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Review Article

Perioperative Nociception and Pain in Dogs and Cats - Part I: An Illustrative Text on Pain Mechanisms, Models and Assessment

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Abstract

Effective perioperative pain management depends on a thorough understanding of pain mechanisms and reliable assessment methods. Pain serves as a vital protective mechanism, acting as an alarm for underlying disease, trauma, or surgical injury. In disease, it prompts investigation of causative factors, while in trauma or surgery, it encourages protection of injured tissues. Inadequate management of acute pain can lead to significant physiological and behavioral consequences, including stress hormone release, increased heart rate and blood pressure, behavioral alterations, delayed wound healing, chronic pain development, and prolonged hospitalization. The purpose of this review is to enhance clinicians' and researchers' understanding of acute pain and nociception in dogs and cats, with particular emphasis on perioperative settings. The physiological processes involved in pain transmission, modulation, perception, as well as peripheral and central sensitization are discussed. Additionally, commonly used subjective and objective pain assessment techniques, including behavioral scoring systems and physiological indicators, are reviewed to support accurate pain recognition. This review is presented in two complementary parts. Part I focuses on pain pathophysiology and assessment, forming the foundation for Part II, which evaluates pharmacological and multimodal analgesic strategies for perioperative pain management.

Keywords: Cats, Dogs, Nociceptive pathways, Pain assessment, Perioperative pain, Sensitization

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Introduction

Pain functions as a natural alarm to an underlying disease, trauma or surgery. In disease, it prompts investigation of the cause(s), whereas in trauma or surgery, it acts as a protective warning to safeguard the injured site against further damage. Absence or inadequate perception of pain may result in undetected disease progression and insufficient protection of injured tissues, ultimately leading to adverse outcomes. Poorly managed acute pain can trigger stress hormone release and is associated with reduced activity, increased heart rate and blood pressure, behavioral alterations, shivering, hypersensitivity, aggression, vocalization, anorexia, weight loss, delayed wound healing, depression, transition to chronic pain, and prolonged hospitalization. Therefore, this review aims to discuss the pathophysiology, recognition, assessment, and management of acute pain in veterinary patients, with emphasis on improving animal welfare and clinical outcomes.

Definition: Pain and Nociception

The official definition of pain by the International Association for the Study of Pain (IASP) is: "An unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage" [1,2].

Clinically, pain can be classified as either acute or chronic. Acute pain results from an injury and ends with wound healing. Chronic pain lasts beyond acute pain or wound healing.

Generally, pain that persists for more than three months is considered chronic pain. This suggests that there is an ongoing slow change over time in the mechanism leading to chronic pain; and that there is not anything that otherwise causes "chronic pain" after three months. Typical example of causes of pain is surgical pain, trauma, or onset of acute disease, such as pancreatitis, whereas osteoarthritis, cancer and periodontal disease are examples of conditions in which chronic pain is seen. Surgical pain that exists beyond the expected time frame of acute pain is called chronic pain [1,3,4]. In animals, verbal communication is not comprehensible across different species, hence, making the task of pain assessment more challenging for clinicians and researchers [5]. It is highly important for a clinician to have a good understanding of pain physiology, pathways, and assessment methodology in order to treat it effectively.

Let us briefly consider why animals do not consciously perceive pain under general anesthesia. If pain perception is absent, one may question why physiological parameters such as heart rate, blood pressure, and respiratory rate increase in response to surgical stimulation during anesthesia, while animals may vocalize due to pain immediately upon recovery.

This phenomenon is explained by the process of nociception, which refers to the neural encoding and processing of noxious stimuli [6,7]. Nociception is distinct from pain in that it does not involve a conscious emotional experience; rather, pain requires processing at the level of the conscious brain. Under general anesthesia, cortical function and the emotional component of pain perception are suppressed. However, nociceptive pathways at the peripheral and spinal level may remain active.

As a result, surgical stimuli can continue to generate nociceptive input, leading to autonomic responses such as increases in heart rate, blood pressure, and respiratory rate, despite the absence of conscious pain perception. Importantly, once the animal recovers from anesthesia and cortical function is restored, this nociceptive input can then be perceived as pain, resulting in behaviors such as vocalization.

Nociception begins at the site of tissue injury, where specialized receptors termed nociceptors transduce noxious stimuli into electrical signals. These signals are transmitted through the nervous system for further processing. Thus, while nociception can occur in the absence of conscious pain, the perception of pain requires intact central processing and does not occur without nociceptive signaling.

Nociceptors

The receptors characterised by free, non-encapsulated nerve endings that detect nociceptive (noxious) stimuli are called nociceptors. These are widely distributed in the skin and deep tissue, with their cell bodies located in dorsal root and trigeminal ganglia [1]. These nociceptors are peripheral terminals of primary afferent neurons. Mechanical, chemical and thermal stimuli can stimulate these receptors. Some nociceptors respond to only one of these stimuli (unimodal), whereas others respond to a variety of stimuli (polymodal) [6,7].

Sensory Nerve Fibers: Three classes of sensory nerve fibers have been described based on anatomy (myelination), function and speed of conduction. Large, myelinated group Aα and Aβ nerve fibers - these are low-threshold fibers with a diameter of 10 μm and detect low-intensity, innocuous (touch, vibration, pressure, and limb movement) stimuli with high velocities of 30 to 100 m/s. Smaller, lightly myelinated Aδ fibers and nonmyelinated and nonmyelinated C fibers - Aδ and C fibers are high threshold fibers and are more predominant (>90%) than low threshold fibers [8]. Aδ fibers conduct at a speed of 5 to 30 m/s and detect sharp pricking pain and result in rapid withdrawal from the stimulus as a primary warning (fast pain). C fibers conduct at speeds of 0.5 to 2.0 m/s and detect slow burning pain (slow pain) and are primarily involved in nociceptive signalling. Both Aδ and C fibers supply to the skin and deep somatic or visceral structures [1]. Silent nociceptors represent a subset of C-fiber afferents characterized by a high activation threshold and minimal or absent responsiveness to mechanical or thermal stimuli under normal physiological conditions. Exposure to inflammatory mediators significantly decreases their threshold, and these previously silent nociceptors become stimulated and sensitive to a variety of stimuli [1,9].

Activation of pain receptors in the peripheral tissue (skin) generates action potentials that are conveyed to the central nervous system (CNS) through Aδ and C nerve fibers. Sharp, pin prick and injurious pain (first pain) are transmitted by fast Aδ fibers. However, dull aching pain (second pain) is transmitted by slow C fibers. The primary sensory neurons enter the spinal cord and synapse with neurons in the dorsal horn. From the dorsal horn, second-order neurons having long axons cross in the

anterior commissure and pass through the spinothalamic tract towards the brain. Some long axons that synapse with C neurons do not cross but pass cranially in the ipsilateral anterolateral spinal pathway. Fibers from the anterolateral spinal pathway terminate in the thalamus from where signals are passed to the sensory cortex and other centers in the CNS, where pain perception takes place. Pain consists of three main components: a sensory-discriminatory component (temporal, spatial, thermal/mechanical), an affective component (subjective and emotional, describing associated fear, tension, and autonomic responses) and an evaluative component, describing the magnitude of the quality (e.g. stabbing/pounding; mild/severe) [1,10-12].

Anatomy of the Spinal Cord

Fig. 1 illustrates the anatomy of the spinal cord which is classified into gray and white matter. In a broader overview, the gray matter is divided into three distinct regions: dorsal horn, intermediate zone, and ventral horn, which are further subdivided into ten undemarcated laminae *(Fig. 1)*. The dorsal horn comprises laminae I to VI, the intermediate zone comprises lamina VII, the ventral horn comprises laminae VIII to IX, and lamina X surrounds the central canal of the spinal cord [13]. In primary afferent fibers, the thinly myelinated Aδ fibers project to lamina I and V, thick myelinated Aβ fibers project to lamina III, VI and V, while, non-myelinated C fibers project to lamina I and II in the dorsal horn of the spinal cord.

Electrophysiological studies have revealed that spinal cord neurons within lamina I generally respond to noxious stimuli, neurons in laminae III and VI respond to non-noxious stimuli, and neurons in lamina V receive both noxious and non-noxious information. Neurons in lamina V are called wide dynamic range neurons (WDR) given their response to a range of intensities from non-noxious to noxious stimuli [14]. Neurons in lamina I and V carry nociceptive information to the thalamus and brainstem via spinothalamic and spinoreticulothalamic tracts. The spinothalamic tract is relevant to the sensory-discriminative aspect of the pain experience, whereas the spinoreticulothalamic tract is more relevant to poorly localized pain. From the brain stem and thalamus, the information reaches the cortical structures [14].

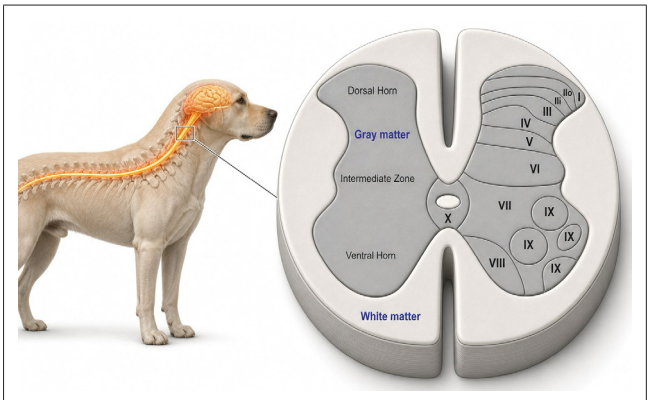

Fig 1. Anatomy of the spinal cord. Classified into gray and white matter. The gray matter is divided into three distinct regions: dorsal horn, intermediate zone, and ventral horn, which are further subdivided into ten undemarcated laminae

Nociceptive Pathway

Nociceptive pathways comprise of ascending and descending pathways. Propagation of noxious information from the site of injury towards the brain occurs through the ascending pathway, whereas movement of information from the brain to the periphery occurs through the descending pathway. This processing of information occurs through the phenomenon of nociception.

Nociception

Nociception is a phenomenon by which intense mechanical, thermal or chemical noxious stimuli are detected by nociceptors, a subpopulation of peripheral nerve fibers [14]. The process of nociception takes place in a few steps: transduction, transmission, modulation projection and perception *(Fig. 2, Fig. 3)*.

When tissue is damaged, noxious stimuli are translated into nociceptive signals through a process called transduction. Transmission is the process by which these signals from the periphery reach the spinal cord. Along the nociceptive pathway, signals undergo both inhibitory

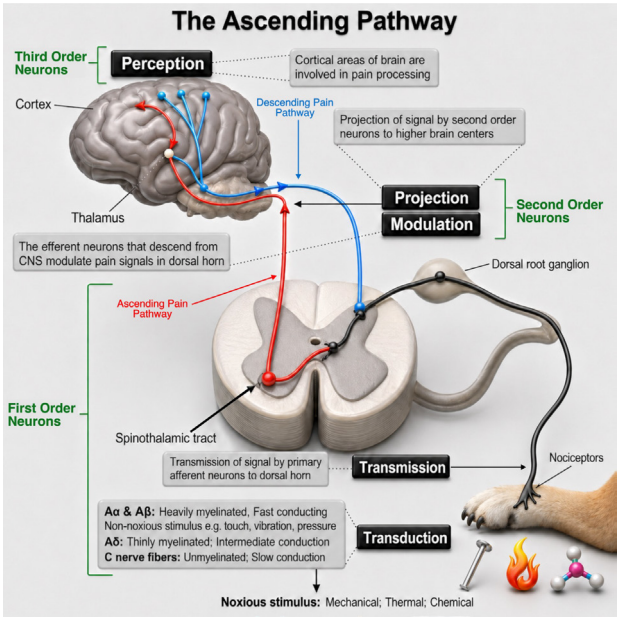

Fig 2. Illustrative summary of the ascending pathway of nociception. When tissue is damaged, noxious stimuli are translated into nociceptive signals through a process called transduction followed by transmission, modulation, projection and perception. Phases of the pain pathway, types of nerves involved, and anatomical regions have been labelled

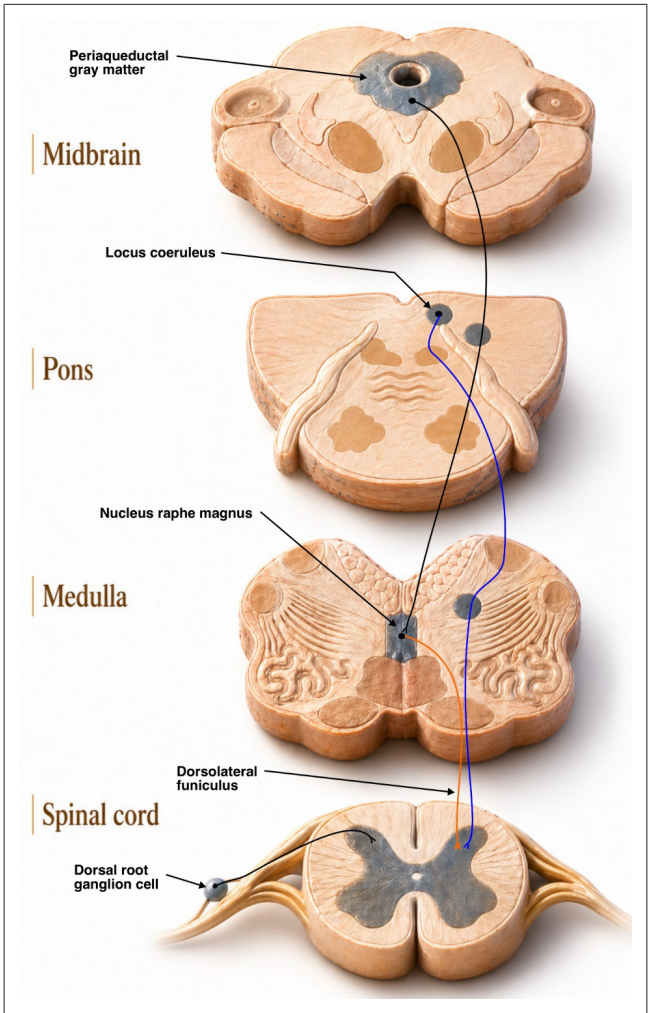

Fig 3. The descending inhibitory pathway. Periaqueductal gray matter (PAG) and rostroventral medulla (RVM) are the main regions involved in the descending inhibitory pathway. Both centers have high concentrations of opioid receptors and endogenous opioids. The PAG receives inputs from brain regions of interest, such as the thalamus, hypothalamus and reticular formation, and sends to the nucleus raphe magnus and locus coeruleus. These centers project to the dorsal horn to inhibit pain transmission

and excitatory processes, collectively termed modulation. This process regulates the intensity and transmission of nociceptive input through mechanisms within the peripheral nervous system, spinal cord, and brain.

Projection refers to the ascending transmission of nociceptive signals from the spinal cord to higher brain centers. This process ultimately leads to perception, the final stage of nociception, in which these signals are consciously interpreted as pain [15].

Transduction: When nociceptors are stimulated by noxious stimuli (heat, cold, chemical or mechanical), this results in a conformational change in the receptor. With this change in the receptor the noxious signals are converted into electric current by activating the opening of depolarizing cationic ion channels or the closing of outward potassium channels [16]. Various molecules, such as members of the transient receptor potential (TRP) cation channel family, acid-sensitive ion channels (ASIC) and purine-activated ligand-operated ion channels, have been identified in the process of transduction. The TRP family members include, transient receptor potential subfamily ankyrin (TRPA), transient receptor potential subfamily canonical (TRPC), transient receptor potential subfamily melastatin (TRPM), and transient receptor potential subfamily vanilloid (TRPV) [17,18] transduce thermal and chemical signals. Certain receptors of the TRP family are activated by a decrease in local pH, or chemical ligand, i.e., capsaicin (the vanilloid constituent that provides the "hot" in hot peppers). The TRPA and TRPM share the role of transducing thermal signals with many two-pore potassium channels (K2P) and $Na_v1.8$ channel [19]. Ischemia and inflammation result in an increase in hydrogen ion concentration, which activates ASIC. Damage to cells leads to excesses of adenosine triphosphate (ATP) that signal to purinergic receptors; P2X is a purine-activated ligand-operated ion channel, and P2Y is a G-protein-coupled receptor. Bacterial lipopolysaccharides and inflammatory cytokines result in expression of the bradykinin-1 (B1) receptor, while the bradykinin-2 (B2) receptor is constitutively expressed. Activation of B1 and B2 forms inositol 1,4,5-triphosphate and diacylglycerol with subsequent increases in intracellular calcium concentration. Activation of bradykinin B_2 receptors leads to the production of prostaglandin E_2 (PGE_2) and prostacyclin (PGI_2), which act to sensitize nociceptors and amplify the nociceptive signal [20]. Piezo channels, TRPA1 and TRPV4, transient Ca^{2+} channels, and at least three K2P channels are among the transducers known to be involved in mammalian mechanotransduction [21-26]. Clinically, activation of these receptors leads to heat hypersensitivity as a result of tissue injury and inflammation, contributing significantly to the development of painful condition.

Recent research has revealed that TRPV1-4 channels play a role in transduction of chemical and thermal stimuli and are expressed in afferent nociceptors, and pain-sensing neurons [27]. Two of the channels, TRPA1 and TRPM8, are activated by cold temperature [28]. These channels have been proposed as a therapeutic target due to their association with heat and cold thermal stimuli. Further research into nociception will facilitate the development of more effective and targeted therapeutic strategies for the management of pain and its associated pathologies.

Transmission: Once the successful transduction of nociceptive stimuli by primary afferent neurons takes place, different voltage-gated ion channels are activated by receptor potentials. Voltage-gated sodium and potassium channels play significant roles in the generation of action potentials, which transmit nociceptive signals to the dorsal horn [14]. Tetrodotoxin (TTX)-resistant channels,

TTX-sensitive channels and voltage-gated sodium channels (Na_v) 1.1, 1.6, 1.7, 1.8 and 1.9 are different types of voltage-gated sodium channels [29]. Tetrodotoxin (TTX)-sensitive channels cause rapid depolarisation and are responsible for acute pain signal transmission. Tetrodotoxin (TTX)-resistant channels are responsible for transmitting slow, burning, or inflammatory pain signals (neuropathic pain). clinically these receptors are related to several chronic pain conditions, manifested as hyperalgesia and allodynia. When the threshold potential is reached, an action potential is generated, allowing propagation of the nociceptive signal along the neuron. This action potential arises through the coordinated opening of voltage-gated sodium (Na^+) and calcium (Ca^{2+}) channels, which mediate membrane depolarization, along with the relative reduction in potassium (K^+) efflux that otherwise contributes to membrane hyperpolarization [20]. Nociceptive signals are transmitted to the CNS via the dorsal horn of the spinal cord. The cell bodies of primary afferent nociceptors are located in the dorsal root ganglia. Within the dorsal horn, primary afferent neurons synapse with secondary neurons and interneurons across different laminae. These connections play a critical role in the integration, modulation, and further transmission of nociceptive information to higher centers of the brain.

Modulation or Descending Inhibitory Pathway: The pain signals in the dorsal horn are modulated by efferent neurons that descend from the CNS. In addition to antinociceptive pathways, descending pathways exist with the ability to modify the pain reaction. The descending pathways include thalamocortical structures, pons of the brain stem, the PAG of the midbrain, the rostroventral medulla (RVM), and the dorsal horn; PAG and RVM are generally considered core regions of this important system *(Fig. 3)* [30,31].

Modulation of plasticity augments sensitivity (excitatory) to incoming nociceptive signals; this modulation takes place after release of excitatory neurotransmitters such as substance P, glutamate, and calcitonin gene-related peptide (CGRP). Augmented sensitivity is facilitated by upregulation of excitatory neurotransmitter receptors (Structural Plasticity) and decreased signal intensity required to induce conformational changes in receptor proteins (functional plasticity). On the other hand, sensitivity of secondary neurons to incoming information is decreased due to hyperpolarization in the postsynaptic nerve terminals or decreased influx of calcium into presynaptic terminals. This decrease in sensitivity (inhibitory) occurs after the release of GABA, endorphins and enkephalins, norepinephrine, and serotonin acting at some serotonin receptors [30,31].

"On" and "Off" cells are the unique populations within the RVM. Off-cells hyperpolarize in response to activation of the spinothalamic tract and decrease the transmission of the streams of nociceptive action potentials in the brain stem [32]. On the other hand, nociceptive information from the spinothalamic tract causes excitation of on-cells and engages parabrachial, hypothalamic, cingulate, insular, and septohippocampal pathways subserving arousal and aversive reactions to pain [33]. These on-cells appear to be important in producing hyperalgesia by maintaining central sensitization after peripheral tissue injury [32,34]. This understanding of on-cells and central sensitization is crucial for managing chronic pain conditions, where hyperalgesia can significantly impact a patient's quality of life.

Projection and Perception: The dorsal horn of the spinal cord possesses bodies of second-order nociceptive neurons. From the dorsal horn, these second-order neurons carry nociceptive impulses through their axons projecting to higher CNS centers, which are responsible for the processing of nociceptive information. The cortical regions involved in the pain processing are the primary and secondary somatosensory cortex and its vicinity in the parietal operculum, insula, anterior cingulate cortex and prefrontal cortex. These are collectively called pain matrices [35]. Imaging studies have revealed that the thalamus, insula, hypothalamus and ventral tegmental area (VTA) are associated with the stimulation of C fibers in response to noxious stimulation in rats [36].

The wide dynamic range and high threshold neurons (spinothalamic tract) pass through the pons, medulla and mid-brain to terminate in specific portions of the thalamus. The somatosensory cortex receives nociceptive signals from the thalamus. The reticular formation also receives collateral branches from the spinothalamic tract. Emotional exhibition of pain and sensory discrimination takes place when the signals pass through these tracts. Emotional components of pain, including somatic and autonomic motor reflexes as well as increased arousal is probably the function of the reticular formation [37,38].

Peripheral nociceptors (Aδ and C fibers), responsible for transduction and transmission are slowed down or blocked by anaesthetics at the periphery. Dorsal root ganglia serve a role of relay station for nociceptive information to the CNS, which is the area of modulation of the nociceptive information. General anaesthetics suppress transmission of nociceptive signals within ascending pathways, including the spinothalamic tract, thereby preventing conscious perception of pain. The brain stem regulates respiration and CVS; anaesthetics have minimal depression effects on these centers compared to the thalamus and cortex, responsible for nociception and pain [39-42].

Mechanisms of Sensitization

Sensitization is defined by the International Association for the Study of Pain (IASP) as the 'Increased responsiveness of nociceptive neurons to their normal input, and/or recruitment of a response to normally subthreshold inputs. Peripheral sensitization refers to an increased responsiveness in the nociceptive neurons in the periphery, whereas central sensitization refers to an increased responsiveness of nociceptive neurons in the central nervous system (CNS) to their normal or subthreshold afferent input [43,44].

Hyperalgesia and allodynia are typical clinical characteristics of sensitization. Hyperalgesia is an exaggerated and prolonged response to a noxious stimulus, while allodynia is a pain response to a low-intensity, normally innocuous stimulus such as light touch to the skin or gentle pressure. Hyperalgesia and allodynia are consequences of peripheral and central sensitization [1].

Mechanism of Peripheral Sensitization

Tissue damage during surgery results in the accumulation of endogenous factors produced from stimulated nociceptors and non-neural cells that reside within or infiltrate the injured area. These non-neural cells include mast cells, basophils, platelets, macrophages, neutrophils, endothelial cells, keratinocytes, and fibroblasts. Neuropeptide substances such as substance P, nerve growth factor (NGF) and calcitonin gene-related peptide (CGRP) are produced after the noxious stimulus has been recognized by the sensory nerve endings of peptidergic nociceptors. The release of substance P and CGRP induces vasodilatation, plasma extravasation, and other effects, thus producing 'neurogenic inflammation' [45]. Discharge of vasoactive substances from mast cells and increased vascular permeability leads to neurogenic edema [46].

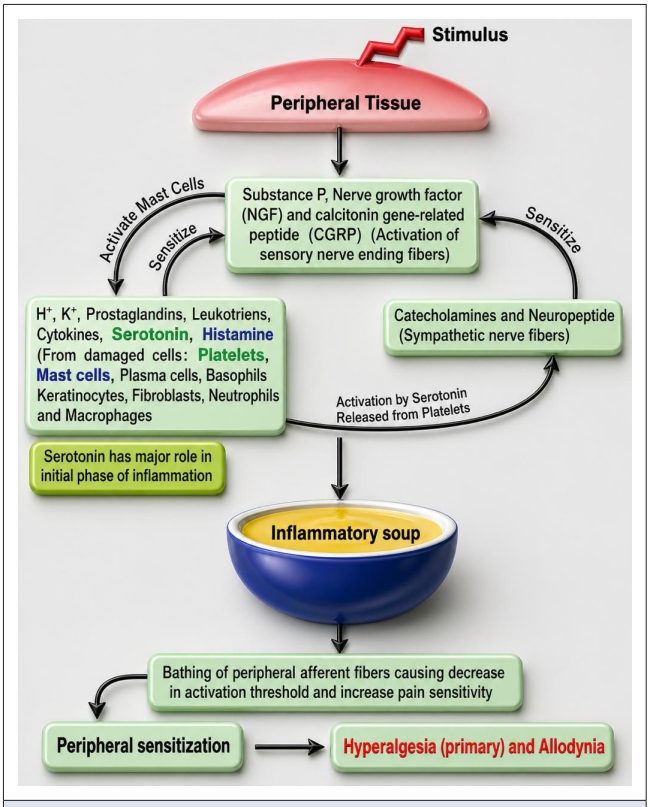

Fig 4. Illustrative summary of peripheral sensitization, where a vicious cycle of neural and non-neural mediators created an inflammatory soup to develop peripheral sensitization. This cascade of events produces an "inflammatory soup", which bathes peripheral nociceptors, consequently decreasing their activation threshold or augmenting their sensitivity to pain, resulting in peripheral sensitization

Histamine and serotonin are also released as a result of increased permeability and act as algogenic substances. Substance P, NGF, kinin and interleukin-I cause degranulation of mast cells, releasing histamine, which acts on sensory neurons to produce pain and itching [47]. This activation of sensory neurons by histamine induces the discharge of neuropeptides and prostaglandins (PGs), resulting in aggravation of inflammation and hyperalgesia. Serotonin released from platelets and mast cells during injury is a major inflammatory mediator during the initial phase of inflammation. Serotonin directly stimulates sensory neurons via activation of the 5HT type-3 ($5HT_3$) receptor. Norepinephrine is released as a result of stimulation of the sympathetic nervous system in response to nociceptive impulses. Norepinephrine accelerates sensitization of the nociceptors, creating another vicious cycle [48]. *Fig. 4* illustrates the vicious and complex mechanism of peripheral sensitization.

In summary, these mediators augment the excitability of nerve fibers, resulting in a decreased activation threshold of nociceptors and, consequently, amplifying the sensitivity to thermal and mechanical stimuli in the surrounding injured tissue. Subsequently, silent nociceptors are also recruited and activated [16]. In addition to neurotrophin injury, these mediators also cause the release of cytokines; among them, interleukin-β (IL-β), IL-6 and tumor necrosis factor α (TNF-α) are the key players in nociception. These cytokines act on nociceptors and contribute to pain hypersensitivity by increasing the production of PGs, bradykinin and extracellular protons [14]. This cascade of events produces an "inflammatory soup" [49]. This inflammatory soup bathes peripheral nociceptors, consequently decreasing their activation threshold or augmenting their sensitivity to pain, resulting in peripheral sensitization [50].

Peripheral sensitization is characterized by allodynia and hyperalgesia. In allodynia, activation thresholds are decreased, resulting in pain from the stimulus that does not normally provoke pain. In hyperalgesia, there is increased pain from the stimulus that normally provokes pain [2]. This hyperalgesia at the site of injury is called

primary hyperalgesia, which is commonly observed following surgery and other types of trauma. However, hypersensitivity at the site of uninjured skin around the wound is called secondary hyperalgesia, which is the function of central sensitization [51]. Activation of silent nociceptors results in mechanical hypersensitivity [10], which is an important feature of inflammatory pain and a result of central sensitization [49].

Mechanism of Central Sensitization

Central sensitization takes place at the level of the dorsal horn. When the noxious stimulus is encoded into an electrical signal, these signals are then transmitted to secondary neurons in the dorsal horn of the spinal cord from the site of injury by primary afferent nerve fibers. The secondary neurons are divided into two classes, "nociceptive specific" or "high threshold" and "wide dynamic range" or "convergent". Nociceptive-specific neurons respond exclusively to noxious stimuli from C and Aδ fibers. Wide dynamic range neurons respond to both noxious and non-noxious stimuli. Bombardment of WDR and nociceptive-specific neurons by afferent C fibers in the dorsal horn of the spinal cord results in a progressive increase in the frequency of firing of second-order spinal neurons at a frequency above 0.5 Hz. This is also known as windup, in which magnesium blocking of the N-methyl-D-aspartate (NMDA) receptors is removed, rendering them for glutamate activation [20].

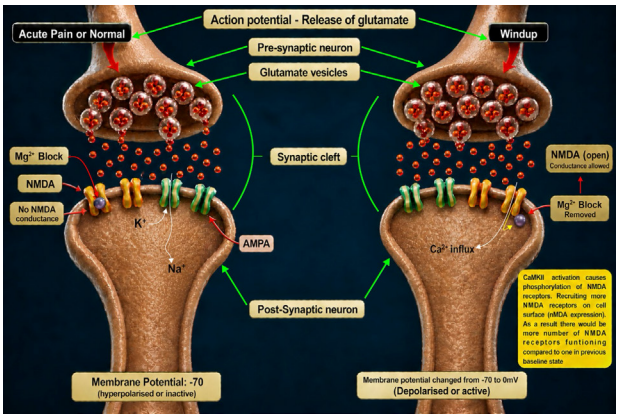

Fig 5. Illustrative summary of central sensitization compared with acute pain or normal nerve stimulus (Bombardment of WDR and nociceptive-specific neurons by afferent C fibers results in a progressive increase in the frequency of firing of second-order spinal neurons at a frequency above 0.5 Hz. This is also known as windup, in which magnesium blocking of the N-methyl-D-aspartate (NMDA) receptors is removed, rendering them for glutamate activation. Glutamate is the main and fast neurotransmitter in the CNS and plays a major role in nociceptive transmission. Glutamate acts at α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors, NMDA receptors, kainate (KA) receptors, and metabotropic glutamate receptors. Glutamate-activated NMDA receptors are involved in the increased synaptic efficiency. Phosphorylation of NMDA receptor activates its mobility from intracellular store to synaptic membrane, enhancing its responsiveness to glutamate by removal of the voltage-dependent magnesium (Mg++) ion block, thereby favoring the open-state configuration)

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Phosphorylation of ion channels and membrane receptors are the post-translational changes caused by activation of the gamma isoform of protein kinase-C. This causes an increase in neuronal excitability for tens of minutes even after the initial stimulus has stopped. This recruits responses to subthreshold stimuli and mediates the change from short-term to long term hyperexcitability of nociresponsive spinal cord neurons The increase in excitation along with a reduction in the inhibition of spinal cord neurons results in central sensitization [10]. However, the neurons in the dorsal horn of the spinal cord are also excited by other second messenger pathways that are activated by brief and intense C-fiber signals [53]. Within seconds of exposure to strong nociceptive stimuli, central sensitization develops, resulting in enhanced nociceptive facilitation that can persist for hours beyond the initial stimulus [54].

There is evidence that stimulation of glial cells within the dorsal horn of the spinal cord also plays a crucial role in the creation and maintenance of pathological pain [55]. When nerve trauma or inflammation occurs, glial cells become activated, which leads to the production of several proinflammatory mediators [56,57]. Crucial pro-inflammatory cytokines include IL-6, IL-1b and TNF-α [56]. Chemokines such as monocyte chemoattractant protein and macrophage inflammatory protein-2 recruit neutrophils and macrophages from the bloodstream into the neurons [57].

Thus, an increased pain state, central sensitization, can be established and sustained by these neuroexcitatory chemicals released after glial cell activation. Central sensitization results in allodynia and hyperalgesia by increasing the receptive field (response area) and tissue sensitivity to thermal, mechanical, and chemical stimuli [58]. The difference between central and peripheral sensitization is that central sensitization allows low threshold sensory fibers (Aβ) to cause pain by increasing the excitability of spinal neurons and produces changes in spinal cord sensory processing. In contrast, peripheral sensitization enables low-intensity stimuli to induce pain by stimulating sensitized Aδ and C fibers. In central sensitization pain signals are produced by low-threshold Aβ mechanoreceptors, which normally produce touch, vibration, and movement pressure (nonpainful) signals. Central sensitization has a crucial contribution to postsurgical hypersensitivity [10].

Thus, surgical trauma contributes to postoperative pain hypersensitivity via peripheral and central sensitization: two different but collaborative mechanisms [59]. In patients undergoing ovariohysterectomy, this phenomenon of peripheral and central sensitization has been demonstrated clinically by an increase in pain score in response to palpation at surgical and distant sites [60-62].

Long-Term Potentiation

Long-term potentiation (LTP) has been reported as an important form of synaptic plasticity in nociceptive spinal pathways, and its mechanism resembles central sensitization. In acute and chronic pain states, it has been suggested as one of the cellular mechanisms contributing to pain amplification. A noxious stimulus is mandatory to develop a typical spinal LTP and therefore speculated to contribute to acute and chronic forms of pain that develop post-operatively or from an initial painful event, neuropathy or peripheral inflammation [63,64].

LTP induction at spinal C-fibre synapses requires a rise in post-synaptic Ca^+ concentration. Post-synaptic Ca^+ rises by opening of the post-synaptic NMDA and Ca^+-permeable AMPA receptors, T-type voltage-gated calcium channels and release of Ca^+ from intracellular calcium stores. All these calcium sources trigger the activation of metabotropic glutamate or neurokinin-1 receptors. Finally, this rise in post-synaptic Ca^+ activates Ca^+-dependent signaling pathways involving calcium calmodulin-dependent protein kinase II (CaMKII), protein kinase C, and nitric oxide synthase (NOS) [65-67].

Currently, the evidence is clear for the post-synaptic Ca^+-dependent form of LTP induction in spinal cord lamina I neurons. Induction of LTP at C-fibre synapses requires activation of group I metabotropic glutamate receptors [68] and co-activation of the NK1 and NK2 receptors. It also requires the opening of ionotropic glutamate receptors of the NMDA type [69-71] and T-type voltage-gated calcium channels [70,71]. Substance P-mediated stimulation of NK1 receptors may directly augment single NMDA channel opening [72] and NMDA receptor-mediated electric signals in lamina I neurons. All this may result into a significant increase in post-synaptic intracellular calcium [70].

In an *in vitro* setting, it has been shown that an increase in post-synaptic intracellular Ca^+ is crucial for LTP induction, and there is a linear correlation between the amount of Intracellular Ca+ increase and the amount of LTP [61]. In a previous study, the data demonstrated that LTP-induced stimuli caused a significant rise in intracellular Ca^+ in lamina I neurons, and this rise was not only observed during slice preparations but also in intact animals [71]. Therefore, it was not surprising that signal transduction involves Ca^+-dependent pathways, including activation of CaMKII, NOS, protein kinase A and C, phospholipase C, inositol triphosphate-3 (IP3) receptors and extracellular signal regulated kinase (ERK), including members of the mitogen-activated protein kinase family (MAPK) [22,70,73-76].

In summary, significant similarities exist between central sensitization and spinal cord long-term potentiation, and both require NMDA receptors and involve key signaling transduction pathways, including extracellular signal-regulated kinase, protein kinase C, Src, and dependence of protein synthesis and gene transcription [64]. The salient features of acute central sensitization are short latency (seconds) by intense, repeated, or sustained nociceptive stimuli, which typically can persist for several hours in the absence of further nociceptive stimulus [77]. However, an additional mechanism may be needed for maintenance of late-phase long-term potentiation (greater than 4 h). Because it is technically challenging to follow long-term potentiation over time, it is unknown if there is ongoing spinal long-term potentiation, months after painful insults [64].

NMDA receptors play a crucial role in the spinal pain process. Noxious and non-noxious stimuli stimulate the sensory dorsal horn neurons through NMDA. This suggests a role for NMDA receptors in nociception, as well as in the development of behavioral hyperalgesia and allodynia. Various NMDA anatagonists reduced thermal hyperalgesia, mechanical hyperalgesia/allodynia, and spontaneous nociceptive behavior. Thus, surgical stimuli can trigger LTP in nociceptive pathways resulting in chronic post-surgical pain (neuropathic pain); clinically, manifested as hyperalgesia and allodynia [78]. Understanding the NMDA, AMPA receptors, calcium dependent pathways, and other molecules involved in LTP, and drugs to antagonize these pathways could be a way forward to manage or prevent chronic pain.

Mechanism of LTP has been reported to be associated with crucial physiological functions such as learning, memory, cue reward-learning and fear in various regions in the brain, for example, in hippocampus, neocortex, thalamo-amygdala and lateral amygdala. Whereas, in the dorsal horn of the spinal cord LTP is associated with pathological pain or chronic pain [79]. Lack of understanding of this association of LTP across various brain regions makes it challenging to design region-specific therapeutic interventions without affecting unrelated areas. Many molecular pathways involved in LTP might have a negative effect on other physiological processes, such as addiction potential, normal sensory processing, memory and learning. These could be possible challenges in translating LTP research into effective clinical therapies.

Assessment of Pain

Relieving the pain and suffering of the animals is the prime obligation of the veterinary clinician and researcher. It has been demonstrated that painful stimuli in humans cause comparable behavioral and physiological changes to those in other nonhuman species [5]. Human verbal and physical communication help clinicians and researchers evaluate pain. The lack of this communication ability makes it difficult and challenging to assess pain. Dogs and cats likely perceive pain in a manner similar to humans due to the presence of comparable, if not highly conserved, neurotransmitters and neural pathways involved in nociceptive processing [5]. Assessment and recognition of pain is an essential part of animal care and veterinary clinical practice. Quantitative assessment of pain in animals is a challenge to both researchers and clinicians and hinders objective measurement of the efficacy of analgesics. The ability to objectively measure pain is a pre-requisite to compare the efficacy of analgesics, which is difficult in animals because they cannot communicate directly [80]. Various models/methods of pain assessment, including behavioural [81], neurohumoral [82] and neurophysiological [83] have been used in veterinary research.

Basic Animal Pain Model

Research studies in humans and animals exploring the mechanism underlying acute pain as well as evaluating the analgesic effects of the drugs require appropriate use of stimulus to instigate the sensation. This stimulus must be non-invasive, repeatable and quantifiable [80,84] to obtain an appropriate response. Electrical, mechanical, thermal and chemical stimuli are four basic types of nociceptive models used to study the analgesic effects of the drugs in question. The end point of these stimuli is the various withdrawal reflexes or behavioral responses [80,84].

Electric Stimulation: A painful electrical stimulus can be helpful to assess the effectiveness of centrally acting agents. Electrical stimuli are non-invasive, quantifiable and reproducible and produce synchronized afferent signals. However, there are some disadvantages of the electrical stimulus, such as it is not a natural stimulus felt by animals in a normal environment. Furthermore, it bypasses the transduction mechanism (circumventing the transduction process at sensory receptors) and directly stimulates all peripheral afferent fibers (Aβ→ Aδ→ C fibers) [85]. Electric stimulation has advantages in studies where effects of the drugs are assessed on the CNS, when administered systematically. Any effect will be of central origin because of the bypassing mechanism of the transduction process, provided that the drug has no effect on peripheral fibers. Variation in the impedance of the tissue being stimulated is another disadvantage; nevertheless, this can be minimized by using a constant current stimulator and monitoring the voltage and current of the stimulus applied. When the electrical stimulus is applied, it initially activates Aβ, followed by Aδ and then C fibers [80]. A double pain phenomenon has been reported in humans, the first or "fast", a well localized, stinging type of pain as a result of activation of Aβ fibers and second or "slow", burning type of pain, more intense and difficult to localize, as a result of activation of C fibers [86].

Various studies have reported the use of electric stimulation to evaluate a particular model and to evaluate the analgesic effects of test drugs. The electric current was used to stimulate the radial and tibial nerves, limbs, oral mucosa and periosteum to evaluate the microstrain gauge algesimeter for quantitative measurement of nociception [87] and validate the noxious stimuli techniques for determining the minimum alveolar concentration (MAC) in dogs [88]. Somatosympathetic reflexes of midazolam [89] and clonidine [90] were observed by electric stimulation of radial and tibial nerves in dogs. Electric stimulus was used on the dental pulp of the anaesthetized dogs to evaluate the analgesic effects of IV and intrathecal morphine compared to saline [91]. To evaluate the analgesic efficacy of low-dose ketamine in conscious dogs, Bergadano et al.[92] used a constant current device with a maximum voltage of 100 V and a maximal current of 40 mA to test a repetitive nociceptive stimulus. To evaluate the effects of tramadol, morphine and parecoxib on electroencephalography (EEG), Kongara et al.[93] used an electric current of 50 V at 50 Hz for 2 seconds on the right hind limb of dogs. A similar stimulus has been reported by Kaka et al.[94] in dogs.

In summary, various models have been developed for the quantitative evaluation of the antinociceptive effects of drugs in dogs. None of the models are ideal for use in dogs, with each having advantages and disadvantages. Nevertheless, these studies have demonstrated the use of electric stimuli in various models to assess the analgesic effects of drugs.

Mechanical Stimulation: In mechanical stimuli, pressure is applied against the surface of an organ to be tested. Application of the pressure initiates a nociceptive response; application of pressure can be progressive or coarse. The animal produces a behavioral response when the stimulus activates the nociceptors. These behavioral responses are the active withdrawal of the limb, guarding the test site or vocalization. The stimulus is withdrawn immediately at the time of an active response [81].

Various models of mechanical nociception have been developed to evaluate the analgesic effects of drugs. Colorectal distension with a balloon was used in rats, a mechanical model of visceral nociception. Avoidance behavior along with reflex activities was recorded by electromyogram from the abdominal muscles; this model also developed tachycardia and increased arterial blood pressure. These parallel parameters were attenuated or even reversed using certain anaesthetic agents [95]. Other similar models were distension of the stomach [96] or duodenum [97,98]. The model of colonic distension has also been reported in rabbits [99,100] and dogs [101].

Other different designs of mechanical devices have been used in dogs while working to quantify pressure applied to toes using a pair of Doyen intestinal forceps [102]; pneumatic device [103,104] and aluminum pliers with concave working ends [105]. The disadvantage of these techniques is that the rate of stimulus application cannot be controlled, and they can only be applied to the toes. In previous studies, an electronic method to measure joint tenderness in rheumatoid arthritis and tenderness of myofascial tissues was described by Atkins et al.[106] and Bendtsen et al.[107] respectively. Barnhart et al.[108] used a pressure device for mechanical pressure in dogs. The device consisted of a pneumatic cylinder powered by compressed nitrogen. When pressurized a bolt (2.54 cm in length) protruded out of cylinder pressing the skin overlying the periosteum of the medial aspect of the proximal tibia with enough pressure to cause transient discomfort to the dog. This method could not demonstrate the antinociceptive effects of morphine.

Advanced Mechanical Models

In electronic measurement devices, the force applied via a rigid probe tip is quantified using an integrated load cell, which converts mechanical force into an electrical signal for precise measurement. A von Frey device works on the same principle and contains a tip that applies a gradually increasing force to the skin surface to create noxious stimuli. In some von Frey devices, fine hairs can also be used instead of a rigid tip that bent at a maximum force, and larger hairs can be used to retest until the subject reacts prior to hair bending. While working on the quantification of pain with a von Frey device on humans [80] scientists have diverted their attention towards developing devices that can be used at the site of pain. Recently, a von Frey device has been used successfully to measure mechanical nociceptive threshold testing for analgesic trials of morphine in dogs [109]. For algometers refer to section- "Objective tools of pain assessment".

Thermal Stimulation: Heat causes selective stimulation of cutaneous receptors, resulting in specific activation of thermal and nociceptive fibers. This type of stimulation (with radiant lamps or contact thermodes and immersion of an animal's limb or tail in a thermostatic bath) results in the asynchronous stimulation of peripheral and central neurons because of the slow speed of cutaneous heating (<10°C/s). Thus, this model is not suitable for the study of a neural phenomenon seen in other sensory systems, where synchronous excitation of fibers is mandatory. However, thermal stimulation using a CO_2 laser thermal stimulator, results in the stimulation of nociceptive receptors only, as it does not actually contact the skin and provides controllable, safe, reproducible and specific noxious stimuli [80].

Various models of heat sources have been developed and are being used in studies evaluating the analgesic efficacy of drugs. Barnhart et al.[108] used the thermal model to assess the antinociceptive action of morphine. There was no significant difference in thresholds between the control and morphine groups. The device was comprised of a linear, ramped-intensity, incandescent bulb housed in a metal cylinder. The device had a slow rate of heating and was dependent on skin contact. These factors may have influenced the consistency and accuracy of the measured responses and should be considered when interpreting the study results. Recently, Hoffmann et al.[110] used a thermal device containing a "thermal probe" as a temperature sensor and "heater element" in a thermally conductive epoxy that can be attached to the clipped thoracic wall of a dog or cat by an elasticated band in the center of an inflatable bladder that provides constant contact between the probe and the skin [111]. The probe can be heated to record the skin temperature at a steady, incremental rate of 0.6°C per second until the dog responds to the thermal stimulation or the 55°C safety cut-out, whichever comes first. This model was used to assess the baseline reproducibility and the effect of levomethadone, acepromazine and fenpipramide.

Chemical Stimulation: Chemical stimulation involves injection of an algogenic substance into the animal tissue. These tests use intraperitoneal and intradermal injections of irritant chemicals, such as formalin, capsaicin, carrageenan, turpentine and complete Freund's adjuvant (CFA), commonly available inflammatory substances with the ability to provoke inflammatory responses and irritating body tissues. This stimulation is very slow,

progressive, of longer duration and different from electric, mechanical and thermal stimuli. The result of this test is a behavioral score rather than the threshold measured. Animal models of visceral and peritoneal pain also use algogenic agents.

In a previous study, an administration of intradermal 0.5 - 15% formalin at 0.5 -15% on the dorsal surface of the fore paw of the rat produced painful behavior. This behavioral posture was graded as "0" for normal posture; "1" for the paw on the ground with no support to the animal; "2" for the paw unable to place on the ground; and "3" for the paw being licked, nibbled, or shaken in response to chemical irritation [112].

The chemical nociceptive model uses the "writhing test" by injecting the algogenic substance intraperitoneally and directly into the hollow organs to produce visceral pain in rats [113,114]. This pain is characterized by whole body movements (particularly of the hind paws), abdominal contractions, twisting of the dorsoabdominal muscles, and decreased motor activity and motor incoordination. The "pain behaviour" produced by these tests is complex and biphasic, characterized by body stretching and contraction of either the flanks or the whole body in the initial phase and predominantly involves abdominal licking and nibbling in the second phase [115,116]. Similar models have been reported for bladder pain with intravesical injection of capsaicin, capsaicin-like substances [103,117-119] and turpentine as an irritant substance [120]. A model of uterine pain was also developed, where intrauterine injection of mustard oil was used as an algogenic agent [121].

In an experimental model, the dogs under anaesthesia were given sodium urate or calcium pyrophosphate crystals in stifle joints to quantify the host response [122]. Toutain et al.[123] used Freund's adjuvant arthritis model of pain in dogs to determine the dose regimen of nimesulide, a COX-2 selective nonsteroidal anti-inflammatory drug (NSAID). In another study [124], efficacy of low-dose ketoprofen was investigated in sodium urate crystal-induced synovitis model of arthritis. In a similar study by McCann et al.[125] a urate crystal-induced synovitis model was used to observe the *in vitro* effects and *in vivo* efficacy of a novel COX-2 inhibitor in dogs.

In summary, the above studies demonstrate the use of animal models of pain using various chemical agents in rats and dogs. The disadvantage of these models is that they may harm the animal because they have unavoidable characteristics once administered [80]. Additionally, chemical models are more invasive and less humane [80]. These models are experimental models of chronic pain. Therefore, distinction should be made from our current discussion on acute perioperative models. Detail discussion of the chronic models of pain is beyond perioperative pain. It is worth to mention here that, the fundamental knowledge on pain studies was developed from rodent models, however, the disparity between nerve fibers composition, density of nerve fibers per skin area, and pain tolerance may lead to key differences in pain response among dogs and cats from rodents [126].

Surgical Models of Pain

Ovariohysterectomy and Castration: Over the last decade, interest in the understanding of the mechanism underlying pain for the benefit of animals has increased. For this purpose, most of the work has focused on acute pain models. Commonly used models for this were heat or pressure [127,128] and clinical pain [129]. Most acute clinical pain studies have evaluated the effects of surgical trauma on companion animals [93,94,128,130]. Among these studies, the most commonly used surgical model of pain is ovariohysterectomy. The Center for Veterinary Medicine of the US Food and Drug Administration (FDA) considered OHE a suitable model to investigate moderate pain in clinical studies of analgesia [129]. Castration and ovariohysterectomy are commonly performed elective surgical procedures in small animal practice [131]. These surgeries produce significant postoperative pain [132]. Several studies have reported ovariohysterectomy, to be a noxious procedure for bitches [129,133,134]. Traction on the mesovarium during ovariectomy is recognized as one of the most potent noxious stimuli, often eliciting pronounced autonomic and hemodynamic responses, and, in some cases, patient movement despite anesthetic depth [135,136]. The efficacy of the drug to be tested can be evaluated more reliably on these elective procedures because these surgeries are performed on healthy animals that are pain free, assuming all the postoperative pain is due to surgery [137].

Pain Assessment Tools

Pain assessment tools can be broadly categorized based on their application in experimental or clinical settings and may be further divided into objective and subjective measures.

Objective tools include neuro-endocrine responses, heart rate (HR), blood pressure (BP), respiratory rate (RR), electroencephalography, heat and mechanical threshold testing and hand-held devices such as algometer and the von Frey device. Heart Rate, BP, RR and pupillary diameter are routinely used parameters of pain assessment intraoperatively as well as postoperatively during the recovery period and even later. Postoperatively, factors such as stress, fear, and environmental noise may also

influence physiological parameters, including HR, BP, and RR, and should be considered and controlled for when interpreting these measurements [138-140].

Objective tools include behaviour and pain scoring scales. Objective markers like HR, BP and RR would be very useful along with behavioural markers for proper pain assessment. Pain scoring scales can be further categorised into unidimensional and multidimensional. Unidimensional scale consists of expressions like No, Mild, Moderate or Severe Pain. Examples of these include simple descriptive (SDS), Numerical Rating Scale (NRS) and Visual analogue scale (VAS). Multidimensional are subcategorised with behavioural or pictorial indicators given a particular score, these include the University of Melbourne Pain Scale, Glasgow pain scale, Colorado pain scale for canine and feline, Feline Grimace and UNESP-Botucatu MCPS. The advantage of multidimensional scales is that they allow pain measurement from more than one aspect; neither specialized skills nor experience is required to use these scales. This could be due to the pain descriptors, acquired from practicing veterinary surgeons, regularly examining dogs and cats for acute pain behavior.

Requirement of busy veterinary practice is that a pain scale should be user friendly, require less skills and knowledge for scoring. The authors' experience with the Glasgow Composite Pain Scale and, more recently, the Feline Grimace Scale has been favorable. In contrast, the Visual Analog Scale was found to be more challenging to apply consistently.

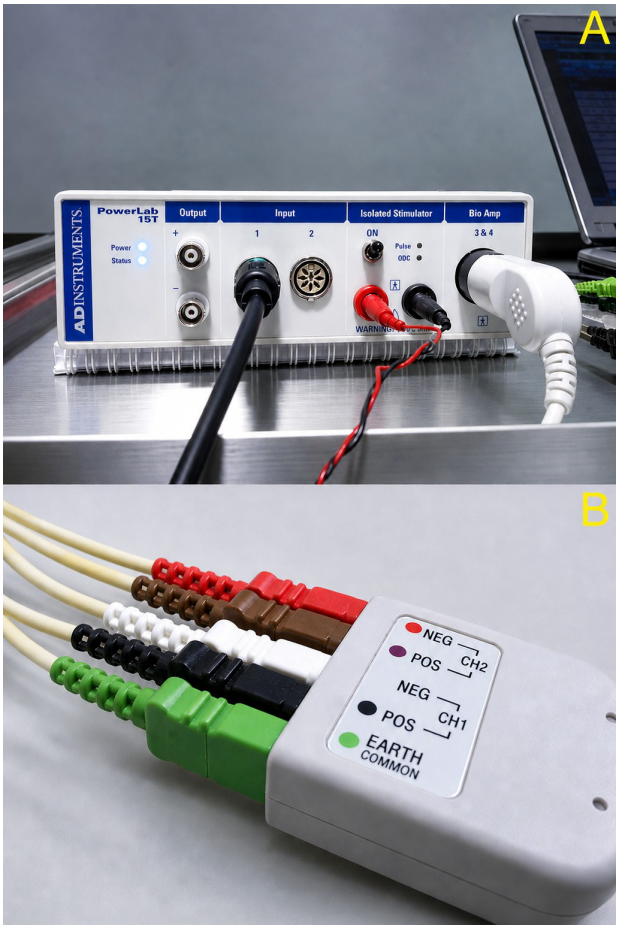

Fig 6. (A) Power Lab Biopotential Recordings system device (Power Lab data acquisition system, AD Instruments Ltd. Sydney, Australia). **(B)** Electrode indicators with channels 1 and 2

Objective Tools of Pain Assessment

Neurophysiological Techniques

Electroencephalography: Electroencephalography (EEG) has been used as an objective tool for the investigation of pain [141]. This is a non-invasive and stress-free technique. Parameters of the EEG for nociception under anaesthesia and pain in conscious animals have received much attention. However, due to the deficiency of standard recording techniques in animals, and complexity in data analysis, the use of EEG in veterinary practice is limited [142].

What is Electroencephalography? The EEG is the real-time graphical representation of the microvolt range tiny but spontaneously produced neuronal signals from the cerebral cortex through electrodes placed on different positions on the head. Neurons having an intrinsic electrical nature to produce electrical and magnetic fields. When recorded at a distance from the source, these fields are termed "far-field potentials", whereas those recorded at a short distance from the source are known as "near-field potentials". The EEG is recorded at distance from the source neurons and therefore is "far-field potential". An action potential transmitted along the nerve fiber approaches a synapse and creates a change in the membrane potential of the postsynaptic neuron, the postsynaptic potential. These changes in the membrane potential of postsynaptic neurons are the main contributors of the EEG [143].

Electroencephalography Recording: The EEG recording apparatus comprises electrodes, a signal recording amplifier and a recording system *(Fig. 6)*. Electrodes connect with the conducting fluid of the tissue and the input circuit of the amplifier. The impedance is crucial for accurate and/or minimum loss of EEG signals. Thus, electrode-tissue impedance is maintained at a level substantially lower than the input impedance of the amplifier to ensure accurate signal acquisition [141,143,144]. Different configurations for the placement of the electrodes have been described, and several studies have reported the configuration described by Mayhew and Washbourne [145] with the positive, negative and ground electrodes as shown in *Fig. 7* [142].

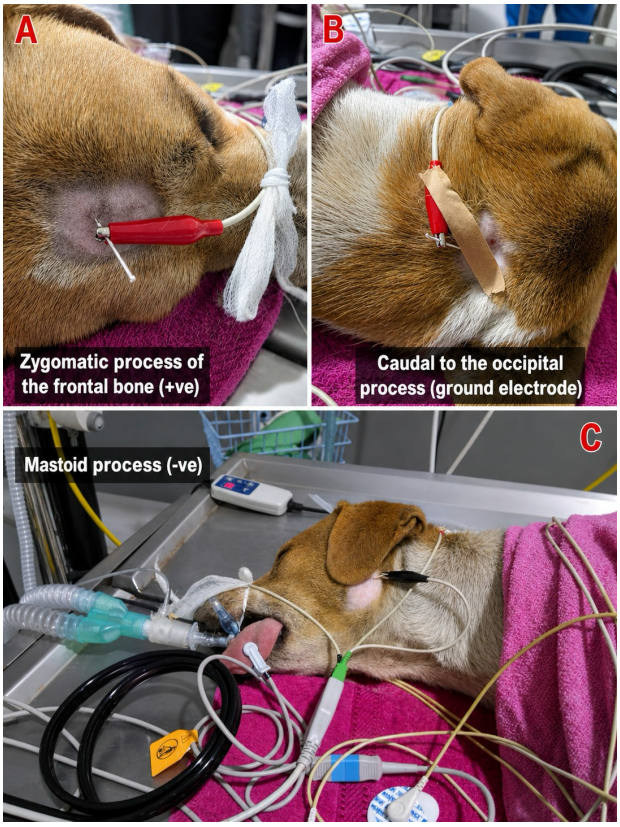

Fig 7. A, B, C: Three electrode configuration to record EEG electrodes in an anesthetised dog

Analysis of the Electroencephalographic Data: The analysis of EEG data is conducted offline at the end of the experiment. Fast Fourier Transformation (FFT) is a mathematical procedure to quantify data within raw EEG signals. The fast Fourier transformation system converts the raw EEG signals to different frequencies characterized by corresponding amplitudes. These EEG frequencies have been reported as delta (0.1 to 4 Hz), theta (4.1 to 8 Hz), alpha (8.1 to 12 Hz) and beta frequency [12.1 to 20 Hz] [142,143,146]. This creates a power spectrum, which is the distribution and derivation of the EEG variables of corresponding frequencies and amplitudes *(Fig. 8)*.

Variables of the Electroencephalography: Total power (Ptot), Median frequency (F50 or MF) and spectral edge frequencies (SEF95%) are the variable extracted from EEG data. These are the most common variables associated with nociception in animals [142,144,146]. "Total area under the cover" is the total power. The median frequency is "the frequency below which 50% of the total power of the EEG is located". Spectral Edge frequency 95% is "the frequency below which the 95% of the total power of the EEG is located" [144].

Arousal or desynchronization is characteristic of the brain's response to unpleasant stimulus, typically causing increase in MF *(Fig. 9)* and a decrease in Ptot [93,94].

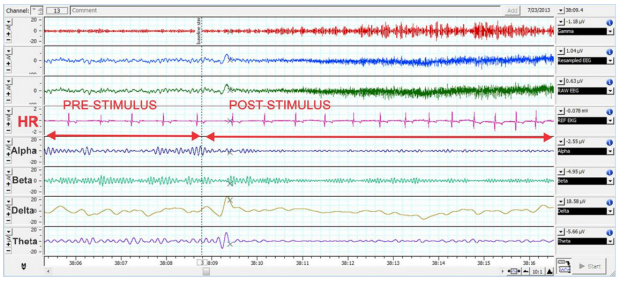

Fig 8. EEG spectrum of a dog under anesthesia, showing visible effects of nociceptive stimulus (pre versus post stimulus) on various waves and heart rate

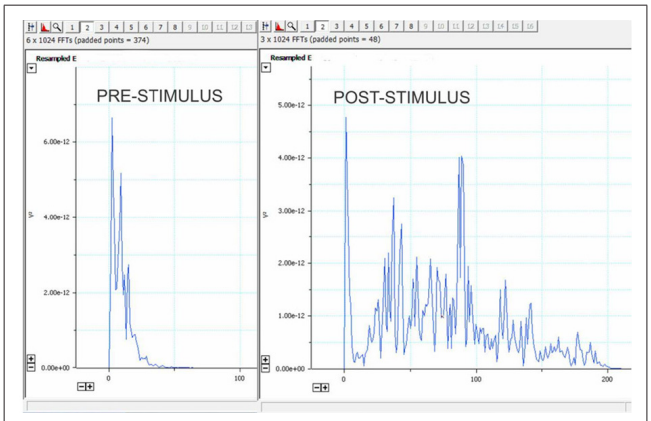

Fig 9. Median frequency of a dog under anesthesia, showing visible effects of nociceptive stimulus before and after stimulus. Note the increase in the area under the curve after stimulus

Noxious stimulus results in a change of the EEG from high amplitude low frequency (seen in anaesthesia, sedation, sleeping or calmness) to low amplitude high frequency waves (resembling that of awakeness or alertness); this shifting of EEG is called arousal or "desynchronization" [144].

Synchronization or paradoxical arousal is another change in EEG associated with noxiousness but is less commonly reported and is mostly described in clinical studies, which used multiple anesthetic and analgesic agents with varying noxious stimuli [147-149]. Synchronization is a shift in the EEG towards high-amplitude and low-frequency activity. Some other clinical studies were unable to demonstrate EEG changes in response to noxious stimuli [150-152]. However, current reports suggest that EEG is a robust tool for well-controlled nociceptive studies [141,142,143,146].

Changes in EEG Spectrum During Nociceptive Studies: It has been accepted that EEG is a direct indicator of the state of responsiveness of the central nervous system under anesthesia [94,142]. Changes in the EEG power spectrum can be used objectively to quantify nociception in horses [153,154], rats [155], deer and sheep [156,157], calves [158] and dogs [141,142]. Median frequency has been reported to be the predominant change in response to noxious

stimuli [93,94,141,142].These lines of evidence suggest that EEG can be used as an objective tool to evaluate the analgesic efficacy of drugs.

Minimal Anesthesia Model (MAM)

Johnson and his co-workers developed a model of anesthesia to study the effects of noxious stimuli on the EEG spectrum [144]. This model was first used in horses to investigate the effects of castration on EEG [153]. In the minimal anesthesia model, animals are anesthetized to a stable plane, but they are capable of showing EEG responses to nociceptive stimulation despite being unconscious. When compared with other studies using painful methods, this model works a negative control group without compromising animal welfare/ethics. This method uses the EEG response to noxious stimulation as a technique to assess analgesia for studies of pain in applied veterinary research. This is a sensitive and quantitative technique of assessing central responses to painful stimulation [144]. A minimal anesthesia model has been used in horses [153,154], sheep [156], red deer [157], pigs [160] and dogs [93,94,141,142].

Somatosensory Evoked Potentials

Somatosensory evoked potentials (SEPs) have been reported to evaluate the function of the nervous system. These are the electric potentials generated at various levels of the nervous system, for example peripheral nerve, plexus, nerve roots, spinal cord segments and the sensory cortex. Most commonly used nerves for the stimulation of pelvic and thoracic limbs are tibial and median nerve, respectively [161-164]. These nerves are stimulated through inserting electrodes or stainless-steel needles into subcutaneous tissue at distal end. Stimulation at proximal to targeted nerve can result in more reflex actions of muscles due to recruitment of more fibers with increased potential. This increased activity of the muscles can be avoided by stimulation of the median nerve just above the carpus and tibial nerve just above the tarsus [151] The cathode (negative electrode) can cause depolarization of the axon membrane and should be placed 2 cm proximal to the anode (positive electrode), to avoid conduction block by anode [162,163]. Recordings of the evoked potential are shown on a computer screen; recordings and waves associated to each limb can be seen in various windows, adjustable for improved visualization. Clinically SEPs are used to evaluate the integrity of sensory pathway conductivity and may therefore be used to examine central and peripheral neurological conditions [163].

von Frey

In electronic devices, an integrated load cell is used to measure the force applied to a rigid tip. A von Frey device consists of a tip that produces painful stimuli by gradual application of force to the skin surface. Some von Frey devices employ larger hairs to retest until the subject reacts before the hair bends, whereas fine hairs are used that bend at a maximum force. While working on quantification of pain with von Frey device on humans [80] scientists have diverted their attention towards developing devices which can be used at the site of pain. von Frey has been used successfully to measure mechanical nociceptive threshold testing for analgesic trials in dogs [110].

Fig 10. Wagner algometer FPX 25 with circular tip used in various studies of pain research in animals

Algometry

Schiffman et al.[165] described a pressure algometer for examining muscle tenderness in patients with myofascial pain syndrome. The use of algometers for mechanical pressure in humans led to the usage of algometers for mechanical nociceptive threshold testing of analgesic trials in dogs [61,129,166]. An algometer consists of a probe with a tip or disc, a load cell, transducer and an amplifier. It is used perpendicular to the skin on the point to be tested. Pressure is applied on the site by gradually increasing force to produce a painful stimulus *(Fig. 10)*. The force exerted to the tip is transferred to a load cell, producing a voltage output. This voltage output is transduced, amplified, and

displayed in newtons or kilograms [61,128,166,167]. These devices have the ability to activate both the Aδ and C fibers, responsible for encoding clinical pain [80]. Algometry can be used in clinical settings to assess post-operative hyperalgesia and in experiments to assess the effectiveness of analgesics [168].

A variety of algometers have been used in humans [169,170] as well as animals [167,171-173] with different objectives. These studies have used various diameters of tips, such as 1 cm probe tip [67,174], 2 mm probe tip [61,132,171], and 0.5 mm probe tip [109]. Due to various tip sizes, the pressure required to initiate the response was different in these studies. For example, pressures were 1.92 N [109], 30.81 N [171], ~38 N [174,175], 38-44 N [167], and 9.94 N at the tibia [61] to provoke a response in these studies.

Regardless of the tip size, whenever an algometer is used in research, one should practice its use in order to avoid incorrect readings and then set a baseline of the particular experimental animal. Each animal would have a different baseline based on animal´s pain tolerance. Before we begin to use the algometer in a clinical setting, there is a need to fix an end-point for a particular species. However, the use of an algometer is rare in clinical settings, due to the fact that behavioural and physiological parameters like HR, BP and RR can easily be applied for pain assessment. On the other hand, algometer is more frequently used in experimental models.

Fixing an end-point of a nociceptive test would be challenging experimentally because of individual or species variation. Peripheral perception may be impacted by nociceptors' distribution, blood flow, and skin thickness [126]. Therefore, to achieve the best outcomes, it is essential to assess nociceptive threshold and determine the end-point for each species and individual. These end-points could be identified through training of the animals before the experiment. Vocalization, limb withdrawal reflex with and without vocalization and guarding of the abdomen were the most common end-points in dogs [166].

Electronic von Frey and algometer have similar working principles. Unlike the von Frey device, the algometer is easy to use and consists of a single, basic handheld unit without any electrical wire or additional parallel units. It can be used easily in the clinical setting in conscious dogs. One of the disadvantages of the algometer use is the inability of the operator to apply a continuous rate of force. Threshold readings could be affected by the rate of force application, for instance, faster rates would result in higher readings [176]. This can be minimized by continuous practice [62] or using a continuous rate of application of force (CRAF) meter for accuracy [61]

Neuro-Endocrine Responses

Endocrine response is stimulated by afferent action potential from the site of surgery. These action potentials travel along sensory nerve roots through the dorsal root of the spinal cord to the medulla to stimulate the hypothalamus. Increases in sympathetic activity and pituitary hormones are characteristic responses to surgery [177]. Norepinephrine from the presynaptic nerve terminals and catecholamines form the adrenal medulla are released as a result of hypothalamic stimulation of the sympathetic autonomic nervous system. Hypothalamic releasing factor stimulates the anterior pituitary, causing the secretion of adrenocorticotrophic hormone (ACTH), growth hormone, prolactin, arginine vasopressin and beta-endorphin [178]. Growth hormone activates protein synthesis and prevents its breakdown, activates glycogenolysis in the liver, promotes lipolysis, and has an anti-insulin effect [177]. Arginine vasopressin, which has a major role as an antidiuretic hormone, is also released from the posterior pituitary. Corticotrophin or adrenocorticotrophic hormone acts on the adrenal cortex and increases cortisol secretion in the plasma within minutes after surgery. Both of these hormones can be measured in the plasma within minutes of surgery [177].

Cytokines: Surgery instigates a series of events, including inflammatory reactions and nociception. Tissue and peripheral nerve injury can result in a local inflammatory reaction. In reaction, the concentrations of various biological mediators, including substance-P, PGs, bradykinins, CGRP and cytokines, can also be elevated in the injured tissue [179], particularly pro-inflammatory cytokines [180,181]. Among various mechanisms of pain, pro-inflammatory cytokines have recently been investigated comprehensively [180,182,183].

Cytokines are small proteins secreted and released by cells. They have specific action on the interaction and communication between cells. Cytokines are produced by cells such as lymphocytes (lymphokines) and monocytes (monokines). Those produced by leukocytes act on other leukocytes (interleukin), and cytokines can also release chemokines to perform chemotactic activities. Cytokines are pleiotropic (various cell types produce the same cytokine or single cytokine acts on different cell types) as well as redundant (same functions stimulated by different cytokines) in their action. They are mostly released in a cascade where one cytokine activates its target cell to produce additional cytokines. They also have synergistic and antagonistic effects. Many cells are responsible for making cytokines, but macrophages and helper T cells are principal cells [184].

Mechanism of Action: Cytokines influence the intracellular modulating processes by binding its specific membrane-bound receptor. A series of phosphorylated constitutively expressed signal proteins takes place inside the cell. These phosphorylated signal proteins travel through

the cytoplasm and contact the nucleus on the condition that they should have a nuclear localization sequence or bind to a protein with their compatible sequence. In the nucleus, they may affect the transcription rate or induce post-translational changes. Proteins that participate in pain are signal transducer and activator of transcription (STAT) [185], c-fos [186,187], Ras/Raf, c-Jun [186] and mitogen activated protein kinase (MAPK) [185,188]. Interestingly, these proteins also take part in the intracellular signaling pathways of several cytokines, including IL-6 [189].

In previous studies of the cellular mechanism of inflammatory pain, it has been established that a well-defined sequential cascade of cytokines/chemokines is followed by the release of PGs and sympathetic amines [190,191]. Classical pro-inflammatory cytokines such as TNF-α, IL-1β and IL-6 play roles in pathological pain [184]. IL-1β and IL-6 can provoke peripheral and central sensitization, resulting in pain augmentation (hyperalgesia) [192,193]. The direct action of cytokines on nociceptive neuronal receptors and ion channels has been described in previous studies, where they regulate neuronal excitability, sensitivity to external stimuli and synaptic plasticity [194].

Exogenous Cytokine Response: In various experiments conducted on animal models, exogenous administration of pro-inflammatory cytokines augmented nociception [190,195-199] and increased neuronal excitability in response to noxious stimuli. In contrast, when the function of these cytokines was blocked with soluble TNF receptors, interleukin-1 receptor antagonist (IL-1ra), anti-IL-1 or anti-IL-6-neutralizing antibodies, it prevented or reversed hyperalgesia and allodynia in an animal model tested for peripheral inflammation and/or injury [182,200-203]. Patients with decreased levels of pro-inflammatory cytokines have reported less severe pain [183,204]. IL-1 and IL-6 are released in both the brain and periphery after exposure to psychological stressors [205].

Cytokine Response to Analgesic: Elevated central and peripheral levels of COX-2 and pro-inflammatory cytokines, especially IL-1β, result in the production of prostaglandin E_2 (PGE2) [206,207]. Pre-emptive epidural analgesia has been proven to reduce postoperative pain and decrease the production of pro-inflammatory cytokines, including IL-1β [183]. Pre-emptive blockade of pro-inflammatory cytokines (TNF-α and IL-6) increased the nociceptive threshold in experimentally induced inflammatory pain [208], reduced morphine requirement and recovery was better after cancer surgery [209] Decreased levels of proinflammatory cytokines have been observed in patients administered with mixture of opiates and local anesthetics in the postoperative period [45].

Role of Cytokines in Neuropathic and Inflammatory Pain: Neuropathic pain results from injury or dysfunction of nerves. Animal models of neuropathic pain has shown involvement of several proinflammatory cytokines such as TNF-α, IL-1β, IL-6, and IL-17. These cytokines have been reported in disc herniation, sciatica, lumbar herniation, spinal cord injury and complex regional pain syndrome (CRPS) [210].

Possible mechanisms for IL-1β, include activation of the dorsal root ganglion neurons and increased spinal cord activity. IL-7 results an increase in the activity of transient receptor protein vanilloid 4 (TRPV4), an ion channel that has been found to mediate mechanical allodynia.

Inflammation is caused by abnormal signaling or production of pro-inflammatory cytokines such as TNF-α and IL-6, IL-1 [211] These cytokines are released by immune cells after tissue injury and inflammation, sensitizing peripheral nociceptors and reducing their threshold of stimulation. This stimulation triggers the action potential travelling to dorsal horn of the spinal cord, synapsing with second order neuron. Glutamate and substance P transmit pain signals to ascending pathway, reaching higher centers of brain involved in perception. At the same time modulation of pain occurs through descending pathways, affecting intensity and perception of pain [212]. Increased sensitivity of nociceptors and exacerbated pain responses are hallmarks of inflammatory pain, indicating involvement of both peripheral and central components of pain. IL-1, and TNF-α play a role in hyperalgesia. Development of therapeutic agents, targeting these cytokines and their signaling pathways would significantly help alleviate pathological pain. This offers a way forward as a therapeutic approach and requires understanding interplay between cytokines and various inflammatory or neuropathic conditions

Anti-inflammatory Cytokines: Just as proinflammatory cytokines and their involvement in pain, nature has provided anti-inflammatory cytokines to keep balance in the immune system by suppressing inflammation and maintaining immune homeostasis. when proinflammatory cytokine levels rise in neuropathic and inflammatory pain, there is decrease in the anti-inflammatory cytokines. Lower levels of anti-inflammatory cytokines have been demonstrated in patients with chronic neuropathic pain conditions, such as complex regional pain syndrome, atypical facial pain, lower back pain, and post-herpetic neuralgia. IL-4, IL-10, IL-13, transforming growth factor beta (TGF- β) [210], IL-35, and IL-37 [213,214] have been reported as anti-inflammatory cytokines. interleukin-10 and transforming growth factor-beta (TGF-β) limit the transmission of pain.

Glatiramer acetate, an immune modulator, has been reported to decrease pain and has been correlated with increase IL-4 and IL-10. Calcineurin, uliastatin,

plasmid DNA, and viral vector indirectly increase IL-10 concentrations and could be therapeutic target for neuropathicpain. Flexibilide, a substance derived from soft coral and delivery of bone marrow stromal cell into the spinal cord of mice has been reported to reduce neuropathic pain Ibudilast, a phosphodiesterase inhibitor originally developed as an asthma medication, suppressed glial cell activation and reduced IL-1β, TNF-α, IL-6 [210]. Botulinum toxin (BoNT), the paralytic neurotoxin produced by the bacterium Clostridium botulinum, reduced pain related to spinal cord injury [215] and peripheral neuropathic pain [216].

IL-35 is an anti-inflammatory cytokine with potent immunosuppressive capacity. IL-35 alleviated pain in autoimmune encephalomyelitis (EAE) mouse model of central neuropathic pain [217] and the streptozotocin rat model of diabetic neuropathy [218] IL-37, suppresses inflammation and immune activation. Inhibits production of IL-1β, IL-6, TNF-α. This anti-inflammatory cytokine has potential therapeutic target for autoimmune diseases and chronic inflammatory conditions [219].

Currently FDA approved TNF-α inhibitors include infliximab, etanercept, adalimumab, certolizumab pegol, and golimumab. These are recommended for painful disorders such as inflammatory bowel disease, rheumatoid arthritis, psoriatic arthritis, and ankylosing spondylitis. Canakinumab, Gevokizumab, and Anakinra, are FDA approved drug against IL-1β, Tocilizumab, Sarilumab, and Sirukumab against IL-6 (Low back and leg pain) and secukenumab for IL-7 and Secukinumab for IL-17. Other cytokine modulators include Emapalumab, Ruxolitinib, Tofacitinib, approved by FDA for various conditions involving pathological pain pathways [211].

Hormones (Adrenaline, Nor-adrenaline, Endorphins, Cortisol): Briefly, several hormonal changes linked with stimulation of the hypothalamic-pituitary-adrenal axis in response to surgical stress have been reported. Of these, adrenal corticosteroids such as cortisol, inflammatory mediators including beta-endorphins and other associated pituitary hormones have been used in previous studies of pain and associated stress assessment [82,128,130,220,221]. This method of changes in plasma concentration of hormones and cytokines is advantageous in the sense that it minimizes observer variability associated with behavioral assessment of pain and is more objective than behavioral assessment. However, there are some disadvantages and limitations [222] of this method, summarised as follows:

1. A single sample collected at a specific time point in an animal experiencing pain may not accurately reflect the level of pain present at that moment. For example, similar biomarker readings observed in control animals without pain and in animals subjected to painful stimuli may reflect the inherent variability of hormonal and cytokine responses. These variations can result from changes in plasma concentrations associated with stress or other non-nociceptive physiological processes.

2. These biomarkers lack sufficient specificity for accurate pain assessment and should therefore be interpreted in conjunction with other validated scoring systems. However, their routine use in clinical practice is limited due to their invasive nature, associated costs, and the need for specialized analysis, and they are consequently infrequently utilized in clinical settings.

Conclusion

It is now well established that surgical trauma results in nociception triggering a cascade of chemical reactions that take place at the primary site and throughout the central nervous system. These chemical reactions produce an "inflammatory soup" that brings changes in the central nervous system, resulting in the development of central sensitization and long-term potentiation manifested clinically as hyperalgesia and allodynia, subsequently developing into chronic pain. Nociception includes sodium and potassium channels that contribute to transduction and transmission. Excitatory neurotransmitters, including glutamate, substance P and CGRP, play key roles in modulation. Glutamate, through activation of NMDA receptors and other excitatory pathways, plays a critical role in the development of central sensitization. The use of multimodal analgesia, involving drugs with different mechanisms of action, can help attenuate these processes and improve postoperative pain management.

Declarations

Availability of Data and Materials: All the data generated are included in the manuscript

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