Chapter-1

Introduction and Literature Review

1.1 Introduction

Pain is defined by IASP as “Pain caused by lesion or disease of somatosensory system”, that involves lesions in peripheral fibres and central neurons resulting in wide variety of complex chronic pain syndromes (Raja et al. 2020). But recently the same has been revised and defined as by IASP “An unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage(Yang et al. 2016; Raja et al. 2020). Pain is a vital signal that serves the purpose of protecting our body against any kind of external or internal injury by activating both reflex and conscious responses(Zaki et al. 2022). These signals of pain are detected by specialized receptors known as nociceptors within our body, and the process is referred to as nociception. The International Association for the Study of Pain (IASP) defines nociception as "the neural processes involved in detecting and interpreting noxious stimuli." Profuse stimuli, like heat, inflammation, necrosis, muscle spasms and several chemical mediators may play a role in triggering the pain sensation (Pahan and Xie 2023). The perception of physical pain is subject to variation depending on the context and which other sensory inputs are being received (Kuner and Kuner 2022). Pain is generally classified into two major categories: acute and chronic. Acute pain is normally sudden in onset, time limited, and motivates behaviors to avoid actual or potential tissue injuries Acute pain usually lasts for less than 7 days but often extends up to 30 days (Wu et al. 2019). Acute pain is protective in nature and has two phases:

an initial alert signal to the brain that lasts for seconds and a sub-chronic phase that can persist for hours or days as the body works to heal (Afridi et al. 2020).

Chronic pain is defined by the international Association for the Study of Pain and International Classification of Diseases (ICD)11 as pain that extends beyond 3 months, irrespective of the cause, and chronic pain is thus considered as a disease condition in its own (Michaelis et al. 2014; Su et al. 2014). Chronic pain requires immediate attention and treatment as it greatly hampers the quality of life and remains most common complaint of outpatients in clinics.

1.2 Prevalence and Impact of Chronic Pain

Approximately 30 % of people suffers from chronic pain worldwide posing an enormous personal and economic societal burden (Dembla et al. 2017; Zhang and Jeske 2020). The occurrence of chronic pain in Asian adults varies, with prevalence rates ranging from 7.1% in Malaysia to 61% in Cambodia and Northern Iraq. Among the elderly population in Asia, the prevalence is even more elevated, ranging from 42% to 90.8% (Zaki and Hairi 2015). It is estimated that over 100 million people, experience chronic pain, costing up to $650 billion a year in medical treatment and lost productivity in the United States alone (Raver et al. 2020). Chronic pain can further be categorized into neuropathic pain, nociceptive pain and inflammatory pain. Specifically, treatments for chronic pain conditions lack precision in targeting the root causes and come with a significant risk of side effects for individuals dealing with pain (Zaki et al. 2022; Pahan and Xie 2023). Furthermore, available approved therapies for chronic pain are associated with substantial adverse effects due to their off- target actions in both the

central and peripheral nervous systems. This necessitates the compelling need for development of new therapeutic approaches with improved effectiveness and a wider range of safety measures to better address the complex challenges associated with chronic pain management.

1.3 Etiology and Pathophysiology of Neuropathic Pain

Neuropathic pain is characterized by spontaneous pain, abnormal hypersensitivity to stimuli (hyperalgesia) and nociceptive responses to non-noxious stimuli (allodynia). Pathophysiology of neuropathic pain is complex and heterogenous factors as viral infections, autoimmune diseases metabolic disorders regulate the central or peripheral nervous system afflicting the same (Xu et al. 2016; Malfanti et al. 2019). Origin of neuropathic pain may be either central or peripheral. Central neuropathic pain is a result of lesion or disease in brain or spinal cord, while peripheral neuropathic pain involves pathophysiological alterations in sensory fibres (C, Aβ, and Aδ) (Bennett and Xie 1988; Wang et al. 2008; Jensen and Finnerup 2014). Any kind of lesion or inflammation as a result of injury or infection, leads to sensitization of peripheral nociceptors which is accompanied by spontaneous discharge resulting in ongoing pain. Peripheral neuropathic pain is gaining concern, with ageing global population, increased incidences of diabetes, and longevity due to treated patients with cancer, HIV and leprosy(Herr et al. 2020).

1.3.1 Diabetes induced Neuropathy

According to a report published revealed that worldwide epidemiological prevalence of neuropathic pain ranges between 6.9% and 10% and is likely to increase

in years to come (Senechal et al. 2007). Though, it is hard to estimate neuropathic pain prevalence in India, due to lack of proper set of data , one of the studies has estimated peripheral neuropathy to lie between 5 to 2400 per 10,000 population in different community of various regions (Trivedi et al. 2017). According to American Diabetes Association Peripheral neuropathy is a common complication of diabetes, affecting up to 50% of adults with diabetes in the US. Whereas with diabetes depending on age, duration of diabetes, glucose control, and type 1 *versus* type 2 diabetes prevalence of peripheral neuropathy may range between 6% and 51% Globally, more than 40 million people with diabetes live with neuropathy. It affects specific regions of the nervous system, leading to morbidity, thereby increasing the economic burden for diabetes care(Serrano Cardona and Muñoz Mata 2013). While diabetic neuropathy can be asymptomatic in up to 50% of cases but is also a leading cause of lower extremity amputations ranging between 40–60% of cases (Hicks and Selvin 2019). In India around 77 million people were living with diabetes in 2019 and by 2045 this will rise to 134.2 million. Diabetic peripheral neuropathy has been found between 26 to 31% in surveys in the Indian diabetic patients. The overall prevalence of DPN was found to be 28.85% from which 88% patients were found to have painful symptoms (Baxi et al. 2020).

1.3.2 Cancer and Chemotherapy-induced Peripheral Neuropathy

Each year, more than ten million people worldwide are diagnosed with cancer. Pain associated with cancer diagnoses is a serious concern and one of the most common symptoms reported by cancer patients. The overall 44.5% cases prevail severe cancer pain while 30.6% of cancer patients experienced moderate to severe pain (Aoki et al.

2008; O'Hearn et al. 2017). According to a study conducted in united states, overall, of the identified 4526 cancer survivors, 1648 (34.6%, 95% CI, 32.7%-36.5%) reported having chronic pain and 768 (16.1%, 95% CI, 14.8%-17.5%) having HICP, representing approximately 5.39 million and 2.51 million cancer survivors, respectively, in the US population (Meng et al. 2011). While in a study conducted in Germany in a sample of 3,745 cancer patients across all tumor entities. In total, 37.9% patients suffered from cancer-related pain and 56.1% suffered from non-specific pain(Broemer et al. 2021). Although consolidated reports on prevalence of neuropathic pain in India remains lacking, recent controlled studies depict under diagnosis and under reporting of chemotherapy-induced neuropathic pain by physicians. A recent study shows a substantial burden of neuropathic pain in cancer patients, with 54% of them experiencing ongoing pain. This higher prevalence in oncology population may be linked to the significant proportion (almost 60%) of patients with metastatic disease(Lin and Lane 2019; Satija et al. 2021). Higher pain prevalence in patients with metastatic disease may be ascribed to the more advanced stage of cancer, leading to a higher likelihood of neuropathic pain due to nerve involvement or damage. This emphasizes the importance of tailoring pain management strategies to the specific needs of cancer patients, particularly those with metastatic disease, to enhance their overall well-being and quality of life.

1.3.3 COVID, a potential cause for Neuropathy

Recent worldwide COVID pandemics has added up to neuropathic pain burden as one of the studies suggest up to 2.3% of hospitalized patients being treated for corona virus reports neuropathic pain. The number may jump up as reports analysed were from

quite an early stage and neuropathic pain development takes time(Colloca et al. 2017a; Mahan et al. 2020).

1.4 Pain Process

Four major processes are involved during pain perception: transduction, transmission, modulation, and perception. While first three parameters transduction, transmission, and modulation being neural processes can be studied objectively. Whereas, pain perception that is, awareness of pain is purely subjective and cannot be directly and objectively measured. **Transduction** is activation of nerve endings in response to any tissue damaging stimuli. **Transmission** refers to the process by which messages travel from the location of tissue damage to the brain regions responsible for perception. **Modulation** refers to the neural process that acts specifically to reduce activity in the transmission system. **Perception** is the subjective and encompasses the coherent and meaningful integration of several sensory messages (Birnbaum et al. 2018).

1.4.1 Anatomy of Pain

Afferent sensory nerves transmit information from receptors located in sensory end organs as skin and tissues to the brain. These receptors detect diverse stimuli, creating an electrical impulses or action potential in sensory nerves. These impulses carrying signals are transduced to the dorsal root ganglion (DRG) in the spinal cord and then synapse with other neurons to reach the brain through the spinothalamic and spinoparabrachial tracts. Primary afferent neurons in peripheral nerve endings perceive various signals in response to different stimuli as mechanical, heat, cold and chemical etc(Steeds 2009). These fibers are of different types and are classified by their

conduction speed and stimulus type as A-beta(Aβ) fibers, C-fibers and A-delta (Aδ) fibers **(Table 1).** Both Aδ and Aβ fibers are found in skin and other superficial organs, whereas C & A-delta fibers (peptidergic/CGRP+) are the main suppliers of deep structures including muscles and joints Aβ fibers are myelinated, fast conducting (>20 m/s) fibers with large diameter and are stimulated by light touch and pressure. Whereas, Aδ fibers are thinly myelinated, and conduct at a slower speed of 2 to 20 m/s compared to Aβ fibers. C fibers, are unmyelinated fibers that conduct at less than 2 m/s. Aδ and C fibers generally activated in response to extensive heat, cold, mechanical, and chemical stimuli and therefore also termed as "polymodal." Since, Aδ fibers are much swifter than C fibers, Aδ fibers elicit the "first pain" sensation which is intense, sharp, tingling in nature, whereas C fibers fetch the "second pain" burning sensation. These primary afferent fibers synapse in dorsal horn of spinal cord (laminae) with second order neurons (nociceptive specific (NS), wide dynamic range (WDR), and low threshold (LR)) NS neurons are activated in response to high-threshold noxious stimuli, while WDR rare sensitive to sensory stimuli, and innocuous stimuli are perceived by LR neurons only(Rozenfeld et al. 2007; Lee and Neumeister 2020). Action potential or electrical impulse hence generated are transmitted through second order neuron to the thalamus via the spinothalamic and spinoreticular tracts. This somatosensory information is processed in thalamus and is transduced to higher brain centers including prefrontal cortex through third order neurons situated in thalamus. Intensity, duration, and location of pain is perceived and integrated in cortex. Descending inhibitory tract and regions of brain as periaqueductal gray (PAG) and nucleus raphe magnus (NRM) the brainstem are involved in alleviating pain (Mokhtarieh et al. 2018).

Fiber Type	Myelination	Conduction Speed (m/s)	Stimulus Type	Location	Sensation Type
A-beta (Aβ)	Myelinated	>20	Light touch and pressure	Skin and superficial organs	N/A (Does not contribute to pain)
A-delta (Aδ)	Thinly Myelinated	2 to 20	Extensive heat, cold, mechanical, and chemical stimuli	Skin and superficial organs	"First pain" - Intense, sharp, tingling
C	Unmyelinated	<2	Extensive heat, cold, mechanical, and chemical stimuli	Main suppliers of deep structures (muscles and joints)	"Second pain" - Burning sensation

Table 1.1 Classification and Characteristics of Peripheral Nerve Fibers

1.4.2 Pathophysiology of Pain

1.4.2.1 Chronic pain, central sensitization, centralized pain

Pain perception is categorized into four major types: **acute pain, nociceptive pain, chronic pain, and neuropathic pain**. Any stimuli capable of producing potential or real tissue damage are referred to as noxious stimuli (e.g., heat, cold, mechanical force, or chemical stimulation) and trigger nociceptors leading to acute pain. Acute pain is often protective in nature and is short lived until the site of injury is healed (Meacham et al. 2017). **Nociceptive pain** is the outcome of the activation of normal physiological pain pathways in response to harmful or noxious stimuli. It serves as a protective mechanism, alerting the body to potential damage and prompting appropriate responses to minimize harm. **Acute pain** transition to inflammatory or **nociceptive pain** when the noxious stimulus persists for a longer duration of time hence, allows nociceptive neurons to release their pro-inflammatory cytokines and

activate responsive cells. **Neuropathic pain** is a result of damage to sensory nerves due to a direct injury or disease such as diabetic neuropathy, shingles, or postherpetic neuralgia (Borzan and Meyer 2017). These repeated or noxious stimulation activate peripheral nociceptors and nociceptive neurons in the dorsal root ganglion (DRG). As a result, large amounts of substance P (SP) and calcitonin-gene-related peptide (CGRP), in DRG, causing enhanced responsiveness and lowered threshold to mediate peripheral sensitization and further transmits pain signals to the spinal dorsal horn (SDH) via the DRG (Dou et al. 2021). Release of substance P and CGRP serves as a predictive signal of actual or potential damage associated with noxious stimulus. Peripheral sensitization can further lead to the development of central sensitization within the spinal cord. Which is primarily caused by an increase in ionotropic and metabotropic glutamate receptors, a decrease in GABA receptors, alterations in sodium and potassium channels, and neuroinflammatory change (Hassanpour et al. 2020).The phenomenon when lamina I neurons of spinal cord are over sensitized to respond to non- nociceptive inputs as a result of neuroinflammatory changes is expressed as allodynia (Chiu et al. 2012).

1.4.2.2 Peripheral sensitization

Peripheral sensitization involves a lowered threshold and heightened response of sensory nerve fibers in the periphery to external stimuli, resulting in increased stimulus-dependent pain known as primary hyperalgesia. It typically occurs after peripheral nerve injury, tissue damage, or inflammation. This heightened sensitivity leads to increased release of neurotransmitters from peripheral endings and spinal cord terminals, intensifying neurogenic inflammation and nociception(Przewlocki and Przewlocka 2001; Shin et al. 2020). Any kind of tissue injury leads to release of a wide

range of signaling molecules is involved in mediating peripheral sensitization, including protons, ATP, prostaglandins (PGE2), thromboxanes, leukotrienes, endocannabinoids, growth factors such as neurotrophins [nerve growth factor (NGF)] and granulocyte- or granulocyte-macrophage colony stimulating factors (G-CSF, GM-CSF), cytokines (IL6, IL1β, TNFα), chemokines, neuropeptides [calcitonin gene-related peptide (CGRP), substance P, bradykinin, histamine], lipids, and diverse proteases (Gangadharan and Kuner 2013). Besides this glutamate, which is the major excitatory synaptic transmitter at central synapses, contributes to sensitization at peripheral nerve endings by binding in a non-synaptic manner to AMPA and NMDA receptors (AMPAR and NMDAR, respectively) to mediate peripheral cell-cell interactions (e.g. upon release from immune cells) or autocrine regulation (e.g. upon release from sensory endings following TRPV1-mediated Ca^{2+} influx(Samineni et al. 2017). Release of these mediators initiate peripheral sensitization either by (1) early post-translational changes in the peripheral terminals of nociceptors, for example, the phosphorylation of the ion channels prolongs depolarization and enhances response by lowering the open threshold or prolonging the open time of channels; or (2) altered gene expression, changing transcription or translation of certain protein (Zhang and Jeske 2020). Shortly after injury ectopic firing in peripheral nerve endings and dorsal root ganglion (DRG) neurons begins in Aδ fibers and later in C fibers. Hyperpolarization-activated and cyclic nucleotide-gated (HCN) channels, part of voltage-gated potassium (Kv) channels, are the main generators of ectopic activity. Changes in channel expression and composition occur throughout the peripheral nerve endings, along the axon, and in DRG due to intracellular second messengers, leading to increased expression of Nav, VGCC, TLR4, TRP, α1-AR, ASIC, and decreased expression of

Kv. In spinal terminals, Aβ fibers sprout in dorsal horn laminae. The autonomic system is involved with upregulation of α1-AR, enhanced adrenergic sensitivity at the injury site and in DRG neurons, and sympathetic fibers sprouting in the periphery and DRG(Chen et al. 2015) .

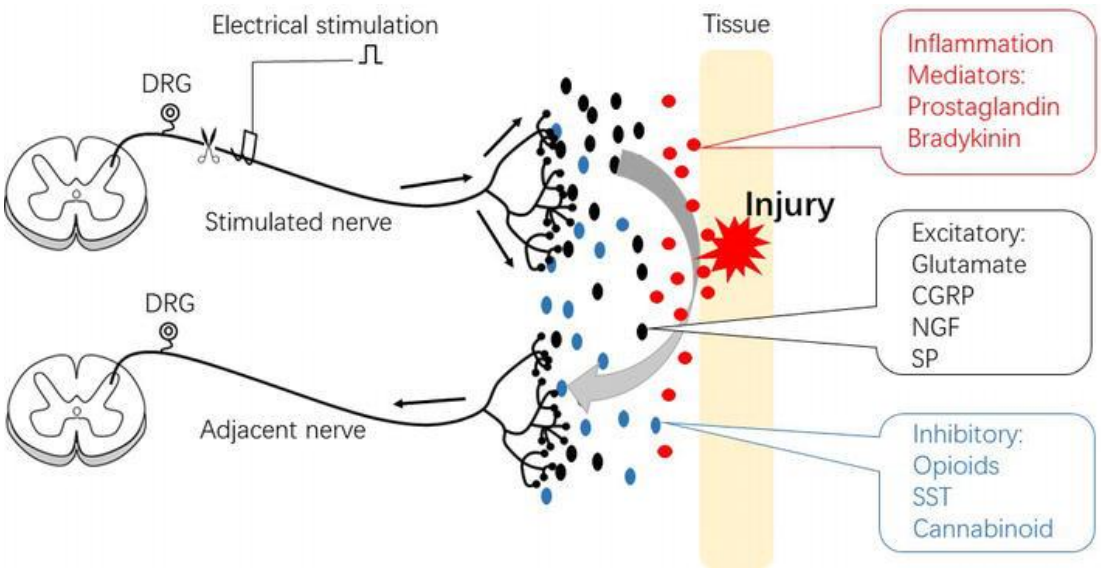

Figure 1.1 Peripheral sensitization: Tissue injury triggers the release of inflammatory mediators and neurotransmitters, further, activating receptors and channels on nearby peripheral nerve terminals leading to peripheral sensitization**.** Reprinted with permission: http://dx.doi.org/10.5772/intechopen.90319.

1.4.2.3 Central sensitization:

Central sensitization is the pivotal physiologic phenomenon underlying the clinical symptoms of neuropathic central pain following peripheral nerve injury (Robert J. Schwartzman, 2001). Under normal circumstances, microglia serve as the sole immunocompetent cells in the nervous system, consistently surveying the central nervous system (CNS) parenchyma to uphold homeostasis. However, following peripheral inflammation, certain microglial cells in the spinal cord undergo alterations in their morphology, functionality, and chemical profile leading to synthesis and release of pro-inflammatory cytokines, among which, IL-1β and TNF-α contribute to the

development of central sensitization by enhancing excitatory and reducing inhibitory currents (Kawasaki et al. 2008). Neuro inflammatory cytokines such as IL-1, IL-6, and TNF-α, favors Inflammation while IL-10, IL-4 and TGF-β are associated with inhibiting inflammation (Bennett Jeanette M,et.al 2018). The major clinical menifestation of nociceptive central pain are (1) hypersensitivity at the site of injury; (2) mechanoallodynia; (3) thermal hyperalgesia (4) hyperpathia (5) extraterritoriality (regional distribution of pain) in the case of complex regional pain syndrome/reflex sympathetic dystrophy and (6) associated neurogenic inflammation, autonomic dysregulation, and motor phenomena that are especially found in complex regional pain syndrome/reflex sympathetic dystrophy. The pain centralization is also mediated via activation of astrocytes on the spinal cord level leading to increased expression of the glial fibrillary acidic protein (GFAP) or astrogliosis (Xu et al. 2019). These pain syndromes exhibit widespread or less precisely localized pain, along with fatigue, mood and sleep disturbances, and a diminished quality of life. They often coexist with other centralized pain syndromes and irritable bowel syndrome. Central pain, more precisely, arises from lesions anywhere along the spino-thalamo-cortical pathway, such as those seen in multiple sclerosis, Parkinson's disease, spinal cord lesions, and thalamic strokes. Central sensitization can contribute to the transition from acute to chronic pain. Reviews indicate that depression, anxiety, fear avoidance, and catastrophizing are linked to the risk of pain becoming chronic(Willis 2001).

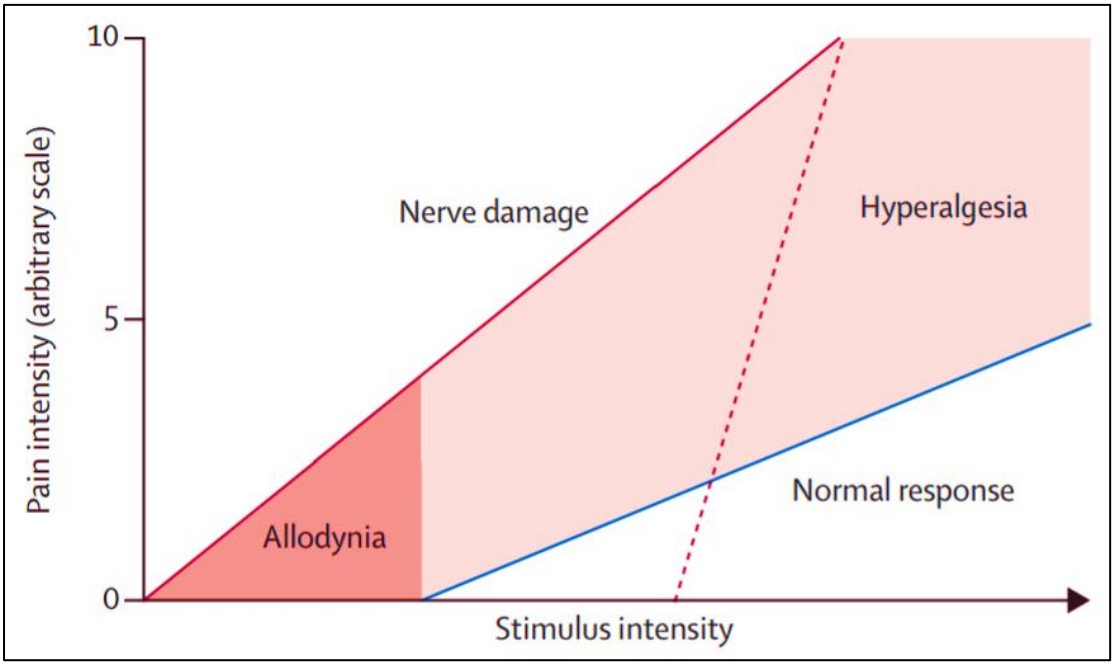

Figure 1.2 Stimulus-response function depicting normal v/s pathophysiological pain (allodynia and hyperalgesia). The normal pain response is shown by the blue line whereas the red line shows the response after nerve damage Reprinted with permission by Elsevier from source reference (Jensen and Finnerup 2014).

1.5 Pain pathway

1.5.1 Ascending Pain Pathway

Pain transmission relies on a balance of excitatory and inhibitory influences in somatosensory neuron circuits across various CNS levels: spinal cord (supraspinal), brainstem (midbrain, medulla, pons), and cortical regions. The spinal dorsal horn (DH) integrates inputs from primary afferent neurons and local interneuron networks, also receiving descending signals from supraspinal centers. Within the ascending system, primary afferent nociceptors are responsible for conveying the noxious information received to the projection neurons in the DH of the spinal cord(Isenberg-Grzeda and Ellis 2015; Borzan and Meyer 2017; Chia et al. 2020). The neurons residing in the dorsal horn of the spinal cord are interneurons rather than second-order neurons and are both inhibitory and excitatory (King et al. 2017; Chen and Heinricher 2019) Following

that, a subset of these projection neurons also known as second-order neurons projects primarily from the lamina I and V of DH acts as origin of multiple outputs and transmit these sensory information up to the thalamus reaching the somatosensory cortex through the spinothalamic tract, thus providing information on the intensity and the location of the noxious stimulus (Westlund 2006). The spinothalamic tract is located in the white matter of spinal cord and consists of two parts—the lateral spinothalamic and anterior spinothalamic tracts, which have different courses of function (Al-Chalabi and Gupta 2019). The lateral spinothalamic tract focuses on transmission of the pain and temperature sensation, while the anterior spinothalamic tract carries information related to the crude touch and firm pressure sensation towards the thalamus in the brain (Mccoy et al. 2011). Other projection neurons connect with the cingulate and insular cortices through the parabrachial nucleus and the amygdala, influencing pain experiences. In this process, ascending information reaches the periaqueductal gray (PAG) and rostral ventral medulla (RVM) in the midbrain, initiating descending feedback systems to regulate spinal cord output. The PAG integrates input from higher brain centers (hypothalamus, amygdala, frontal lobe) and ascending nociceptive input from the dorsal horn (DH). It governs nociceptive information processing in the DH through projection neurons to RVM and dorsolateral pontine tegmentum (DLPT). The PAG/RVM pathways heavily express endogenous opioid and cannabinoid systems, along with neurotransmitters like serotonin (5-HT) and norepinephrine (NE) (Yam et al. 2018).

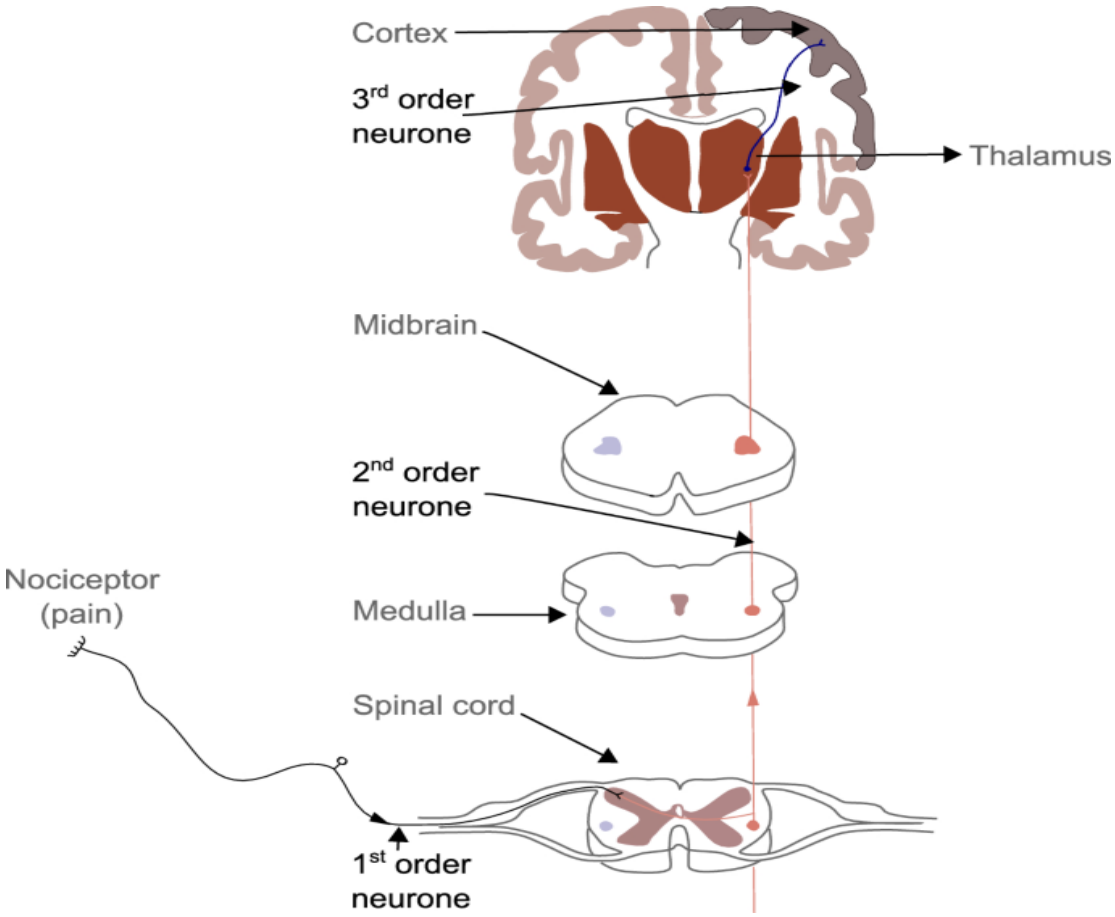

Figure 1.3 Ascending Pathway: Three sensory neurons convey pain information in an ascending signaling pathway. Reprinted with permission: Creative Commons Attribution 4.0 International (Basbaum et al. 2009).

1.5.2 Brain regions involved in the experience of pain

Various pathways within the central nervous system (CNS) contribute to the processing of pain. Human brain imaging studies consistently reveal the activation of cortical and subcortical networks in response to pain, encompassing sensory, limbic, and associative regions. Notably, the primary somatosensory cortex (S1), secondary somatosensory cortex (S2), anterior cingulate cortex (ACC), insula, prefrontal cortex (PFC), thalamus, and cerebellum are frequently activated by noxious stimuli. Additional regions, such as the nucleus accumbens and amygdala, likely receive nociceptive input through spinoparabrachial-amygdala projections, along with the

periaqueductal grey (PAG), which receives nociceptive input through spinoreticular pathways(Bang et al. 2009). The somatosensory cortices (S1 and S2) encode sensory features like pain location and duration. In contrast, the anterior cingulate cortex (ACC) and insula, traditionally associated with the emotional brain, play a key role in encoding the emotional and motivational aspects of pain. Lesions in these regions alter emotional responses to pain, and imaging studies reveal a link between emotional/motivational aspects of pain perception and neural activation in the ACC and insula (Bushnell et al. 2013). The thalamic nuclei are involved in the sensory discriminative and affective motivational components of pain (Ab Aziz and Ahmad 2006) In chronic pain conditions, there is a modified activation pattern in various brain areas that amplifies the overall pain response.

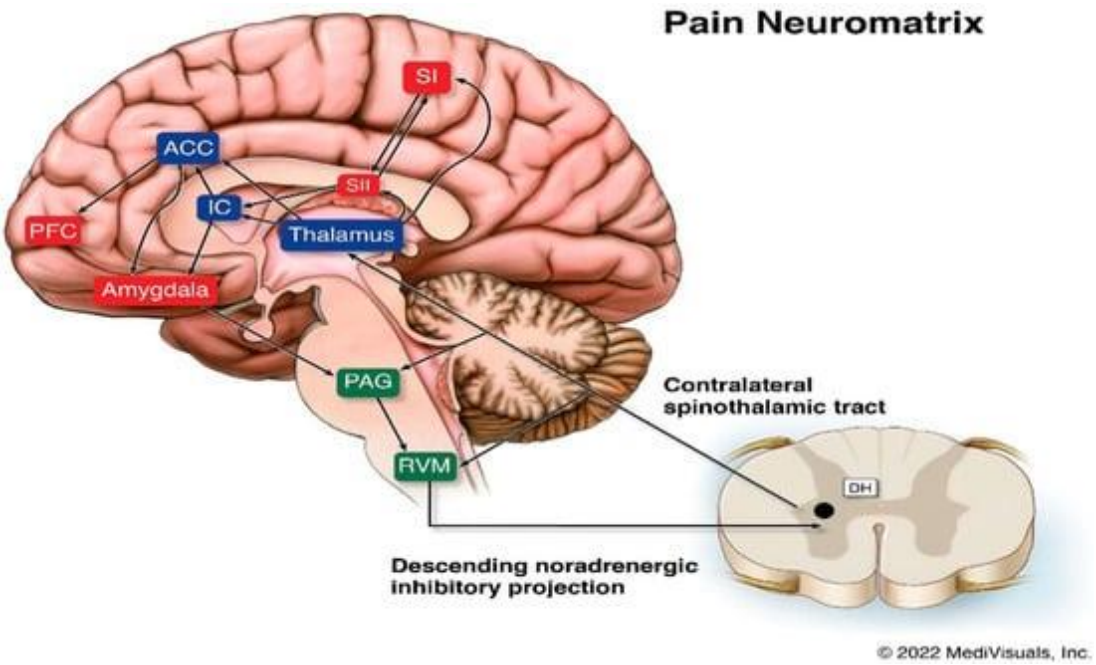

Figure 1.4 Neurobiology of pain processing. Reprinted with permission: Creative Commons Attribution 4.0 International (Laumet et al. 2015)**.**

1.5.3 Descending pain pathway

Midbrain and medullary sites exhibit precise control over nociception in both directions. The periaqueductal gray (PAG), influenced by higher brain centers, can trigger a potent analgesic effect. Meanwhile, the rostroventromedial medulla (RVM) has the capacity to either enhance or inhibit nociceptive inputs, serving as a crucial relay in the regulation of descending pain facilitation(Navratilova et al. 2020). Together, these structures along with dorsal horn of the spinal cord offer a mechanism for cortical and subcortical sites to impact nociception (Iyengar et al. 2017). The descending pain circuit is largely modulated via the neurotransmitter system including serotonergic, opioid, and noradrenaline (Wainger and Brenner 2017). The analgesic impact of the periaqueductal gray (PAG) doesn't directly connect to the dorsal horn but operates through an intermediary known as the "rostral ventromedial medulla" (RVM). The RVM, situated around the raphe magnus and adjacent reticular region near the facial nucleus, is identified as the primary output node in descending pain modulation. Similar to the PAG, electrical stimulation of the RVM induces potent analgesia. The RVM, densely connected to the PAG, projects diffuse bilateral connections to the dorsal horn at multiple levels. The PAG-RVM system stands out as the most extensively studied circuit for descending pain modulation, emphasizing its functional significance (Bang et al. 2009).

1.5.4 N-methyl-D-aspartate (NMDA) Receptors

The N-methyl-D-aspartate (NMDA) receptor is a receptor of glutamate, the primary excitatory neurotransmitter in the human brain and plays an integral role in synaptic plasticity. NMDA receptors typically comprise of four subunits two NR1 and

two NR2 sub units, with NR2 having four (A-D) sub type. Each Sub unit composition of NMDAR is encoded by a different set of gene and display its differential expression and regulate various physiological and pharmacological functions across CNS (Ma and Quirion 2014; McCutcheon et al. 2020). A vast range of scientific reports suggests that long-term potentiation (LTP), generated by AMPA and NMDA receptor activation, with critical involvement of the NR2B subunit, plays a key role in the development of spatial memory in the hippocampus (Günaydın et al. 2020; Uniyal et al. 2021b). Increased expression of NR2B subunit of NMDAR in anterior cingulate cortex, or DRG of spinal cord is responsible for persistent inflammation and consequent development of chronic pain thereby modulating behavioral responses at peripheral level (Lee et al. 2012; Liang et al. 2021). While at the level of spinal cord, neuropeptides as substance P and neurokinin A or NMDA receptor activation is required for the elicitation of spinal LTP. Activation of NMDAR is extensively reported to facilitate allodynic and hyperalgesic behavior in response to noxious as well as non-noxious stimuli, regulated by NMDA- Calcium calmodulin signaling pathway, consequently playing an integral role in initiation or maintenance of persistent chronic painful conditions (Zhou et al. 2011; Gieseler et al. 2013; Lagatta et al. 2018). NR2B subunit of NMDA receptor significantly contributes to LTP in spinal cord (Zhuo 2009) and is involved in mediating chronic pain, regulated by phosphorylation of PSD -95, and CREB in dorsal horn of spinal cord in CCI injured nerve animals. Improved pain behaviour on i.t. injection of NR2B antagonist is consistent with evidence for NR2B being actively involved in development of neuropathic pain (Lee et al. 2012). Direct antagonizing NMDAR or blocking NR2B exerts a vital impact on recovering chronic neuropathic pain conditions by modulating synaptic plasticity at the molecular level and hence improved

behavioural pain conditions (Jiang et al. 2018; Guo et al. 2019) Studies suggest NR2B subunit execute a major contribution towards initiation and maintenance of neuropathic pain during the early phases of injury followed by establishing a long lasting enhanced spinal excitability(Jiménez-Aspee et al. 2016). But before NR2B subunit to exert its effect, on DRG or central neurons, it is required for freshly processed subunit to be trafficked into the extra synaptic dendrites and get localized in to synapse to activate NMDAR and this transport of cargo is carried by various kinesin proteins (Zhong et al. 2020).

1.6 Kinesin Proteins: Their Structure, and Function, and Role in Pain Modulation

KIFs also known as kinesin superfamily proteins are microtubule-dependent molecular motors that transport wide range of cargo as neurotransmitter receptors, cell signaling and adhesion molecule and mRNAs in-between different compartments of mammalian neurons which are crucial for synapse development and plasticity, (Verhey and Hammond 2009; Ali and Yang 2020; Fan and Lai 2022). Based on phylogenetic analysis KIFs are classified into 15 kinesin families, kinesin-1 to kinesin 14B. Kinesin proteins, encoded by 45 genes in both mice and humans. Intracellular transport stands as a fundamental mechanism essential for cellular functionality, survival, and morphogenesis. Within this intricate framework, the Kinesin superfamily proteins (KIFs) emerge as pivotal molecular motors orchestrating the directional movement of diverse cargos, including membranous organelles, intricate protein complexes, and messenger RNA (mRNA) molecules(Guillaud et al. 2003; Lawrence et al. 2004). These molecular motors share a highly conserved globular motor domain responsible for microtubule binding and ATP hydrolysis, the fuel powering their movement. (Fan and

Lai 2022). Based on the pathway preferred, motor proteins are grouped into two classes (1) motor protein which travels through actin-myosin (actin-based motility) and (2) motor proteins that travel through microtubule, i.e., kinesins and dyneins (tubulin based motility) (Guillaud et al. 2003). **(Fig 1.5).** Kinesins, remains an evolutionary conserved class of motor proteins, using dynamic polar filaments of αβ-tubulin known as microtubules, as their neuronal highway and play a crucial role in various biological contexts(Asaoka et al. 2019). This conserved catalytic region known as 'motor or head' domain is responsible for ATP hydrolysis and microtubule binding. Globular motor domain consists of a catalytic core which is linked to a short region 'neck linker' that helps to bring the ATP-dependent conformational changes within the catalytic core and defines the direction of movement along a microtubule track (Iftinca et al. 2021).

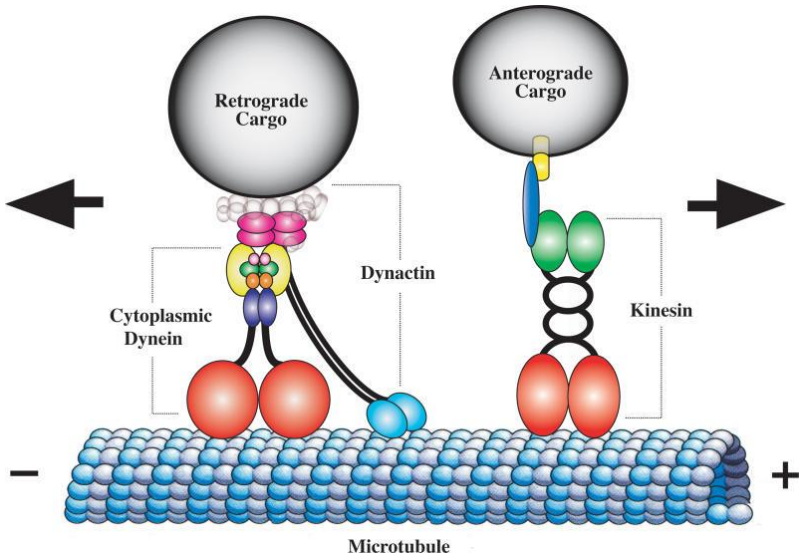

Figure 1.5 Kinesin motor protein transport along microtubule. Reprinted with permission: Creative Commons Attribution 4.0 International (Jason E. Duncan, 2006).

Kinesin also contains a stalk domain with a coiled-coil structure that is required for dimerization, and a tail domain that serves to bind specific cargoes. Majority of the kinesins have their motor domains present on the N-terminus (named as N-kinesins) and move towards the fast-growing end (the plus-end) of the microtubule. Since microtubules in the axon are polarized with plus-end on the distal side, kinesins mediate the anterograde transport in axon. In contrast, because the microtubule polarity in proximal dendrites is mixed, kinesins can transport materials bidirectional in dendrites(Yoshihara et al. 2021).

Importance of kinesin extends beyond basic cellular functions, and a large number of growing evidences implicate kinesins in the complex process of pain perception and modulation. Kinesin's Role in NR2B Subunit Trafficking and NMDA Receptor Function Multiple kinesin nano proteins have been reported to be involved in trafficking of NMDA receptors (2003 Laurent Guillaud). Multiple kinesin nano proteins have been reported to be involved in trafficking of NMDA receptors. (2003 Laurent Guillaud). NMDAR, mainly its subtypes NR2A and NR2B, regulates the NMDA channels. Several reports suggest involvement of NR2B in regulation of nociceptive effects in thermal, neuropathic and inflammatory pain through central sensitization(Vinet and Zhedanov 2011; Shantanu et al. 2021; Uniyal et al. 2022a). KIF3B knockout mice displays a raised LTP a lower LTD governed by direct and indirect interaction of KIF 3B with NR2A and NR2B subunit of NMDA receptors respectively and is responsible for post translational modification (2021 Ruolin Fan). KIF 5B binds to the carboxyl tail of NR2b and inhibits calcium influx at extra synaptic level by modulating the targeting and signalling of NMDAR at post synaptic levels.

(2019 Raozhou Lin). Besides this KIF11 and KIF 21a are also reported to be actively involved in trafficking of NR2B sub unit of NMDA receptors.(Uniyal et al. 2021b) (Fig. 1.6)

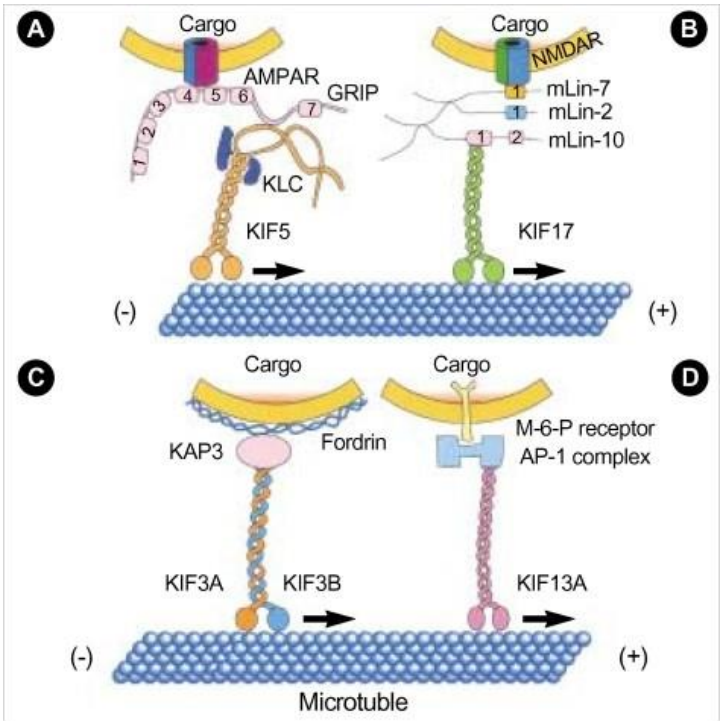

Figure 1.6: Involvement of Kinesin motor proteins in vesicle transport. Reprinted with permission: Creative Commons Attribution-Non Commercial 3.0 Unported (Seog et al. 2004).

1.6.1 KIF17: A Key Mediator in NR2B Subunit Trafficking and pain modulation

KIF17 belongs to kinesin 2 family and is involved in transportation of N-methyl-D-aspartate (NMDA) receptor subunit 2B (NR2B) along microtubules by forming a complex with mLin10 and GluN2B (Uniyal et al. 2022b). Plenty of scientific communications reveals downregulation of KIF 17 and associated suppression of NR2B results in attenuation of chronic and as well as neuropathic pain symptoms (Zhou et al. 2020). The KIF 17 directly binds to the postsynaptic density-95/disc large/zona

occludens-1 (PDZ) domain of Mint 1 (mLin-10) which further get assembled with a large scaffolding protein complex containing the mLin-2, mLin-7, and NR2B subunit. Finally, the cargo is carried by KIF17 using ATP driven motor from the cell soma to the synaptic terminal along the microtubule. The expression of KIF17 and NR2B is found to be associated with chronic pain (Setou et al. 2000; Hartrick 2005; Xia et al. 2020). It is reported that KN93 inhibitor of calcium/calmodulin-dependent protein kinase II is able to alleviate bone cancer pain in mice and the effect was shown to be mediated through inhibition of of KIF17/NR2B trafficking (Uniyal et al. 2022b). Kinesin superfamily protein 17 contributes to the development of bone cancer pain by participating in NR2B transport in the spinal cord of mice (Zhou et al. 2020). While, pain hypersensitivity induced by bone cancer in mouse was effectively ameliorated by Intrathecal injection of peptide Myr-RC-13 by suppressing the expression of kif 17 and NR2B at the level of spinal cord (Kang et al. 2016). Number of reports suggest overexpression of KIF 17 at spinal level in neuropathic pain conditions. The cyclic AMP response element-binding protein (CREB) modulated neuropathic pain behavior in CCI mice were attenuated by CREB antisense oligonucleotide administered intrathecally. KIF 17 levels were over expressed after injury and downregulated after treatment in spinal cord, whereas, KIF 5 levels remain unchanged (Zhang et al. 2020a).

1.7 Pharmacotherapeutics for Chronic Pain: Efficacy and Constraints

Managing chronic pain poses a significant challenge in the healthcare system, demanding multimodal approaches distinct from the limited tissue injury seen in acute pain. Over the past few decades, various analgesics have shown severe side effects, creating substantial barriers to effective pain management. A broad array of

pharmacological options exists for chronic pain. Nonopioid analgesics like nonsteroidal anti-inflammatories (NSAIDs), opioids, antiepileptic drugs, antidepressants like tricyclic antidepressants and SNRIs, topical analgesics, muscle relaxers, N-methyl-D-aspartate (NMDA) receptor antagonists, and alpha 2 adrenergic agonists are employed according to location and severity of pain.

1.7.1 Nonsteroidal anti-inflammatory drugs (NSAIDs)

Anti-inflammatory drugs (NSAIDs) are among the most widely used medications, available both through prescription and over-the-counter channel. NSAIDs have been widely used for managing pain and inflammatory conditions, particularly in acute and chronic orthopedic pain such as osteoarthritis, ankylosing spondylitis, and rheumatoid arthritis, as well as postsurgical pain (Bunch and Qian 2017). In patients with chronic nociceptive pain (e.g. tendonitis, osteoarthritis, or back pain), a shorter course of NSAIDs can be considered. COX2-selective and nonselective nonsteroidal anti-inflammatory drugs (NSAIDs), which display both analgesic and anti-inflammatory properties, are useful options for the management of chronic pain (Ho et al. 2018).

NSAIDs exert their actions by inhibiting enzymatic activity of the COX enzymes. COX-1 and COX-2, acting as bifunctional enzymes, facilitate a COX reaction converting arachidonate and oxygen into the cyclic endoperoxide PGG2. This is followed by a hydroperoxidase reaction, reducing PGG2 to PGH2 (Thirunavukkarasu et al. 2017). The unstable intermediate PGH2 then spontaneously rearranges or undergoes enzymatic conversion by specific synthases to various biologically active prostaglandin (PG) isoforms. The overall regulation of PG type and quantity in a cell

or tissue is influenced by the expression levels of COX-1, COX-2, and terminal synthase enzymes. However, use of these agents is limited by side effects, specifically adverse gastrointestinal (GI) and cardiovascular (CV) events (Togay et al. 2021).

1.7.2 Antiepileptic

Antiepileptics exerts their effect by modulation of voltage-gated ion channels, including sodium, calcium, and potassium channels; by enhancement of γ-aminobutyric acid (GABA)-mediated inhibition through effects on GABAA receptors, the GABA transporter 1 (GAT1) GABA uptake transporter, or GABA transaminase; through interactions with elements of the synaptic release machinery, including synaptic vesicle 2A (SV2A) and α2δ; or by blockade of ionotropic glutamate receptors, including α-amino-3-hydroxy-5-methyl-4-isoxazole-propionate (AMPA) receptors (Morley et al. 2020). Gabapentin and pregabalin act on the α-2-delta subunit of voltage-gated calcium channels leading to reduced release of substance P, glutamate, and noradrenalin. Most common side effects of anti-epileptics include dizziness, somnolence memory loss, edema, dry mouth leading to dropout (Ullah et al. 2021).

In patients with neuropathic pain, antiepileptic drugs represent the first-line treatment. Antiepileptic drugs as Gabapentin and pregabalin show evidence of efficacy in long-term nerve pain (diabetic neuropathy, postherpetic neuralgia, central neuropathic pain, fibromyalgia) and significantly reduce pain in some individuals. But are also associated with common side effects with about 1 in 4 finding them intolerable and discontinuing the medication (Fu et al. 2020). Antiepileptics exerts their effect by modulation of voltage-gated ion channels, including sodium, calcium, and potassium channels; by enhancement of γ-aminobutyric acid (GABA)-mediated inhibition

through effects on $GABA_A$ receptors, the GABA transporter 1 (GAT1) GABA uptake transporter, or GABA transaminase; through interactions with elements of the synaptic release machinery, including synaptic vesicle 2A (SV2A) and α2δ; or by blockade of ionotropic glutamate receptors, including α-amino-3-hydroxy-5-methyl-4-isoxazole-propionate (AMPA) receptors (Bodenant et al. 2008). Gabapentin and pregabalin act on the α-2-delta subunit of voltage-gated calcium channels leading to reduced release of substance P, glutamate, and noradrenalin. Most common side effects of anti-epileptics include dizziness, somnolence memory loss, edema, dry mouth leading to dropout (Khan et al. 2021).

1.7.3 Antidepressants

Although not initially designed as analgesics, antidepressants are recognized for their analgesic effects in chronic pain. While they lack direct antinociceptive effects, antidepressants are considered first-line drugs for neuropathic pain and fibromyalgia. Tricyclic antidepressants (TCA) and serotonin noradrenaline reuptake inhibitors (SNRI) are antidepressants with specific analgesic effects, with TCAs being long-established and SNRIs relatively new. However, selective serotonin reuptake inhibitors (SSRIs), commonly used for depression, are not effective against chronic pain (Hideaki Obata, 2017). Among tricyclic antidepressants, amitriptyline (25–125 mg/day) shows the better number needed to treat for 50% patient relief as compared to duloxetine (30–60 mg/day). Venlafaxine is not commonly used because it has low activity on the noradrenaline pathway with a dosage of lower than 150 mg per day(Maletic et al. 2017). Antidepressants, along with pregabalin/gabapentin (voltage-dependent calcium channels α2δ subunit ligands) are used as first-line drugs for treating neuropathic pain.

These drugs modulate the neurotransmitter availability in synapse but still lack the clear mechanistic elucidation in case of chronic pain (Nugroho et al. 2014; Di Pierro et al. 2015; Obata 2017).The pharmacologic effects of antidepressants involve binding to noradrenaline and serotonin (5-HT) transporters. Reuptake of these neurotransmitters is inhibited, leading to increased levels of noradrenaline and 5-HT in the synaptic cleft (Chatterjee 1998; Butterweck et al. 2000). The most common adverse effects under treatment with antidepressants are dry mouth, dizziness, nausea, headache, and constipation followed by palpitations, sweating, and drowsiness. Each antidepressant displays distinct risk profiles of adverse effects. Amitriptyline, mirtazapine, desipramine, venlafaxine, fluoxetine, and nortriptyline showed the highest placebo effect-adjusted risk of adverse effects. Risk for withdrawal due to adverse effects is seen highest in desipramine followed by milnacipran, venlafaxine, and duloxetine (Chahal et al. 2020; Khan et al. 2023).

1.7.4 Opioids

Opioids, widely employed and highly effective for severe pain relief, have recently faced scrutiny due to escalating levels of abuse and overdose, prompting concerns about their widespread use (Zhang et al. 2012). Most commonly prescribed opioids includes codeine, morphine, oxycodone,tramadol and buprenorphine,etc. for treating chronic pain conditions like low back pain, osteoarthritis, and neuropathic pain (Obata 2017). These compounds act on the endogenous opioid system, consisting four G protein-coupled receptors (mu, delta, kappa, and nociceptin) and four major peptide families (β-endorphin, enkephalins, dynorphins, and nociceptin/orphanin FQ. opioids interact with μ, κ, and δ receptors at the presynaptic level, initiating distinct actions. Upon receptor binding, the α subunit of the G protein inhibits the adenylate cyclase

(AC) pathway, leading to reduced calcium channel activity and subsequent glutamate release. Simultaneously, the same channel is inhibited by the βγ subunit. Buprenorphine stands out as a unique drug with partial agonist activity on μ receptors and antagonist activity on κ receptors. Opioids are recognized for their ability to inhibit calcium channels and activate potassium channels, leading to hyperpolarization at the postsynaptic level. In 2013, it became evident that the increased prescription of opioids was accompanied by a parallel surge in opioid-related harms, such as addiction, overdose, and death. This prompted a reassessment of early clinical trials, highlighting their short duration, restricted dosing, and inability to capture evidence on longer-term harms (Stannard 2018). In susceptible individuals, repeated exposure to opioid analgesics may lead to heightened pain sensitivity, known as hyperalgesia. This can result in inappropriate increases in opioid doses, further worsening the pain. The development of opioid tolerance, requiring dose escalation, contributes to the emergence of hyperalgesia (Uniyal et al. 2020).

1.7.5 NMDA Receptor Antagonists

The glutamate N-methyl-D-aspartate receptors (NMDARs), particularly those situated in the dorsal horn of the spinal cord, play a crucial role in nociceptive transmission and synaptic plasticity. They have been traditionally identified as a focal point for addressing neuropathic pain in therapeutic interventions (Zhou et al. 2011). NMDA antagonists such as ketamine, dextromethorphan, memantine, and amantadine, are emerging as novel therapeutics for pain managemen (Sang 2000). NMDA receptors (NMDARs) play a role in pain linked to peripheral tissue or nerve injury. These receptors are present in both the enteric nervous system and the peripheral nervous system onsequently, NMDA antagonists target peripheral NMDARs, potentially

serving as analgesics (Zhou et al. 2011). These medications target central sensitization and mitigate neuronal excitotoxicity by inhibiting NMDARs at both spinal and supraspinal levels. While they have demonstrated efficacy in preclinical settings, the use of NMDAR antagonists in clinical pain management is limited, and they often result in various central nervous system (CNS) toxicities. The adverse effects arise from the antagonism of NMDARs, causing modifications in their basal physiological functions and cross interactions between intracellular signaling.

1.7.6 Topical Medication

A recent systematic review of neuropathic pain treatments gave a weak recommendation for the use of high-dose capsaicin topical patch. They concluded with moderate quality of evidence that the efficacy of topical lidocaine was also weak (Dey and Vrooman 2021).

1.7.7 Emerging Therapies

Neuromodulation for pain employs electrical or chemical stimulation to modulate the nervous system and relieve chronic pain resistant to conventional treatments. Techniques include Spinal Cord Stimulation (SCS), disrupting pain signals via implanted electrical impulses, Deep Brain Stimulation (DBS) regulating neural activity in specific brain areas, Transcutaneous Electrical Nerve Stimulation (TENS) using low-voltage impulses to block pain signals, and Intrathecal Drug Delivery (IDD) pumping medication directly into the spinal cord (Alorfi 2023).

1.7.8 Alternative Pharmacological Interventions for chronic pain management

Complementary and alternative medicine (CAM) is widely embraced, with

around 33% of US adults using it, and rates soar to 70%-90% for those with chronic conditions like cancer or fibromyalgia. CAM treatments, including acupuncture and massage therapy and herbal treatment, are prevalent. Even if a CAM therapy doesn't eliminate pain but reduces its perception, as in mind-body therapy, it can significantly enhance a patient's quality of life within a comprehensive care program (Bauer et al. 2016). Physical activity and exercise can reduce the severity of pain and improve function, quality of life, and mental health (Dey and Vrooman 2021). Over time, specific CAM treatments like herbal medicine can transition into conventional practices with supporting scientific data. Pain is a common reason adults turn to CAM, especially when traditional treatments are discontinued due to adverse reactions. Herbal medicine, used by nearly twenty million Americans, generates over 1.5 billion dollars annually with 25% annual growth. The global market was valued at USD 71.19 billion in 2016. About 60% of individuals with arthritis or musculoskeletal pain explore CAM. According to WHO, herbal medicine involves plant-derived products affecting eicosanoid metabolism. Rigorous studies are crucial to validate their effectiveness and safety for integration into clinical practice and treatment guidelines (Jahromi et al. 2021).

1.7.9 Global Prevalence of Herbal Medicine used for Chronic Pain

The use of plants for medicinal purposes, particularly as analgesic drugs in folk medicine, has a rich history predating modern medical sciences in developing countries (Martins et al. 2017). Pain is an enormous problem and it is estimated around 20% of adults suffering from severe to chronic pain globally and 10% are newly diagnosed with chronic pain yearly. Based on recent studies high prominence of pain and pain-related

conditions remain the leading cause of disability and disease burden globally (Aremu and Pendota 2021). Ethnomedicinal practices globally involve approximately 70,000 plant species, and WHO estimates around 80% of the world population still relying predominantly on plant-based drugs, mitigating self-medication side effects (Karunamoorthi et al. 2013). It is reported that more than 70% of the developing world's population still depends on the complementary/alternative systems of medicine, also known as traditional medicine. for example, up to 80% of the population in Africa, 71% in Chile, and 40% in Colombia, more than 80% of South Asia's 1.4 billion people have no access to modern health care; they rely instead on traditional medicine using native species (Karunamoorthi et al. 2013). The exploration of medicinal plants for new analgesic combinations is expanding, holding promise for discovering therapeutic agents capable of inhibiting, decreasing, or relieving pain (Uritu et al. 2018). Phytochemicals used in herbal medicine for pain relief have gained significant attention. Research indicates efficacy in pain reduction with compounds like lipoic acid, curcumin phytosome, and piperine complementing conventional treatments. Clinical trials suggest the effectiveness of herbal decoctions, such as Xiaozhi decoction, in relieving postoperative pain. Recent evidence highlights anti-nociceptive and anti-inflammatory properties of the methanolic root extract of C. ficifolius. Incarvillateine (INCA), derived from the Chinese herb Incarvillea sinensis, exhibits potent nonopioid antinociceptive action through A3AR mediation (Chen et al. 2021).

1.7.10 Plants used for chronic pain management: List of plants used for pain management

Substance	Animal Model	Mechanisms of Actions
Pterodon pubescens Benth	Partial sciatic nerve ligation (PSNL) in mice	Inhibition of proinflammatory cytokines, glutamatergic receptors, TRPV1, and TRPA1 channels
Shanzhiside methylester	Spinal nerve injury (SNI) in rat	Microglial β-endorphin expression via p38 MAPK signaling
Emblica officinalis	Streptozotocin (STZ)-induced diabetes in rat	Modulation of oxidative–nitrosative stress
Rubia cordifolia	Paclitaxel-induced neuropathic pain in rat	Involvement of GABA or antioxidant mechanism
Ocimum sanctum	Vincristine-induced neuropathic pain in rat	Decrement of oxidative stress and calcium levels
Acorus calamus	Tibial and sural nerve transection (TST) in rat	Anti-inflammatory, antioxidant, and neuroprotective actions
Acorus calamus	Chronic constriction injury (CCI) in rat	Anti-oxidative, anti-inflammatory, neuroprotective, and calcium inhibitory actions
Acorus calamus	Vincristine-induced neuropathic pain in rat	Anti-oxidative, anti-inflammatory, neuroprotective, and calcium inhibitory actions
Salvia officinalis	Vincristine-induced neuropathic pain in mice	Anti-inflammatory effects
Curcumin	CCI in mice	Descending monoamine system (coupled with spinal β2-adrenoceptor and 5-HT1A receptor)
Curcumin	CCI in rat	Decrement in the serum level of COX-2
Phyllanthus amarus	CFA, PSNL in mice	Anti-inflammatory action
Cannabis sativa	CCI in rat	Mediated by vanilloid receptors TRPV1
Momordica charantia	TST in rat	PPAR-gamma agonistic activity, anti-inflammatory, & antioxidative effects
Lappaconitine	CCI in rat	Regulating the purinergic signaling system at DRG level

Substance	Animal Model	Mechanisms of Actions
Saffron's extracts and safranal	CCI in rat	Antioxidant effects
Nigella sativa and thymoquinone	STZ-induced diabetic in rat	Antioxidant actions
Naringin	STZ-induced diabetic in rat	Antioxidant and antiapoptotic activity
Quercetin	STZ-induced diabetic in mice	Modulation of the opioidergic system

Table 1.2 Commom medicinal plants reported to to be effective in alleviating neuropathic pain conditions (Forouzanfar and Hosseinzadeh 2018). Reprinted with permission: Creative Commons Attribution-NonCommercial 3.0 Unported.

1.8 *Sida cordifolia* Linn. for neuropathic pain management

Sida cordifolia Linn. is a flowering herb found all over tropical and subtropical areas of India and Sri Lanka. The plant is also known as "Bala" or "*country mallow"* and belongs to family Malvaceae.

Figure 1.7: *Sida cordifolia* Linn.

1.9 *Sida cordifolia* Linn: A traditional herb for management of neuropathic pain

1.9.1 Scientific Classification of *Sida cordifolia* (Jain et al. 2011).

- Kingdom – Plantae
- Division – Angiosperm
- Class – Eudicots
- Order – Malvales
- Family – Malvaceae
- Genus – Sida
- Species - *Sida cordifolia*

Plant is commonly known as country mallow (English), Bala (Hindi), Kurumthotti (Malayalam) and ayurvedic names are Vatyâlaka, úîtapâki, vâtyodarâhva, bhadraudanî, samangâ, samâmsa and svarayastikâ (Latha and Radhakrishnan 2015). The entire plant is densely covered with persistent, soft, white stellate hairs, imparting a felt-like texture and reproducing primarily through seeds.

Stems: Yellow-green, erect or ascending, 50 to 100 cm long, slender, and densely hairy.

Leaves: Yellow-green, alternate, with stout stalks bearing two equal stipules at the base. The leaves are oblong-ovate, featuring a felted surface. Individual stalks measure 3 to 8 mm long, formed in leaf axils close to the stem apices. The calyx is 5-lobed and hairy, while the corolla is 5-lobed and joined to the staminal tube at the base.

Fruit: A hard, dark brown capsule, 3 to 5 mm in diameter, smooth, with up to 20 barbed bristles on the top. It separates into 10 two-awned mericarps that act as seeds.

Seed: Dark brown, wedge-shaped, smooth-coated, 2 to 2.5 mm long, with two apical

awns armed with backward-pointing bristles.

Flowers: Dark yellow, often with darker orange centers, either solitary or in groups of different ages.

Root: A robust branching taproot over 1 m long with several surface lateral (Parsons, W.T. Cuthbertson 2000)

1.9.2 Traditional medicinal uses of *Sida cordifolia*

It is well recognized herb in traditional Indian system of medicine and is used in several formulations for treatment of pitta disease stress, and pain in joints (Patel et al. 2022). Traditionally it is used for the treatment of ulcers, wound, boils and fever (Kumar et al. 2019) stomatitis, asthma, and nasal congestion (Franzotti et al. 2000). It is also used as a diuretic agent, for the treatment of Parkins cold plateon's disease (Galal et al. 2014), and neurodegenerative diseases including Parkinson's disease (Galal et al. 2014). Ayurvedic eminence of *Sida cordifolia* is reflected by its frequent description in Vedas, Samhitas and Nighantus (Patel et al. 2022). Traditionally roots of plant are used as tonic to strengthen the central nervous system, and treat neurological disorders such as hemiplegia, facial paralysis, cervical spondylosis, neurosis. Along with this there are several mentions where roots are used for treating neuropathic pain conditions as neuralgia, and sciatica (Justino et al. 2020; Srinivasan et al. 2022). Roots of plant has been reported to possess several therapeutic activities as hypoglycemic, Analgesic and anti- inflammatory (Ravi Kanth and Diwan 1999) diuretic effect (Galal et al. 2014), antibacterial (Kumar et al. 2023), antistress and adaptogenic activity (Sumanth and Mustafa 2009), Antiperoxidative and neuroprotective (Swathy et al. 2010),

nephroprotective (Makwana et al. 2012), Analgesic (Momin et al. 2014), antioxidant (Swathy et al. 2010).

1.9.3 Phytochemistry of *Sida cordifolia*

The chemical composition of *Sida cordifolia* has been reported in several phytochemical studies and the major constituents include Alkaloids, flavonoids, steroids, ecdysteroids, monoterpenes, coumarins, aliphatic and amino acids (Ranjani et al. 2015).

Alkaloid

Ψ-(peudo)-ephedrine was isolated from the S. cordifolia (Ghosal et al. 1975). From the roots of S. cordifolia, β-phenethylamine, ephedrine, Ψ-ephedrine, S-(þ) Nb-methyltryptophanmethyl ester, hypaphorine, vasicinone , vasicine, and vasicinol was reported (Ghosal et al. 1975). Whereas, from the aerial parts of the plant , new alkaloids, 50 -hydroxymethyl-10 -(1,2,3,9-tetrahydro-pyrrolo [2,1-b] quinazolin-1-yl)-heptan-1-one, 1,2,3,9-tetrahydro-pyrrolo [2,1-b] quinazolin-3-ylamine , 50 -hydroxymethyl-10 -(1, 2,3,9-tetrahydropyrrolo [2, 1-b] quinazolin-1-yl)-heptan-1-one , 2-(10 -amino-butyl) indol-3-one , and 20 -(3H-indol-3-ylmethyl)-butan-10 –ol have been isolated (Sutradhar et al. 2006, 2007).

Flavonoid

Three new flavonol C-glycosides: 3'-(3",7",-dimethyl-2",,6",-octadiene)-8-C-β-D-glucosyl-kaempferol 3-O-βD-glucoside, 3'-(3",7",-dimethyl-2",,6",-octadiene)-8-C-β-D-glucosylkaempferol 3-O-β-D-glucosyl [1→4]-β-D-glucoside, and 6-(3"-methyl2"-

butene)-30 -methoxyl-8-C-β-D-glucosyl-kaempferol 3-O-βD-glucosyl [1→4]-β-D-glucoside 3, 30 -(3",7",-dimethyl-2",,6",-octadiene)-8-C β-D-glucosylkeampferol 3-O-β-D-glucoside were isolated from the aerial parts of S. cordifolia (Sutradhar et at., 2007). Further, two new flavones, 5,7-dihydroxy-3-isoprenylflavone and 5-hydroxy-3-isoprenyl Flavon from the chloroform extract of S. cordifolia were isolated (Sutradhar et al. 2007).

Ecdysteroids

Sidasterone A and Sidasterone B (Ghosal et al. 1975).

Aliphatics

(10E, 12Z)-9-Hydroxyoctadeca-10,12- dienoic acid, Coronaric acid (Gangwar et al. 2021).

Some other constituents like choline , betaine have also been isolated from arieal parts of *Sida cordifolia* (Ghosal et al. 1975) and Di-(2-ethylhexyl) phthalate.

1.9.4 Marketed formulations of *Sida cordifolia*

Bala mula siddha dugdha, the medicated milk of bala, is efficacious in healing ulcers and wounds in the urinary tract, mitigating bleeding in urine, and alleviating dysuria. **Baladhyam Gritham, Yakshmani**, a ghee formulation, is prescribed for conditions such as fever, T.B, bronchitis, headache, and migraine. Chakrapani Datta suggested its consumption by adding two parts of milk (Jain et al. 2011).

Chandana balalaksadi taila, a medicated oil containing bala, is applied through massage to relieve pain and swelling in vata disorders and muscular weakness in children. Well-known formulations like **Mahanarayana taila, Balati taila,**

Prabhanjana Vimardhana, and **Ksheera-bala taila** incorporate this herb. These oils are topically used for massaging sore muscles, joints in arthritis and rheumatism, and in conditions like sciatica and neuritis of legs.

Additionally, **Dhanvantari tailam**, containing Bala among 47 other substances, is prepared through 21 and 101 times boiling. *Sida cordifolia*, in the form of a 4:1 extract at 400 mg (equivalent to 1600 mg), is available in 60 capsules, with a recommended dosage of 1-2 capsules per day for its various health benefits.

Commercial products such as **Garnell** *Sida cordifolia* (90 vegicaps), **Reflex** *Sida cordifolia* Complex (for weight loss), and **Chocolate Banana** *Sida cordifolia* 1600mg (slimming pills) are also marketed**. CNP Nutritional Supplements** offers *Sida cordifolia* in the form of 120 tablets.

1.9.5 Preclinical studies on *Sida cordifolia*

Extracts from different parts of *Sida cordifolia* exhibit effective reducing power and free-radical scavenging activity while root extract exhibited superoxide-scavenging activity and inhibited lipid peroxidation in rat liver homogenate (Dhalwal et al. 2005; Momin et al. 2014; Subramanya et al. 2015). Methanoloic extract of *Sida cordifolia* roots contain polysaccharides that exhibit immunomodulatory properties, along with rosmarinic acid that exhibited antibacterial activity (Iqbal et al. 2022). Ethanolic extract of the roots of S. cordifolia indicated analgesic potential by inhibition of writhing at the dose of 500 mg/kg at the dose of 500 mg/kg (Momin et al. 2014). Crude ethanolic extract from the leaves of *Sida cordifolia* was able to produce pronounced antinociceptive activity on orofacial nociception (Konaté et al. 2012). The significant

antimicrobial activity of CHCl3, MeOH and aqueous extracts of S. cordifolia leaf, root. Whole plant extract from *Sida cordifolia* mediated iron-oxide nanoparticles (α-Fe_2O_3 NPs) hold potent antibacterial activity against various gram positive and gram negative bacteria (Pallela et al. 2019). Ethanolic extract of *Sida cordifolia* showed strong bactericidal effect against certain common foodborne bacteria as Escherichia coli, Listeria monocytogenes and Staphylococcus aureus (Kumar et al. 2023). 50% ethanolic extract of *Sida cordifolia* root depicted potent antioxidant and antiinflammatory activity against quinolinic acid induced neurotoxicity (Indira and Swathy 2010). Ethyl acetate fraction, and residual fraction from the leaves of *Sida cordifolia* demonstrated strong anti-inflammatory profile when evaluated by quantitation of prostaglandins (PG) PGE_2, PGD_2, PGF2α, and thromboxane B_2 in the supernatant of lipopolysaccharide (LPS)- induced RAW 264.7 cells (Martins et al. 2017). Methanolic extract of *Sida cordifolia* aerial parts have displayed anti-pyretic and anti-ulcerogenic properties in rats at an oral dose of 500 mg/kg. Pyrexia induced by TAB vaccine was significantly reduced along with significant anti-ulcerogenic effect against aspirin and ethanol induced damage (Schwartzkroin 1999). The analgesic and anti-inflammatory activities of new alkaloid, 1,2,3,9-tetrahydro-pyrrolo [2,1-b] quinazolin-3-ylamine isolated from *Sida cordifolia* produced significant increase in the tail flick latency in radiant heat tail-flick method in Carrageenan induced rat paw edema. 50 % ethanolic extract of the roots of *Sida cordifolia* has a potent hepatoprotective action against alcohol-induced toxicity, which was mediated by lowering oxidative stress and by down-regulating the transcription factors and hepatotoxicity markers as alanine aminotransferase, aspartate aminotransferase and γ-glutamyl transferase (Rejitha et al. 2012) The aqueous extract *Sida cordifolia* showed

a significant inhibition of carrageenin-induced rat paw edema at a dose of 400 mg/kg administered orally. Besides this latency period for mice in the hot plate test was increased and number of writhes produced by acetic acid was inhibited. Effects of aqueous acetone extracts of *Sida cordifolia* produced significant dose dependent analgesic effects on acetic acid writhing test and hot plate method in animal model (Konaté et al. 2012). *Sida cordifolia* have also been reported to be effective in rheumatism and nervous disorder as well as Parkinson's disease (Sutradhar et al. 2007).

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