cells, as well as from the peripheral terminals of activated nociceptors themselves; so called neurogenic inflammation (Chiu et al., 2012).

These mediators include prostaglandins, leukotrienes, bradykinin, serotonin, histamine, CGRP, substance P, purines such as ATP, protons, free radicals, lipids, cytokines, chemokines, and neurotrophins such as nerve growth factor (NGF) (Yaksh et al., 2015). Sensitisation may then occur by one of 3 mechanisms; i) direct activation of cation channels causing nociceptor activation, ii) activation of intracellular regulatory pathways via G-proteins to indirectly alter (e.g. phosphorylate) membrane proteins, or iii) alterations to the transcriptional phenotype of the cell (Dawes et al., 2013). In the case of cytokines and chemokines, while some evidence exists to suggest a direct action on nociceptors, their proalgesic action most likely arises from the strengthening of the inflammatory response and consequent release of other mediators.

A number of factors present in the 'inflammatory soup' have received particular attention in terms of the development of therapeutics to target inflammatory pain and peripheral sensitisation. Prostaglandins, leukotrienes and thromboxanes, collectively termed eicosanoids, are thought to sensitise nociceptors rather than activate them directly (Pethő and Reeh, 2012) but the inhibition of prostaglandin synthesis by cyclooxygenase inhibitors is a common and efficacious approach to pain treatment (KuKanich et al., 2012). Inhibition of other lipid inflammatory mediators, such as the soluble epoxide hydrolase pathway shows promise (Guedes et al., 2017). Antagonism of the prostaglandin E2 receptor has also proved to be an efficacious treatment for osteoarthritis pain in dogs (Rausch-Derra et al., 2016), which may have a reduced risk of adverse effects compared to conventional cyclooxygenase inhibition. Nerve growth factor is released during inflammation and acts via the tyrosine kinase (TrkA) receptor expressed on nociceptors to produce hyperalgesia. A potential therapeutic approach to pain that is being investigated is the use of monoclonal antibodies to NGF to reduce its action (Lascelles et al., 2015).

Transmission of the Stimulus

Once the noxious stimulus has been transduced at the periphery it must be transmitted as an action potential to the central nervous system. Voltage-gated sodium and potassium channels are involved in the generation of this action potential. Different classes of sodium channels, such as Nav 1.1, Nav 1.6, Nav 1.7, Nav 1.8 and Nav 1.9, are expressed in sensory neurons, with the later three being predominantly expressed in nociceptors. Recently, mutations within the Nav 1.7 channel have been shown to underlie dramatic insensitivities to pain in human subjects (Cox et al., 2006) while gain of function mutations here have been shown to cause painful disorders. As sodium channel subtypes seem to be differentially expressed in nociceptive and non-nociceptive primary afferents, there has been interest in these targets for the development of novel analgesics (Emery et al., 2016). Voltage-gated calcium channels are involved in neurotransmitter release at central and peripheral terminals. Calcium channels are composed of α1 pore forming subunits and α2 modulatory subunits. The α2δ subunit is highly expressed in C nociceptors particularly after nerve injury and is an analgesic target of gabapentin (Li et al., 2006).

Integration in the Dorsal Horn of the Spinal Cord

The dorsal horn of the spinal cord is the site of the first synapse of the primary afferent neuron and is a site of tremendous modulation and integration of sensory information before it is projected to the brain. Given the remarkable heterogeneity in the neurobiology of nociceptive primary afferents, we are at an early stage in terms of our understanding of how these inputs both transmit specific stimuli and how this is deciphered by the CNS thereby resulting in a pain percept. The dorsal horn undeniably plays a major role in this processing and whist the theoretical frameworks for pain processing are beyond the scope of this review, those interested readers are referred to an excellent review by Moayedi and Davis (2013).

The grey matter of the spinal cord is divided into laminae based on cytoarchitectonic criteria (fig 1) (Rexed, 1952). The superficial dorsal horn receives the majority of nociceptive afferent input and is composed of laminae I and II. In this area the dorsal horn contains 4 basic neural components: the central terminals of primary afferent neurons, excitatory and inhibitory interneurons, projection neurons and descending modulatory axons (Todd, 2010).

Primary afferents terminate in the dorsal horn in a well-ordered fashion determined by fibre type within a somatotopic arrangement. The input to the dorsal horn is also stratified by somatosensory modality. Aδ nociceptors end mainly in lamina I, peptidergic primary afferents arborize mainly in laminae I and II outer whereas most non-peptidergic C fibres form a band occupying the central part of lamina II (Fig 1). Non-nociceptive afferents such as Aβ tactile fibres end mainly in the deeper laminae III-V (Abraira et al., 2017). Within this arrangement synapses with second-order neurons may have simple single synaptic arrangements or may form complex synaptic glomeruli which give rise to numerous synapses and receive axo-axonic inhibitory inputs from local interneurons (Ribeiro-da-Silva and Coimbra, 1982). Primary afferents all use glutamate as their principle neurotransmitter and hence all synapses with second order neurons are excitatory. These second order neurons receiving synapses may be interneurons which form complex circuits within the dorsal horn, or projection neurons.

Visceral nociceptive primary afferents arborise in a unique way in the dorsal horn and this pattern of innervation, alongside the fact that the viscera are comparatively sparsely innervated, underlies the often diffuse and poorly localised nature of visceral pain. These afferents project extensively to both superficial and deep laminae (I, II, V & X) but notably spread out over several spinal segments and may decussate on the opposite side of the cord (Gebhart and Bielefeldt, 2011). Viscerosomatic convergence is common, such that almost all second order spinal neurons receiving visceral input also receive somatic input from skin or

muscle. This provides an explanation for the phenomenon of referred pain, where visceral nociception is not perceived at the site of origin, rather at an adjacent or distant somatic site (Cervero, 1994). A good example of this is pain felt in the shoulder which results from gas accumulation in the abdomen and consequent diaphragmatic irritation following laparoscopy.

Projection Neurons; the Output from the Dorsal Horn

For a noxious stimulus to be perceived as painful, it must first be projected to higher centres in the brain. Nociceptive specific projection neurons are concentrated in lamina I and scattered through laminae III-VI. Projection neurons in deeper laminae (V) may not be specific to noxious stimuli and are termed wide dynamic range neurons (WDR) as they respond to a broad range of input and encode stimulus intensity (Sikandar et al., 2013). Despite their importance, projection neurons only comprise around 5% of the cells in the superficial dorsal horn (Spike et al., 2003).

The axons of these projection cells cross the midline, travel in ascending spinal white matter tracts and innervate various brainstem and thalamic nuclei. The white matter tracts involved in the projection of nociceptive information vary depending on species; the spinothalamic tract would appear to be most important in humans and primates, the spinocervicothalamic tract predominant in carnivores, and the spinoparabrachial tract most important in rodents (Dostrovsky and Craig, 2013). The brainstem and thalamic nuclei receiving projections include the medulla (caudal ventrolateral medulla (CVLM) & the rostral ventromedial medulla (RVM)), the parabrachial area (Pb), the periaqueductal grey (PAG), the nucleus of the solitary tract (NTS) and the thalamus (Figure 2). Each of these areas is believed to code for specific dimensions of the pain experience (West et al., 2015). The parabrachial area is thought to be particularly important in terms of the affective component of pain as its output provides for a rapid connection to the amygdyla and hypothalamus. The thalamus has been

associated with the sensory-discriminatory aspects of pain due to its connections to the somatosensory cortex. Both the PAG and CVLM are thought to be upstream of other brainstem areas that control powerful descending inputs to the spinal cord (see below).

The majority (80%) of lamina I projection neurons express the neurokinin 1 receptor (NK1r) upon which substance P acts. This receptor has attracted considerable interest as selective ablation of the cells expressing NK1r reduces hyperalgesia in inflammatory, cancer and neuropathic pain models (Mantyh et al., 1997) including in studies conducted in clinical canine patients (Brown and Agnello, 2013).

Synaptic Mechanisms and Plasticity

As mentioned above, all synapses from primary afferents onto second order neurons are excitatory and use glutamate as a neurotransmitter. Although glutamate is the primary neurotransmitter, it can be co-localised with the neuropeptides substance P and CGRP, which also play a role in nociceptive signalling (De Biasi and Rustioni, 1988). Glutamate acts on three ionotropic receptors; the kainate receptor, the alpha-amino3-hydroxy-5-methy-4-isoxazoleproprionic acid (AMPA) receptor, and the N-methyl-D-aspartate (NMDA) receptor. It also acts via the metabotropic glutamate receptor (mGlu).

Synapses within the dorsal horn display activity-dependent plasticity in response to prolonged or high intensity noxious input (Sandkühler, 2009). A result of this inherent plasticity is facilitation of the signal such that the information relayed to higher centres is not coupled to the intensity or duration of the peripheral stimulus (Latremoliere and Woolf, 2009). This phenomenon is commonly termed central sensitisation and results in hyperalgesia and allodynia. Central sensitisation can be readily and rapidly elicited in human volunteers, thus is important in the physiology of acute pain as well as being commonly present in many chronic pain syndromes in humans (Woolf, 2011). The desire to avoid the induction of

central sensitisation also underlies the desire of many anaesthetists to practice preemptive analgesia for surgery. Here, central sensitisation is reduced by the use of analgesics to block the intraoperative nociceptive barrage, thus reducing the magnitude of postoperative acute pain. While this concept has proved controversial and difficult to prove in the human pain literature (Katz et al., 2011), evidence in veterinary species is encouraging (Lascelles et al., 1997) and the concept has been validated experimentally (LaMotte et al., 1992).

Multiple mechanisms underlie central sensitisation but it is well established that the NMDA receptor plays an integral role as its antagonism inhibits activity-dependent plasticity (Bergadano et al., 2009; Dickenson and Sullivan, 1987). The NMDA receptor is usually blocked by a Mg^{2+} ion at resting membrane potential. When the post-synaptic neuron undergoes sustained depolarisation due to the actions of glutamate and also SP and CGRP, this blockade is lifted and glutamate can activate the receptor. This results in a greater influx of Na^{+} and Ca^{2+} and thus amplification of the signal. Subsequent maintenance of central sensitisation occurs due to increases in activity of a number of second messenger systems as a result of an increase in cytosolic Ca^{2+}. Increased activity of intracellular kinases serves to phosphorylate receptors, recruit new receptors and alter gene expression resulting in altered synaptic responses and continuation of sensitisation (Sandkühler and Gruber-Schoffnegger, 2012).

Conversely, a number of endogenous mechanisms may reduce synaptic transmission; the three principle opioid receptors (mu, delta and kappa) are present in high concentrations both pre and post-synaptically in the dorsal horn. Pre-synaptically, opioid receptor activation reduces neurotransmitter release via reducing calcium influx and this is the major mechanism of their analgesic action here (Kohno et al., 1999). It should be noted that opioids also exert a significant effect at supra-spinal levels, particularly at the level of the rostral ventromedial

medulla (RVM). Additional endogenous modulation of noxious inputs to the dorsal horn may occur via descending mechanisms, or inhibitory circuits as discussed below.

Spinal Cord Circuitry

The concept of the dorsal horn as a site of modulatory circuits for noxious signals dates back to Wall and Melzack's gate control theory (GCT) of pain (Melzack and Wall, 1965). GCT proposed that the extent to which a stimulus produced pain was not just a function of the magnitude of the signal in nociceptive specific primary afferents, rather this activity could be modulated at the level of the spinal cord by non-nociceptive afferents. At the centre of the gate control circuit lies an inhibitory interneuron which can be activated by the large fibres resulting in feed-forward inhibition of the action system. Nociceptive specific primary afferents not only activate the action system but also reduce the activity of the 'gating' inhibitory interneuron. It has been demonstrated that the selective inactivation of large populations of inhibitory interneurons in the dorsal horn results in spontaneous pain and itch behaviour and hyperalgesia (Duan et al., 2014; Foster et al., 2015). While these studies and many others previously provide support for the important role of spinal circuitry, the actual circuits involved may be significantly more complex than originally proposed (Mendell, 2014; Peirs and Seal, 2016) containing both excitatory and inhibitory interneurons. The precise identities of spinal neurons and circuits that transmit and gate pain related information remain largely unknown (Zeilhofer et al., 2012).

One of the major barriers to identifying circuits that modulate noxious information is the difficulty in defining functional populations among the interneurons (Todd, 2017). As already stated there are two main types; inhibitory and excitatory interneurons. Inhibitory interneurons use γ-aminobutyric acid (GABA) or glycine as neurotransmitters although many co-express both (Todd, 2010) while excitatory interneurons use glutamate as a

neurotransmitter. A number of classification schemes for superficial dorsal horn interneurons have been suggested and electrophysiological techniques can be used to identify subtypes by input or firing patterns (Yasaka et al., 2010). However, a morphological classification scheme (Grudt and Perl, 2002) has become a widely adopted classification. This describes four morphologically distinguishable interneurons: islet, central, radial and vertical cells. Some of these cells types have been associated with specific roles in nociceptive circuitry (Lu et al., 2013; Lu and Perl, 2003) but this classification scheme leaves a substantial proportion of cells unclassified (Yasaka et al., 2007). An alternative approach to classification has been to use neurochemical markers such as neuropeptides, receptors, enzymes and calcium binding proteins that are expressed by discrete populations of interneurons. GABAergic interneurons have recently been divided into non-overlapping subpopulations defined by expression of galanin/dynorphin, neuropeptide Y, neuronal nitric oxide synthase and parvalbumin and these appear to be functionally as well as neurochemically distinct (Polgár et al., 2013; Duan et al., 2014; Bourane et al., 2015; Petitjean et al., 2015). Excitatory interneurons have also recently been divided into distinct groups based on their expression of somatostatin, substance P, gastrin-releasing peptide and protein kinase C gamma (PKCδ) (Gutierrez-Mecinas et al., 2016, 2017).

Based on current knowledge, it is difficult to rationalise clinical or therapeutic decisions using our evolving knowledge of circuitry. However, manipulation of spinal circuits can powerfully influence nociceptive transmission in many ways. In particular circuits involving excitatory interneurons underlie the potential for touch information to be able to access nociceptive specific projection neurons via heterosynaptic facilitation, hence causing allodynia (Graham et al., 2007). Recent studies have demonstrated the important roles of excitatory interneurons expressing somatostatin (Christensen et al., 2016; Duan et al., 2014), PKCδ (Lu et al., 2013) and vesicular glutamate transporter 3 (VGLUT3) (Peirs et al., 2015)

in the generation of heightened pain states. Adequate inhibitory control of these pathways by the GABAergic or glycinergic interneuron population is important for prevention development of hyperalgesia and allodynia (Foster et al., 2015) . It is thought that a number of clinical chronic pain states may be due to disinhibition of spinal cord circuits. Quite why this may occur is not entirely clear but a promising mechanism may be due to downregulation of the neuronal potassium-chloride co-transporter (KCC2). This mechanism has been suggested to be driven by the microglial response in the dorsal horn seen after injury (Coull et al., 2005). While this mechanism is probably more important in chronic and neuropathic pain, it may still be important in acute inflammatory pain (McMahon and Malcangio, 2009). Interestingly, the spinal neuroimmune interactions underlying this sensitisation appear to differ between males and females in experimental studies, and this may underpin differences in clinical pain sensitivities between sexes (Mogil, 2012).

Descending controls

Descending control pathways from brainstem regions project to the dorsal horn of the spinal cord. They represent a mechanism through which the transmitted nociceptive signal may be facilitated - enhancing the pain experienced, or inhibited - reducing pain. They provide a top-down mechanism whereby cognitive, emotional or autonomic factors can regulate pain processing at the dorsal horn (Bannister and Dickenson, 2017). One can see the potential advantages of antinociceptive mechanisms which can be engaged during 'fight or flight' situations for example. Inputs from many brain regions activate areas of the PAG and this in turn feeds into the RVM and locus coeruleus (LC). From here neurons project bilaterally to the dorsal horn where they release 5-hydroxytryptamine (5-HT) and noradrenaline (NA). Noradrenaline acts via α2-adrenoceptors resulting in antinociceptive effects. The situation with 5-HT is somewhat more complex as it can result in anti- or pro-nociceptive effects through action at either the 5-HT_7 or 5-HT_3 receptors respectively (Dogrul et al., 2009).

Recent evidence points to RVM neurons releasing 5-HT having a predominantly facilitatory effect on nociceptive transmission (Cai et al., 2014). Notably, descending facilitation rather than inhibition predominates overall in young animals. This dominance alongside numerous other neurobiological changes may underlie the excitatory dominance that is a feature of pain in neonates and infants (Fitzgerald, 2015).

One consequence of this descending pain modulation system is that one pain can inhibit another, e.g. an ear pinch may reduce the pain from a toe pinch. This phenomenon is termed diffuse noxious inhibitory controls (DNIC) and this can be easily evoked and quantified in animals and man, thus allowing measurement of the activity of the descending pathways (Bannister and Dickenson, 2017). Reduced DNIC is indicative of altered descending modulation which may be predictive of the actions of analgesic drugs and the degree of pain morbidity after injury (Yarnitsky, 2010).

Representation of Pain in the Brain

As outlined above, information from the spinal cord is projected to various centres in the brain and the resultant neuronal activity gives rise to the multidimensional experience that is pain. Functional imaging studies in humans and animals have increased our knowledge of the brain areas involved hugely (Borsook and Becerra, 2011; Guillot et al., 2015; Tracey and Mantyh, 2007). This combined set of brain regions is often referred to as the “pain matrix" and encompasses areas involved with sensory-discriminative, affective and cognitive aspects.

However, the pain matrix is not entirely specific to pain in the way the visual cortex, for example, is to sight. Instead, none of the regions are unique to pain and many are involved in other aspects of perception and behaviour (Tracey and Johns, 2010). Recently the dorsal posterior insula, and its analogue in other animals, has been suggested as a fundamentally important site for tracking the intensity component of a noxious stimulus at a cerebral level

(Segerdahl et al., 2015). However, while similar regions are implicated in cerebral pain processing and perception in both animals and humans (Thompson and Bushnell, 2012), it is currently unknown how the complex interplay between these areas determines what constitutes a painful experience across the phylogeny. Cognitive and affective top-down regulation of pain via the 'pain matrix' also underlies the placebo and nocebo effects (Tracey, 2010) which are reliant, at least in humans, on prior experience and expectation. The investigation of true placebo effects in animals is at a very early stage (Muñana et al., 2010).

Conclusions

Acute pain is common as a result of surgery, illness or injury and is undeniably unpleasant. Our knowledge of the circuits and cellular and molecular mechanisms underlying acute pain and plasticity is expanding rapidly. In parallel to this explosion of interest in nociceptive mechanisms, researchers are developing novel and highly specific pharmaceutical and genetic techniques to precisely manipulate biological systems. This knowledge should enable the development of new therapeutics and approaches to treating pain based on mechanistic principles with increased efficacy and fewer side effects; ultimately enabling veterinarians to treat pain and the resultant suffering in animals more effectively.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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Figure Legends

Fig 1.

The dorsal horn of the spinal cord is divided into laminae. Different classes of cutaneous primary afferents have specific patterns of innervation of the dorsal horn. The figure demonstrates the stratification of the dorsal horn by somatosensory modality with those afferents carrying impulses resulting from noxious cutaneous stimuli primarily innervating laminae I and I, and those conveying touch signals projecting to the deeper laminae. (C-LTMR stands for C-low threshold mechanoreceptor.)

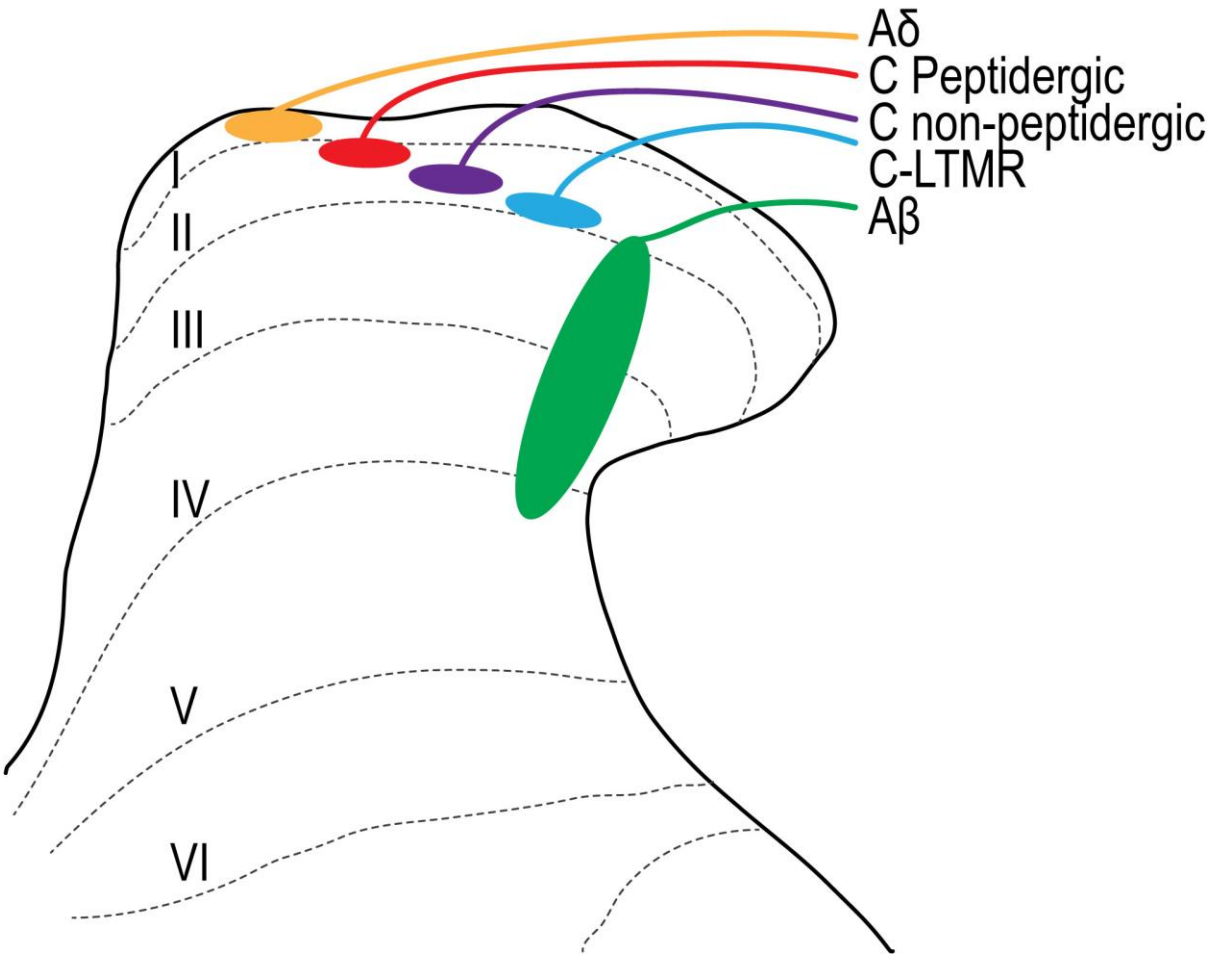
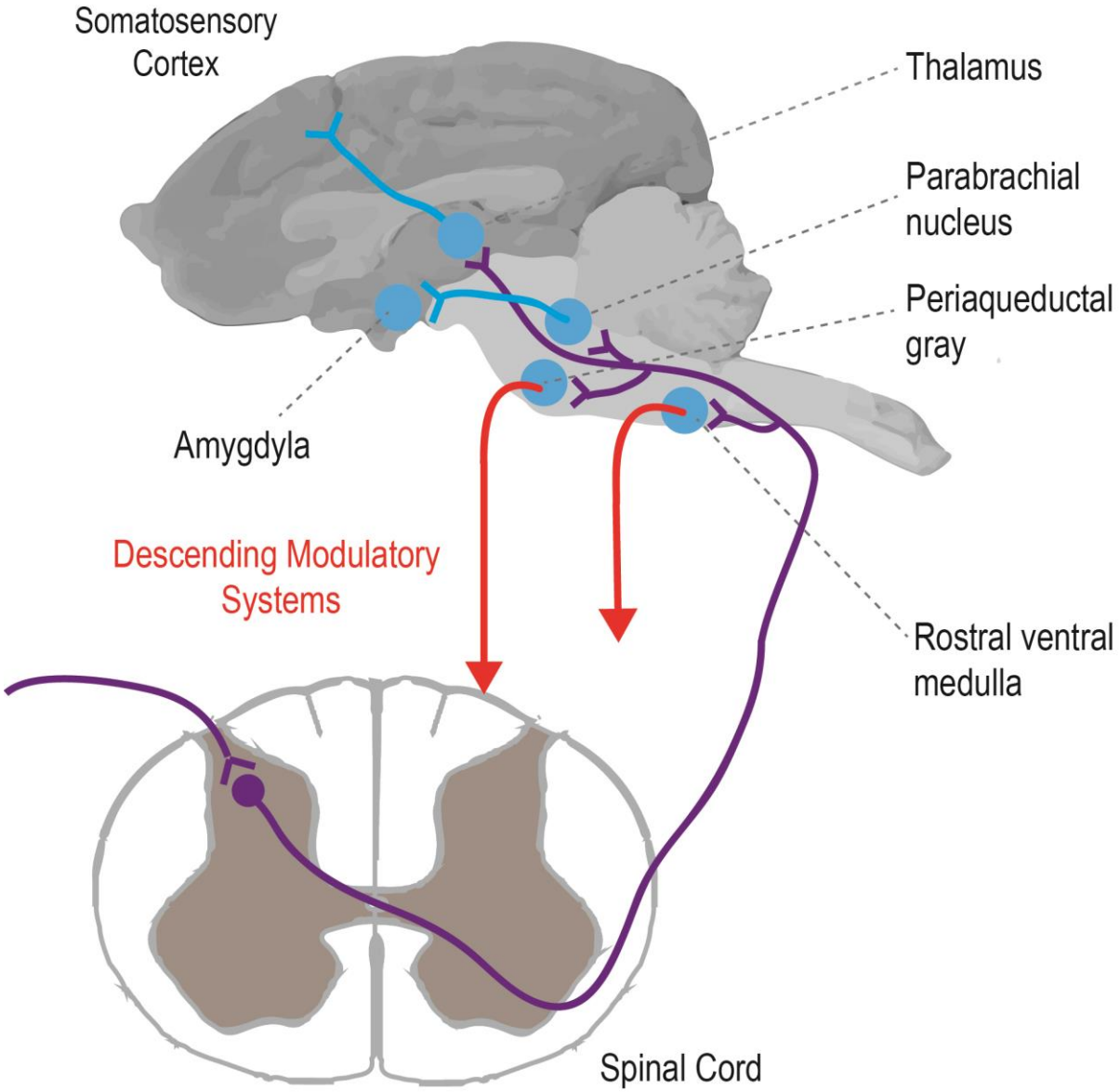


Fig 2.

Projection neurons arise from the dorsal horn of the spinal cord. These innervate various higher centres responsible for aspects of the pain experience including the thalamus and the parabrachial nucleus. Neurons of the ventral medulla and midbrain periaqueductal gray are also engaged which may activate descending feedback systems. The simplified figure shows the sites of primary terminations from projection neurons. Initial activation of these areas forms the basis of a resultant coordinated activity in a multitude of brain regions which comprise the 'pain matrix'.

The figure shows a single projection neuron, in this case a spinothalamic tract (STT) neuron. Note - species differences exist in the white matter tracts for the projection of nociceptive information as detailed in the text.



Tables

Table 1 – The classification of primary afferents by fibre diameter and conduction velocity.

Classification	**Diameter**	**Myelin**	**Conduction velocity**	**Sensory Function**
Aβ	Large (6–12 μm)	Yes	>35 m/s	Touch
Aδ	Medium (1–5 μm)	Thin	5-35 m/s	'Fast' pain
C	Small (0.2-1.5 μm)	No	<2.0 m/s	'Slow' pain