Chapter-1

Introduction and Literature Review

1.1 Introduction

The International Association for the Study of Pain (IASP) defines pain as "an unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage" [1]. The information about 'sensation' or 'feel' in an organism such as touch, pain, pressure, temperature, etc. is encoded by the somatosensory system. The peripheral nerve endings arising from the primary sensory neurons of dorsal root ganglion and trigeminal ganglion act as the receptive field and detect any innocuous or noxious thermal or mechanical stimuli [2]. Acute pain resulting from tissue injury acts as an alarm for the body and serves a protective function. However, acute pain can transit to a chronic state during disease condition due to maladaptation in neuronal circuitry at spinal and supraspinal regions. Chronic pain is associated with significant distress and suffering which hampers the quality of life of patients and imposes a huge burden on the healthcare system. International Classification of Disease (ICD) given by World Health Organization (WHO) defines chronic pain as the pain that persists for more than three months of duration [3]. Everyone experiences pain at some point during their whole life whereas, in terms of seeking medical care for the treatment of pain the worldwide prevalence ranges from 1% to 76%, and varies demographically [4]. The economic burden of chronic pain accounts for more than that of cardiovascular diseases and cancer whereas the available treatment options for chronic pain are limited and are ineffective in a

significant proportion of patients [5]. In addition, most approved therapies are associated with considerable adverse effects that result from their off-target activity at central and peripheral nervous system [6]. Thus, there is an urgent need to develop the novel therapeutics with higher efficacy and broader safety profile.

1.2 Normal v/s pathophysiological pain

Pain is a defensive mechanism of our body system that gets initiated by various stimuli and is required for the wellbeing of an individual [2]. In common terms, sensory neurons that detect various noxious stimuli are called nociceptors and pain signaling across the sensory pathway is known as nociception [7]. Nociceptors consist of particular proteins known as transducers, that encode features of the intensive damaging stimuli and convert them to electric signals. The information in the form of electrical activity carries up to the central nervous system where it can elicit both sensational pain which we called nociceptive pain as well as withdrawal responses [8]. Acute or short-lasting pain alert our body system against a damaging stimulus which does not exist once the stimulus is taken away, whereas, chronic pain is unpleasant suffering that persists even in the absence of stimuli which is no longer a symptom of a disease but itself is a disorder [2]. Figure 1 represents the physiological and pathophysiological pain as the former is shown by blue lines which is a normal response to certain intensity of stimuli, whereas the latter is explained in terms of allodynia and hyperalgesia that are enhanced pain responsiveness to the innocuous or noxious stimuli [9]. Alteration to the pain pathway causes hypersensitivity and as result allodynia and hyperalgesia develop. Allodynia; in *Greek* allo means “others and” odynia is a term “denoting pain”. As per the IASP allodynia is pain due to a stimulus that normally does not provoke pain

and under this condition the normal modality is non-painful but the response is painful. Whereas on the other hand hyperalgesia (denoted in red lines) is increased pain due to the application of a painful stimulus and under this condition, the suprathreshold stimulation shows a high intense pain [7]. Thus, we can conclude that normal pain is physiological in nature and required for survival of the organism, while chronic pain is pathophysiological in nature and requires immediate attention.

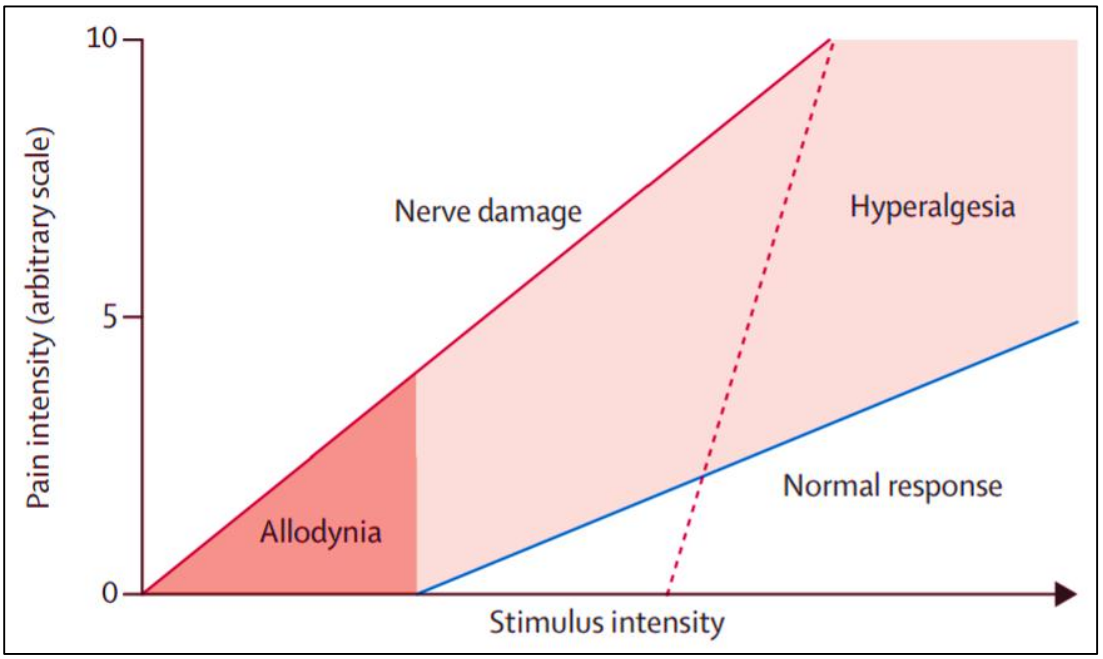

Figure 1.1 Stimulus-response function depicting normal v/s pathophysiological pain (allodynia and hyperalgesia). Reprinted with permission by Elsevier from source reference [9]. The normal pain response is shown by the blue line whereas the red line shows the response after nerve damage.

1.3 Epidemiology of chronic pain

The prevalence of chronic pain is more than 30% worldwide according to some reports and the rate of the disease varies demographically [4]. A metanalysis has suggested that the prevalence of chronic pain is about 18% in developing countries ranging from 5.5% to 65%. In India, the prevalence is 19.3% with a higher proportion in females estimated at 25% [10]. In the United Kingdom, it was reported to be around

40% whereas in the United States of America the prevalence was estimated at 20.4% [4]. The underdiagnosis, unawareness, and limited healthcare infrastructure are the key factors behind a misinterpretation of the chronic pain prevalence. Several risk factors are associated with the prevalence of chronic pain; Socio-demographical like age, gender, ethnicity, and cultural background, socio economic status, employment, and occupational factors; Clinical and biological such as pain, comorbidity, emotional state, surgical interventions, sleep disorders, and genetics; Lifestyle factors including smoking, alcohol consumption, physical activity, nutrition; Miscellaneous consisting of interpersonal violence, traumatic experience, history of abuse etc. [11,12]. The different populations have a distinct rate of chronic pain distribution as literature demonstrates a higher prevalence of chronic pain in the elderly population as compared to that of the young population [12]. Women are more susceptible to the pain as compared to the man, and on a similar note the higher prevalence is observed in military veterans, lower socio-economic background people, and rural area population [12,13].

1.4 Pain ontology

Pain can be classified into four broad categories namely, 1) nociceptive pain 2) inflammatory pain 3) neuropathic pain and 4) dysfunctional pain [14]. The ***nociceptive pain*** occurs due to the high threshold noxious stimulus and remains till the stimulus is present and, there are no additional long-term changes in pain receptor activity and higher-order neuron which makes it protective [15]. Examples of nociceptive pain are acute trauma or procedural pain. The ongoing component of pain is not present in nociceptive pain and it only involves the sensory component of pain [14].

Inflammatory pain arises due to a tissue injury that activates a series of cascades leading to the release of inflammatory mediators followed by sensitization of nociceptors [14]. This led to the subsequent change in CNS and pain transmission gets facilitated. Examples of inflammatory pain are postoperative pain and arthritis.

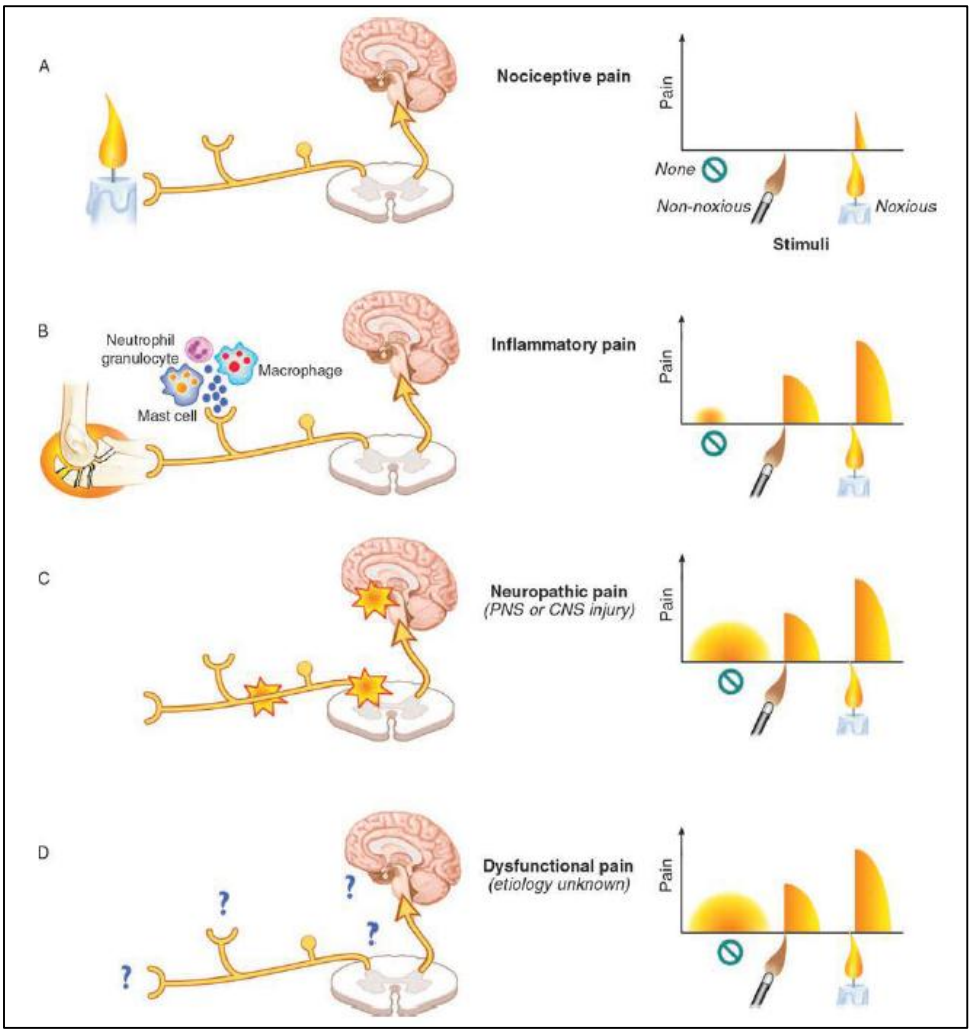

Figure 1.2. Illustration representing the different categories of pain. A) Shows Nociceptive pain B) Inflammatory pain C) Neuropathic pain and D) Dysfunctional pain. Reprinted with permission by McGraw Hill LLC from source reference [14].

Neuropathic pain is another subtype, defined as "pain arising due to a lesion or disease of the somatosensory nervous system" [16]. Examples are diabetic neuropathy, postherpetic neuralgia, thalamic stroke, chemotherapy-induced peripheral neuropathy, etc. Both the inflammatory and neuropathic pain involves the ongoing component of

chronic pain and also elicit a response to the non-noxious stimulus [17]. Despite the similar facilitation of pain signaling, both conditions have different mechanisms of development. Inflammatory pain can act as both protective and devastating while neuropathic pain is a disease that is painful only. D***ysfunctional pain*** is a clinical condition that might involve no stimulus to induce pain-like behavior such as irritable bowel syndrome and fibromyalgia [18]. The prominent features of dysfunctional pain are chronic symptoms either confined to a specific site or widespread, and abnormal sensitivity to pain.

1.5 Functional anatomy of pain

A simple characterization of the sensory nervous system involved Aβ, Aδ, and C fibers based on their levels of myelination, size of fiber, and size of the cell body. But this cannot explain the complex sensory nervous system explicitly as it fails to justify the complexity of neurons encoding a diverse range of stimuli such as pin prick at a particular tissue site or direction of the wind. However, the research in past decades has enlightened the field with the use of high cutting-edge techniques such as electrophysiology, immunohistochemistry, optogenetics and cellular interaction at DRG, spinal and supraspinal regions.

1.5.1 Primary afferents

The peripheral sensory nervous system is classified as the visceral sensory system and somatosensory system. The visceral sensory system consists of general visceral afferents that innervate lungs, trachea, gastrointestinal system, liver, pancreas, etc [14]. Whereas the somatosensory nervous system consists of general somatic afferents that carry the information about touch, pain, and position of extremities [19].

Sensory neurons are also known as primary afferents, are originated from the neural crest which can give rise to the bipotent progenitors that can develop sensory neurons and satellite glia cells [20]. These shreds of evidence support the neurogenesis after injury to the somatosensory nervous system. The glia cells nurture the myelin sheath over the sensory neurons but this is not found continuously throughout the axonal length. By this mechanism, the gap between the myelin sheath provides provision for the penetration by neurotransmitters and macromolecules that participate in cellular signaling [14]. Similarly, the blood vessels of sensory ganglions are lined by endothelium which is not uniform and gives rise to the mixed coating that allows the extravasation of macro molecules [14,19,20]. This is the reason that sensory neurons are exposed to the inflammation, toxins, and macromolecules present in the systemic circulation. However, the CNS residing neurons are separated from the blood circulation via the blood-brain barrier. Nociceptors are the specialized group of peripheral sensory neurons that responds to the noxious touch, heat, or chemical stimulus [21]. These neurons are pseudo unipolar with a single axon which is bifurcated further forming a central and peripheral branch [22]. The cell bodies of all sensory neurons reside in dorsal root ganglion (DRG) except that the trigeminal proprioceptive neuron whose cell body is present in CNS [22]. The cell body in DRG gives rise to the peripheral and central axon that terminates in the target organ and spinal cord respectively [20]. The electrical signals travel from peripheral axonal endings to the central counterparts in the dorsal horn of the spinal cord.

A simple understanding of the somatosensory nervous system explains A-δ and C-fibers role in nociception and Aβ fibers in touch. The myelinated A-δ and unmyelinated C-fibers are nociceptors that detect the high threshold stimulus whereas

A-δ fibers detect the acute and precisely located pain [23]. There are two major types of nociceptors, one is Aδ which is medium diameter myelinated fiber responsible for acute or fast pain, and another is C which is small diameter unmyelinated slow pain mediating fiber [23]. The Aδ fiber is further divided into type I and type II Aδ fibers. Type I is high threshold mechanical nociceptors that respond to the chemical and mechanical stimuli but has a higher threshold for the heat stimuli (>50°C) [2]. Type II Aδ fibers have a lower threshold for heat stimuli thus are responsible for the first acute response to noxious heat. Whereas the threshold for mechanical stimuli in type II Aδ remains high. The C fibers are polymodal nociceptors as they consist the subset of neuronal population that responds to heat and mechanical stimulation [24]. Interestingly, the heat-sensitive and mechanical insensitive unmyelinated fibers that are termed silent nociceptors develop mechanical sensitivity under injury-like conditions. These nociceptors are more responsive to chemical stimuli (e.g., capsaicin) as compared to the heat or mechanical ones [2]. DRG has all the essential enzymes for the synthesis of glutamate which travels toward the spinal residing neuronal endings [25]. Moreover, it has been found that the peripheral terminals of neurons also release neurotransmitters and modulate the functioning at the peripheral sites including inflammation. The gamma-aminobutyric acid (GABA) also gets synthesized in DRG and act as the gate for the signals modulation at soma connecting the central and peripheral counterparts of axons [25,26].

1.5.2 Spinal cord

The primary afferent nerve fibers project into the dorsal horn of the spinal cord where they form a synapse with the second-order neurons. The grey matter in the spinal cord is divided into 10 laminas among which lamina I-V makes the dorsal horn of the

spinal cord [27]. Different afferent fibers projections separate into the spinal cord in distinct laminas; Peptidergic C fibers project to the lamina I and dorsal part of lamina II, whereas nonpeptidergic C fibers to the ventral part of lamina II; Aδ fibers extent to the lamina I and lamina V and finally the Aβ fibers projects to the lamina III, IV, V and ventral part of the lamina II [2]. On the basis of this anatomy of the spinal cord, the functional anatomy is derived that includes; noxious stimulation travels to the lamina I, innocuous stimulation is perceived by the lamina II and IV, and lamina V response the mix stimulation [14]. The dorsal horn of the spinal cord plays a critical role in integrating pain signals and higher transmission of the same toward supraspinal regions [27,28]. Nonneuronal cells such as microglia also participate in pain processing by releasing various intracellular mediators which modulate the signal intensity.

The *Gate control theory* of pain given by Melzack and Wall in 1965 suggest that "non-noxious inputs close the gates for noxious inputs, which prevents pain sensation from travelling to the central nervous system"[29]. The dorsal horn of spinal cord is composed of different laminas that receives the inputs from distinct afferent fibers including C, Aδ and Aβ fibers. According to the gate control theory the non-nociceptive fiber can interfere with the signal transmission of nociceptive fibers through activation of the inhibitory interneurons in spinal dorsal horn which lead to the reduction in transmission cell activity and inhibition of pain signaling [30]. Thus, the theory explains the phenomenon behind rubbing and gentle scratching of the painful site to eliminate the unpleasant feeling.

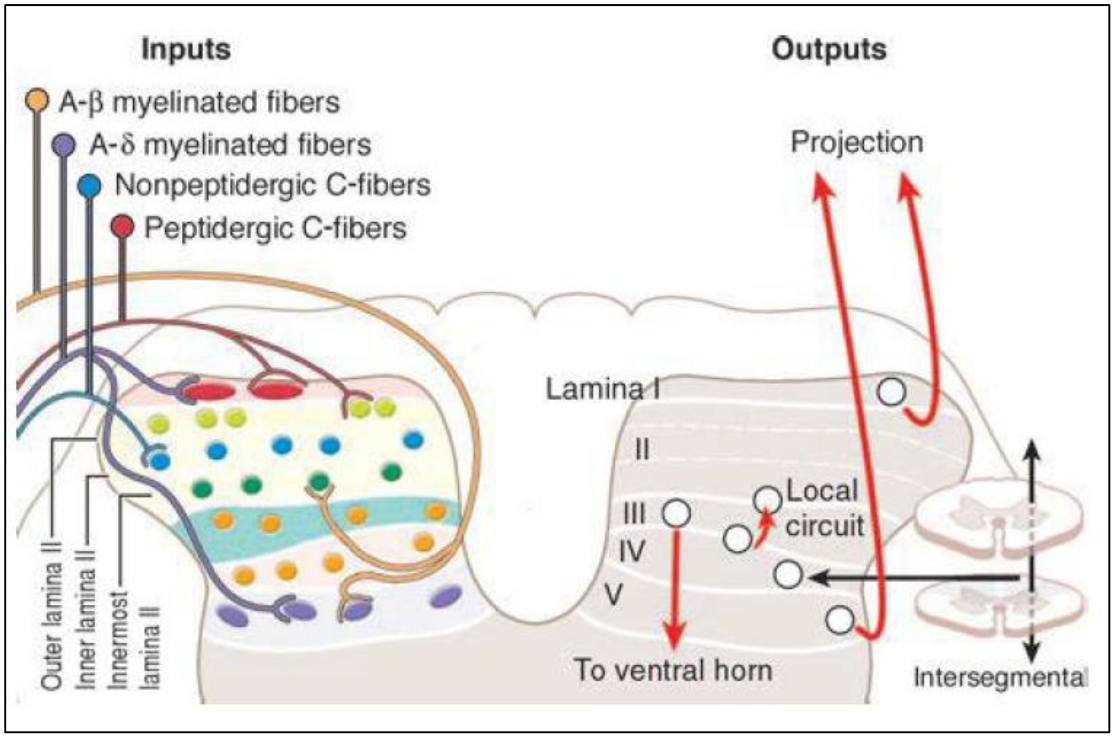

Figure 1.3 Spinal organization of nociceptive central terminals. Reprinted with permission by McGraw Hill LLC from source reference [14].

1.5.3 Ascending pain pathway

The neurons residing in the dorsal horn of the spinal cord are interneurons rather than second-order neurons and are both inhibitory and excitatory [14]. The second-order neurons projects primarily from the lamina I and V are the origin of multiple outputs in form of ascending pathways consisting of spinothalamic and spinoreticulothalamic tract that carry information to the thalamus and brainstem respectively [31]. The nociceptive information travels from the spinal dorsal horn to the brainstem (parabrachial region, periaqueductal grey, catecholamine cell region, and brainstem reticular formation) via spinobulbar track [32].

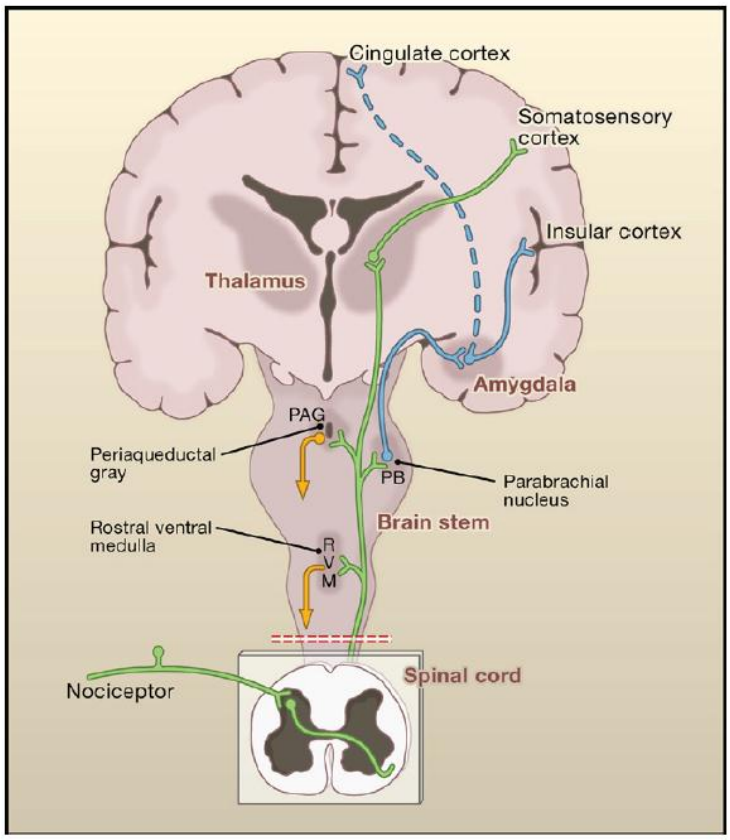

Figure 1.4 Neurobiology of pain processing. Reprinted with permission by Elsevier from source reference [2].

The periaqueductal grey region balances the input of ascending pathway to the output across descending pathway, whereas the parabrachial region forms a rapid connection amygdala which processes the information associated with aversive stimuli [14]. Projections to the posterolateral thalamus carry discriminative information about the stimulus and arise from the terminals of the column/medial lemniscus pathway, whereas, the medial thalamic nuclei receive bring information about the affective component of pain [31].

1.5.4 Brain

The most complex part of pain processing occurs in the brain as the pain has sensory as well as affective components thus the interpretation and processing of pain depend on emotional state, arousal status, and cognitive state [33]. Key brain areas

involved in pain processing are the somato sensory cortex, anterior cingulate cortex, insular cortex, prefrontal cortex, and thalamus [34]. The lateral thalamic nuclei carry perpetual and discriminative information and project toward the sensory cortex [14]. Whereas the projections from the medial thalamic nuclei project to limbic and frontal brain regions and process the affective pain information [33]. Under chronic pain condition, there is an altered activation pattern across the different brain areas which facilitate the total gain of pain response.

1.5.5 Descending pain pathway

The descending pain pathway is composed of Periaqueductal grey, rostroventral medulla, and dorsal horn of the spinal cord [35]. Higher brain centers including the amygdala, cortex, and hypothalamus send inputs to the descending pain pathway regarding fear and hyperalgesia [31]. The descending pain circuit is largely modulated via the neurotransmitter system including serotonergic, opioid, and noradrenaline [14]. The periaqueductal grey project fibers to the rostroventral medulla which further extends to the dorsal horn of the spinal cord via the dorsal part of the lateral funiculus [33]. The rostroventral medulla comprises of dorsal raphe nucleus and adjacent reticular formation. Distinct cells are present in the rostroventral medulla including ON, OFF, and NEUTRAL that modulates the pain signal and results in the inhibition of facilitation of the same [36]. Under chronic pain condition there is a decrease in activity of descending inhibitory pain pathway due to reduced synthesis of GABA or loss of inhibitory interneurons [37].

1.6 Molecular mechanisms of pain

The overall mechanisms of pain can be subdivided into five broad categories; 1) detection 2) conduction, 3) transmission, 4) modulation and 5) perception [14]. As

discussed previously normal or baseline pain is known as nociceptive pain. Different receptors are involved in the detection of pain not only the type of stimuli but intensity as well [2]. A temperature of more than 43°C is perceived as noxious which activates C fibers and Aδ fibers presenting transient receptor potential vanilloid 1 (TRPV1) ion channels [38]. Among TRP family members TRPV1 is the most studied receptor responsible for heat detection above 42°C which is close to the noxious threshold of pain sensation. Other members of the TRP family also participate in the detection of temperature such as TRPV2 which gets activated above 52°C, TRPV3 and TRPV4 activate 25°C to 35°C [38]. Cold stimuli are also sensed by TRP channels including TRPM8 (sense menthol and temperature about 28 °C) and TRPA1 nociceptors [39]. The detection of mechanical noxious stimulus is still being characterized by researchers and the recent progress in the field has suggested the involvement of piezo channels in the same [40]. Mechanosensation is also perceived by the TRPV2 and TRPA1 receptors suggesting their polymodal nature in stimulus detection. TRPV1 and acid-sensing ion channels (ASICs) sense the protons which are associated with acid microenvironment-related pathophysiology [14].

The next phase is pain conduction which is mainly done by opening and closing different ion channels such as voltage-gated calcium channels and voltage-gated sodium channels which modulate the neuronal firing throughout the nervous system. Nociceptors are equipped with NaV1.1, NaV1.6, NaV1.7, NaV1.8, and Nav1.9 which are involved in the modulation of neuronal firing patterns [41,42]. The transmission phase of pain is carried out by the release of neurotransmitters that produces depolarization of post synaptic neurons [43]. The phase is majorly carried out in the spinal cord, especially the dorsal horn part which carries numerous interneurons and axonal projections. Glycine and γ-aminobutyric acid, glutamate, serotonin,

neuropeptide Y, brain-derived neurotrophic factor, etc. are the major mediators and modulators of pain transmission across the CNS.

Modulation and perception are another important phase of pain mechanisms [34]. Modulation of nociceptive transmission is an adaptive process which involves both excitatory and inhibitory signaling. In modulation phase the brain alters the intensity of the signal depending upon environmental condition and the circumstances surrounding the pain initiation. The interneurons modulate the pain signals by regulating the release of substance p and glutamate at the presynaptic terminals of primary afferents [44]. This in turn lead to the suppressed post synaptic excitotoxicity at the terminals of second order neurons. Moreover, the modulation of pain is carried out by the variety of neurotransmitters including serotonin, norepinephrine and enkephalins [44,45]. The perception of pain depends on the neuronal encoding in spinal and supra spinal brain regions [14]. At supraspinal levels the pain is perceived as more than a nociceptive signal pattern and an appropriate emotional and motor response is initiated. The action potential traveling through spinothalamic ascending pathway are decoded by thalamus, sensorimotor cortex, insular cortex and anterior cingulate cortex [14]. Whereas, action potential ascending through spinobulbar pathway is decoded by hippocampus and amygdala.

1.6.1 Neurobiology of chronic pain

Any damaging stimuli that persist for long-duration cause accumulation of cytokines and release of a variety of mediators from immune cells that participate in the neurobiology of chronic pain. In preclinical studies, the mechanism of chronic pain can be distinguished as inflammatory and neuropathic, based on the model of induction such as complete Freund's adjuvant and nerve injury [14]. Involvement of ion channels

including TRP family members, sodium channels, calcium channels etc. across the peripheral nervous system is one of the most prominent features of chronic pain neurobiology. Moreover, at central level it involves heightened transmission in ascending pain pathway and suppressed activity across the descending inhibitory pain pathway [37]. Some other common features of chronic pain pathophysiology are activation of C fibers and Aδ fibers, peripheral sensitization, and central sensitization [2,14,16]. The term sensitization defines as the lowering of threshold and enhanced response of nociceptors to supra-threshold stimuli and spontaneous activity [46]. The sensitization at peripheral or central levels is key pathophysiology of chronic pain and a brief about them is discussed below.

1.6.2 Peripheral sensitization

Peripheral sensitization is the increased responsiveness of nociceptive neurons in the periphery due to the continuous presence of a stimulus and several signaling pathways are associated with this phenomenon [43,47]. After tissue injury release of different mediators occurs at the site including bradykinin, nerve growth factors, adenosine tri-phosphate, histamine, interleukins, etc. These mediators stimulate the ion channels present on the nociceptive terminal by direct (phosphorylation) or indirect mechanisms (prostaglandin pathway) [14,47,48]. Stimulation of nociceptors initiates the peripheral sensitization often accompanied by cytokines storm, protein phosphorylation, and ion channel activation which in chronic terms modify the gene transcription [2,14,48,49]. Activation of G protein-coupled receptors (GPCR) or serine/threonine kinases such as protein kinase A and protein kinase C occur which further activate the downstream signaling and modulate cellular activity especially the electric impulse across the PNS. The vicious cycle of inflammation and nociceptor activation gets developed due to continuous stimulation of ion channels (e.g., TRPV1,

$Na_V1.9$) and the release of inflammatory mediators. Another mechanism of peripheral sensitization is altered intracellular signaling and change in substrates for activation which is independent of altered nociceptive threshold and relies upon the heightened sensitivity and cross-interactions of pathways inside the cell.

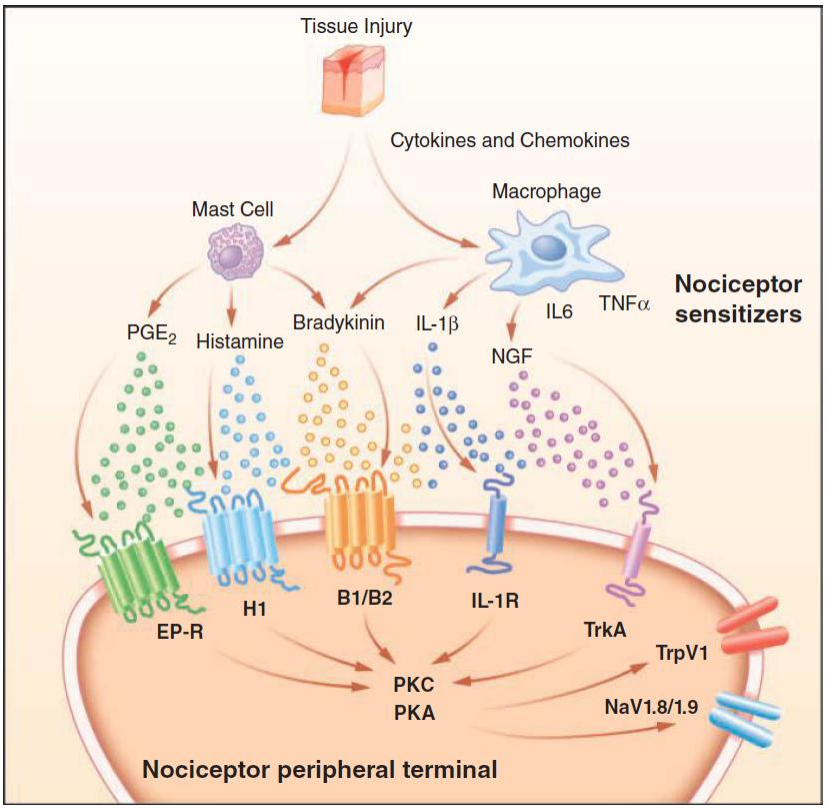

Figure 1.5 Peripheral sensitization under chronic pain. Reprinted with permission by McGraw Hill LLC from source reference [14].

1.6.3 Central sensitization

Central sensitization is a phenomenon that occurs in spinal cord and brain regions and increases pain responsiveness [50,51]. This feature of chronic pain is very unique as, unlike peripheral sensitization, it can persist without the presence of the stimulus and is adequate independently to develop hypersensitivities [14]. In presynaptic sites of the central nociceptor terminal activation of various cytokine and-

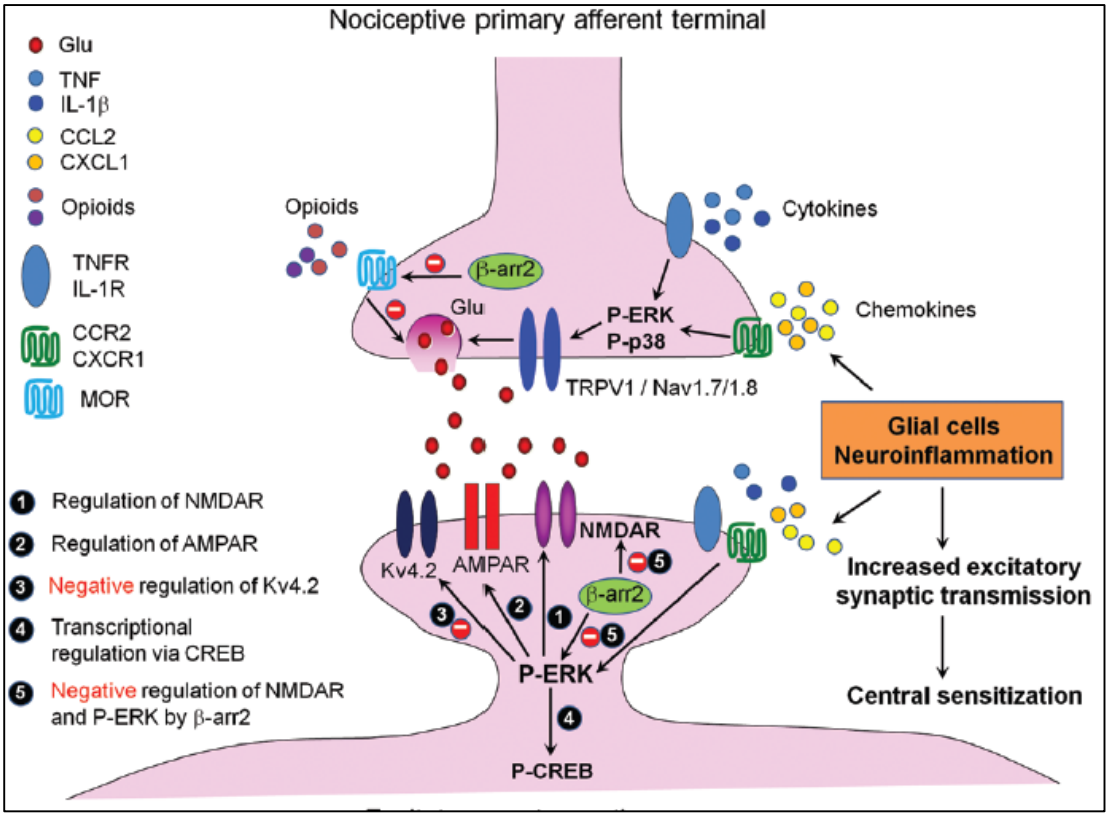

Figure 1.6. Molecular mechanism of central sensitization in chronic pain. Reprinted or adopted with permission by American Society of Anesthesiologists from source reference [50].

chemokine receptors, and ion channels occur that result in the initiation of ERK and p38 MAPK pathway which in turn facilitate the release of glutamate into the synaptic cleft [50]. These series of events activate NMDA and AMPA receptors with simultaneous inhibition of potassium channels (e.g., Kv4.2 and Kv1.2), which further mediates several downstream pathways thereby inducing the nociceptive gene expression [14,52,53].

Temporal windup or summation are the terms used to define the central sensitization where repeated stimulation led to persistent pain even after the removal of stimuli. Glutamate and its receptors N-methyl-d-Aspartate (NMDA participate in the development and maintenance of central sensitization which is Ca++ impermeable under normal conditions but due to certain mediators (e.g., Nerve injury associated

molecules) the Mg+ block is removed and calcium enters the cell causing the neuronal excitability [53]. The process is maintained by activity of various enzymes such as MAPK, PKA, PKC extracellular signal-related kinase (ERK) and Src [50]. Ion channels such as Kv4.2 also promote the NMDA induced excitotoxicity as the ERK inhibits these channels which resist the neuronal homeostasis. Apart from this different cytokine, prostaglandin, BDNF, substance P also excite NMDA receptors and promotes thermal and mechanical hypersentivity. Microglia, astrocytes, axonal degeneration, immune cell infiltration, protein phosphorylation and enhanced intracellular trafficking are other key mediators of central sensitization [36,43,50,51].

1.7 Neuropathic pain

The pain developed in the peripheral or central nervous system due to lesions and/or disease to the somatosensory nervous system is referred to as neuropathic pain, affecting 7 to 10% of the general population worldwide [16]. The symptoms include intense burning, tingling or electrical sensation, numbness, and extreme sensitivity to non-noxious stimuli, which can likely become chronic if not managed properly [16]. Peripheral tissues, lower back, and neck are the most commonly affected areas experiencing neuropathic pain [54]. The distribution pattern includes length-dependent, distal peripheral neuropathies with progressive sensory loss in distal-proximal regions. Clinically, 'Glove and stocking' distribution is the key characteristic of peripheral neuropathic pain where the hands, forearms, calves, and feet are most prominently affected [55]. Chemotherapy, diabetes, nerve trauma, HIV infection, and Guillain-Barre syndrome are some associated disorders that generally involve painful chronic neuropathy [56]. Other symptoms accompanying neuropathic pain are anxiety,

depression, and sleep disturbances which further deteriorate the patient's quality of life [57,58]. The complexity of symptoms and the diverse pathophysiological situations, different pain etiologies, and genetic predispositions pose a great burden to the development of effective treatment for neuropathic pain. Broadly neuropathic pain can be classified into, peripheral and central neuropathic pain. The myelinated A-fibers (Aβ and Aδ) and unmyelinated C fibers are predominantly involved in the pathology of the peripheral neuropathic pain altering the electrical properties of sensory neurons causing imbalance between excitatory and inhibitory signaling which develops spontaneous and stimulus-evoked pain experiences [16,59]. Primarily, the damage caused to the first-order neurons increases the number of sodium and calcium ion channels thus increasing its firing potential. The enhanced depolarization leads to abnormal discharges resulting in the spontaneous nature of pain. Moreover, in chronic neuropathic conditions, alterations in the central pain processing leading to central sensitization have also been reported, causing hypersensitivity of spinal neurons and reduction in the activation thresholds [60].

An increase in excitation and facilitation with a loss of inhibitory signaling at the periphery shifts to the sensory pathways causing a state of hyperexcitability which in due course of time mediates neuropathic pain condition to a chronic state [61,62]. Enhanced response due to increased excitability of spinal neurons results in lowering the threshold of mechanosensitive Aβ and Aδ fibers activating second-order nociceptive neurons for a given stimulus thereby causing central sensitization [54]. Specifically, the continuous release of excitatory amino acids and neuropeptides leads to postsynaptic changes in second-order nociceptive neurons by activation of NMDA and AMPA receptors. In clinical and pre-clinical studies, physical allodynia and

enhanced sensory thalamic neuronal activity have been explained by these second-order changes [61]. But still, many more aspects of neuropathic pain remain elusive and investigations are required to fully understand the functional changes in neuronal and non-neuronal cells, such as microglia and astrocytes within the spinal cord, which have a vital role to play in the development of hypersensitivity. Abnormal activity in primary afferent fibers due to infection such as HIV and leprosy has also been investigated to have a role in the pathophysiology of the neuropathic pain followed by peripheral nerve injury [63]. Among all the other factors that contribute to the development of neuropathic pain the voltage-gated calcium and sodium channels are the most prominent in contributing to the increased excitability of DRG neurons in neuropathic pain [64], [4,65].

1.8 Inflammatory pain

Pain resulting from increased spontaneous excitability of peripheral nociceptive fibers in response to tissue injury or inflammation is referred to as inflammatory pain [66,67]. The prevalence of inflammatory pain is high as 350 million people suffer from the most common phenotype which is arthritis and joint disease worldwide [68]. The signs and symptoms of inflammatory pain include redness, swelling, and increased sensitivity. In the case of inflammatory pain in joints, stiffness and limited or restricted movement are generally observed [69,70]. Under the general pain conditions, signs and symptoms may vary depending on the intensity and localization of the stimuli but in the case of inflammatory pain spontaneous hypersensitivity is observed. Furthermore, enhanced response to noxious and non-noxious stimuli i.e. hyperalgesia and allodynia are some additional features of inflammatory pain [69,70]. The etiology of

inflammatory pain is fundamentally different from neuropathic pain as it generally offers a protective mechanism to warn the body about noxious stimuli and promotes tissue regeneration, but the problem arises when the pain fails to subside over a normal period [71]. Several molecular factors are responsible for the manifestation of inflammatory pain among which the ion channel-based mechanisms are the most prominent, facilitating hypersensitivity and its spontaneous nature of the disorder [72].

A wide range of chemical and biochemical factors, also known as inflammatory mediators released in response to injury into the extracellular space which recruits immune cells and stimulates nociceptors [71]. These inflammatory mediators have a vital role in the development and maintenance of pain and cytokines, ATP, substance P, bradykinin and prostaglandins are some of the examples popularly known as algogens or endogenous substances for inducing pain [73,74]. Pro-inflammatory cytokines, TNFα, IL-1, IL-6, and IL-8 may directly or indirectly stimulate nociceptors and sensitize the peripheral sensory neurons (Matsuda et al., 2019). Enzymes such as COX-1 and COX-2 present at peripheral tissues, when activated by cytokines in response to tissue injury, lead to the synthesis of prostaglandins which are prominently known to activate nociceptors hence, contributing to pain [71,75]. COX-dependent pathway has also been shown to elevate the levels of Nav1.7 and sensitize sensory neurons to other mediators such as bradykinin, which is an important early contribution to the inflammatory cascade [76]. Other factors promoting inflammatory pain include neurogenic factors such as substance P and CGRP in the central as well as distal terminals of peripheral sensory neurons [71]. Synergistic interaction between different neurogenic factors at synaptic terminals greatly contributes to nociception during inflammation. Substance P has also been documented to not only stimulate the

production of cytokines and prostaglandins PGE2 but also induce activation of immune cells such as T cells, monocytes, and neutrophils [77]. Peripheral inflammation results in the activation of a non-neuronal cell population such as spinal microglia and astrocytes in both acute and chronic cases which plays a vital role in the activation and recruitment of macrophages in the DRG but not in the spinal cord in inflammatory pain conditions [69]. Pain sensation arises from high-intensity stimuli, often inducing the innate immune activation which leads to the release of mediators that facilitates activation of C fibers through a number of stimulation of receptors expressed on the afferent terminals of the sensory neurons. Unlike neuropathic pain, here C fibers have a dominant role in the development and maintenance of pain [71]. During prolonged inflammation, calcium influx in neurons increases leading to enhanced phosphorylation of proteins and activation of the secondary messenger system [67]. Many inflammatory mediators such as prostaglandins, bradykinin, etc. have been well documented to sensitize TRPV1 at the nerve terminals near the zone of tissue damage or inflammation mainly through Gq/11 coupled signaling pathway [67]. Pharmacological interventions such as opioids and NSAIDs are rather found to be sensitive in inflammatory pain conditions, unlike the neuropathic state. Interestingly, other agents such as gabapentin and NMDA antagonist show almost similar efficacy in both pain states [72].

1.9 Pharmacotherapeutics for the treatment of chronic pain and their limitations

The treatment for chronic pain is a big challenge in the healthcare system as it requires multimodal strategies, unlike acute pain which has limited tissue injury. Moreover, for the past few decades severe side effects are observed with different analgesics which has created a substantial barrier to the pain management.

1.9.1 Nonsteroidal anti-inflammatory drugs (NSAIDs)

Nonsteroidal anti-inflammatory drugs (NSAIDs) are the most commonly used analgesics by different age groups people with a higher proportion is used by elderlies. These drugs are used against a variety of acute pain conditions such as injury, sprains, headache, menstrual cramp, surgical pain, etc., and also used for chronic pain such as rheumatoid arthritis and osteoarthritis. The NSAIDs are prescribed by the medical practitioner to reduce inflammation and other mediators of peripheral sensitization [6,78]. This category of drugs acts majorly through cyclooxygenase (COX inhibition) and thereby suppressing the prostaglandin synthesis. Although the drugs are effective but due to raising concerns about their contraindications and adverse effect reporting, their use is being restricted in clinics [79]. The major side effect associated with NSAIDs use is gastric ulcer development which has led researchers to identify selective COX-2 inhibitors which have a lower risk of gastrointestinal side effects as compared to the non-selective blockers. But gastric bleeding is associated with chronic use of all cox inhibitors, along with the cardiovascular risks due to which few drugs were removed from the market (e.g., rofecoxib) [6]. Acetaminophen which is considered a safer and potent NSAID analgesic in the acute treatment model works through the inhibition of COX-3. Liver toxicity and nephropathy are the risks associated with its use thus patients with a history of alcohol and tobacco use are prescribed lower doses of acetaminophen [6,80].

1.9.2 Anticonvulsants

Anticonvulsants are used in a variety of chronic pain disorders, especially from neuropathic origin. Under neuropathic pain activation of different ion channels occur

such as voltage-gated sodium channel which modulates the neural firing [6]. Carbamazepine, oxcarbazepine, topiramate, gabapentin, pregabalin, and lamotrigine are the most commonly used anticonvulsant used in chronic pain management including diabetic neuropathy, trigeminal neuralgia, etc. [6,81]. Gabapentin and pregabalin are frequently prescribed for the management of chronic pain and both drugs modulate the intracellular Ca++ levels with additional activity on substance p and CGRP. The most common side effects of anticonvulsant are sedation, dizziness, memory loss, edema, dry mouth, etc. which again puzzled the clinicians and scientist to limit their use or not [6].

1.9.3 Antidepressants

Antidepressants are another class of drugs used for chronic pain treatment especially belonging to the tricyclic antidepressants (TCAs) and serotonin-norepinephrine reuptake inhibitors (SNRIs) [4,82]. These drugs modulate the neurotransmitter availability in synapse but still lack the clear mechanistic elucidation in case of chronic pain. Studies have suggested the involvement of descending pain pathway which involves serotonin and norepinephrine mediated inhibition of pain transmission. Other mechanisms reported are blockade of NMDARs and voltage-gated sodium channels. Amitriptyline, nortriptyline, and desipramine are tricyclic antidepressants used as a first-line treatment for neuropathic pain [6]. The common adverse effect observed with this class of compounds are conduction block or slow conduction velocity in cardiac events thus are contradicted with anti-arrhythmic (Class I) medications. Monitoring cardiac activity by electrocardiography is recommended when prescribing TCAs [83,84]. Confusion and hallucination are other adverse effects coupled with the use of TCAs. SSRIs such as venlafaxine, duloxetine, milnacipran are

prescribed for pain management but they carry serious side effects including suicidal tendencies, liver damage, weight gain, withdrawal symptoms, etc.

1.9.4 Opioids

Opioids are the most potent and efficacious class of analgesics but despite their proven therapeutic efficacy, they have recently been degraded to third-line of therapy for the management of chronic pain in clinics [65,85]. Morphine, methadone, tramadol, tapentadol, buprenorphine, etc. are the most commonly prescribed opioids for pain therapeutics [6]. The opioid crisis is a major threat of the 21st century with a remarkable juxtaposition of use and abuse. The reason behind this is the development of potential side effects (respiratory depression, constipation, sedation, withdrawal symptoms, addiction, etc.) and tolerance after repeated dosing [85]. Opioid tolerance is the major limiting factor leading to the treatment withdrawal, severe side effects due to dose escalation, and sometimes even death of the patients. Every day more than 90 people die due to the overdose of opioids in America and a similar trend has been seen across the globe [86]. Research on opioid tolerance shifted towards the central nervous system (CNS) based adaptations because tolerance is much more than just being a cellular phenomenon. Thus, neurobiological adaptations associated with opioid tolerance are important to understand to set newer pain therapeutics. These adaptations are associated with alterations in ascending and descending pain pathways, reward circuitry modulations, receptor desensitization and down-regulation, receptor internalization, heterodimerization, and altered epigenetic regulation.

1.9.5 NMDA antagonists

NMDA antagonists such as ketamine, amantadine, and dextromethorphan are emerging as novel therapeutics for pain management [6]. These drugs act on central sensitization and prevent neuronal excitotoxicity via inhibiting the NMDARs at spinal and supraspinal levels. Despite their proven preclinical efficacy, the NMDARs fails to treat pain condition in clinics adequately and produce several CNS toxicities. The adverse effect mainly got precipitated as antagonizing the NMDARs lead to the modifications in basal physiological role of these receptor systems and cross interactions between intracellular signalings.

1.9.6 Other pharmacological treatment

Other strategies available for the management of chronic pain are low dose naltrexone, topical agents (diclofenac, lidocaine, and capsaicin), skeletal muscle relaxants (baclofen, tizanidine, cyclobenzaprine, on botulinum toxin A, cannabinoids, etc. which still require further studies to establish a better therapeutic approach such as combination therapy [6].

1.10 Kinesin superfamily proteins and their role in intracellular trafficking

The cellular system work as a factory where proteins are utilized as machines to perform any action and maintain balanced outputs. Intracellular trafficking is the most critical process that maintains cellular integrity and homeostasis. Cells are so tiny and use a simple diffusion process to transport small molecules such as gases and glucose to reach their destinations. But the intracellular transport of large protein molecules requires an active process which is done by microtubule-based master motor

proteins such as dynein and kinesin [87,88]. Directional transport in polarized neurons from the cell body to the axonal terminal is highly essential for basal physiological activity and cell survival [88]. Kinesin superfamily proteins (KIFs) are ATP-guided microtubule selective molecular motors that are responsible for the trafficking of cargo to the synaptic membrane. They utilize the chemical energy and convert it to mechanical energy that generates a motile force that helps to move kinesins along the microtubule tracks. As per the HUGO Gene Nomenclature Committee (HNGC) database so far 46 types of kinesins in humans and other mammals are identified [89]. The kinesins are categorized into 14 families based on their protein structure. These families can be organized into three groups namely; N-kinesin, M-kinesin, and C-kinesin as their motor domain is located in N-amino terminals, middle region, and carboxy terminals respectively [88]. Adaptor or scaffold proteins are required to facilitate the kinesin-cargo binding and transport in some cases. Kinesin structure contains a motor domain that facilitates the mechanical transport and a stalk and tail domain that is used for binding of kinesin to their respective cargos [88,90]. N-kinesins are involved in the anterograde movement of protein cargo, retrograde transport is mainly carried out by dynein but some C-kinesin also perform the intracellular transport in the same direction. Kinesins are involved in the transport of synaptic proteins, mitochondria, and axonal and dendritic of specific proteins. Kinesins use *fast axonal transport* (50-200 mm/day) to transport the cellular organelles whereas cytoplasmic proteins are transported through *slow axonal transport* (0.1-3 mm/day) [88].

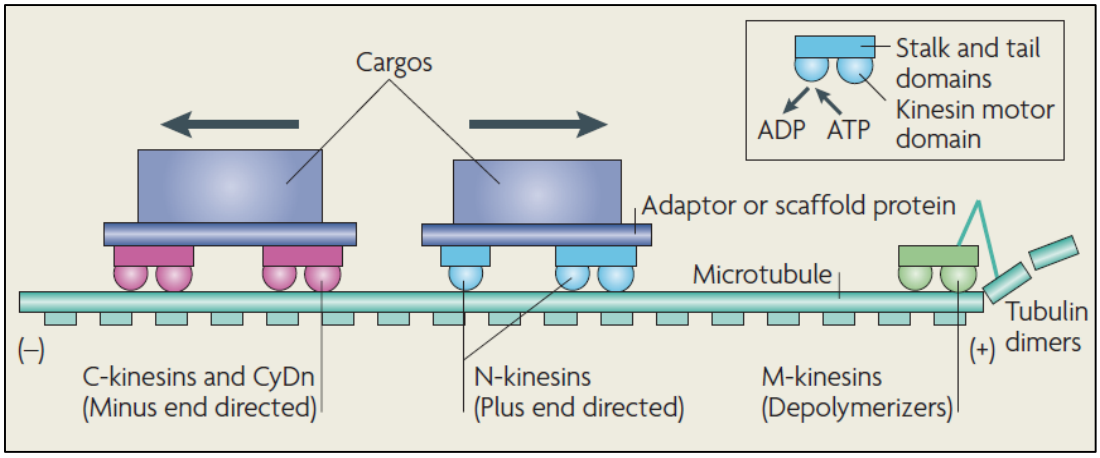

Figure 1.7 Mechanism of kinesin mediated cargo transport. Reprinted with permission by Nature Research from source reference [88].

1.11 Kinesin nanomotors mediated trafficking of NMDA-loaded cargo as a novel target in chronic pain

Research from the past few decades focused on the neurobiology of chronic pain has substantially increased the understanding of the disease. Various ionotropic channel receptors are widely implicated in the pathophysiology of various disorders and hold the glory of therapeutical implications. The recent decade in pain research has witnessed a shift from the discovery of direct exogenous ligands to alternate strategies, such as modulating receptor synthesis, maturation, and transport processes [91–93]. The role of the N-methyl-D-aspartate (NMDA) receptors system in chronic pain is well supported with pre-clinical evidences [94–97]. However, the direct blockade of NMDA receptors failed in clinical trials for chronic pain due to the alteration in basal physiological role of these receptors and off targeting induced severe side effects [98]. This suggests that targeting NMDA receptors after their insertion into the synaptic membrane may not be an optimal approach. Meanwhile, the potential of this receptor system cannot be ignored due to the high rate of success in basic research studies [96,99–101]. Thus, developing an indirect approach to target this receptor system could

provide a new series of therapeutics for the management of chronic pain. We have previously demonstrated the generalized trafficking of different proteins that contributes to chronic pain via kinesin motors [102]. Here, we reviewed the literature on the neurobiology of NMDA receptor trafficking through specific kinesin motor proteins and its implication in chronic pain management. Kinesins are motor proteins that are involved in the anterograde transport of receptors and proteins. Kinesins move along microtubules to transport cargoes containing vesicles, mRNAs, and other cellular materials mostly in the anterograde direction [102–104]. These nanomotors also regulate the trafficking of NMDAR across the microtubule tracks and make them functional by insertion into the synaptic membrane [105]. Cargos are large protein complexes filled inside vesicles by Golgi bodies and transported by motor proteins into the synapses. The NR2B subunit of the NMDA receptor is trafficked by kinesins hence making the receptor system functional at the synaptic level.

1.11.1 NMDA receptors system: A key player in the pathophysiology of chronic pain

NMDA receptors are ionotropic, excitatory, heteromeric glutamate receptors. In the central nervous system (CNS) of mammals, fast synaptic transmission is mediated by α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors and kainite receptors while the slow synaptic transmission is mediated by NMDA receptors. NMDA receptors have been widely implicated in the transmission of both nociceptive and neuropathic pain [106]. The NMDA receptors are abundantly distributed in the pain pathway and are responsible for synaptic plasticity corresponding to chronic pain [107]. Painful stimuli cause the activation of afferent myelinated Aδ and unmyelinated C fibers that cause a release of glutamate along with substance P and neurokinin A in the

dorsal horn of the spinal cord. This makes the postsynaptic neurons fire that project signal to the supraspinal structures of the CNS. The thalamus acts as a relay station and distributes the afferent signals to different cortical regions specifically the anterior cingulate cortex (ACC) and the insular cortex (IC) that are responsible for the unpleasant feeling of pain. Hence, as a consequence of these series of events the endogenous pain modulation mechanisms are activated.

1.11.2 NMDA mediated central sensitization across the ascending and descending pain pathways

Central sensitization is a key term used to demonstrate the pathophysiology of chronic pain. The neuronal transmission during central sensitization gets rapidly increased among the excitatory neurons while the activity of inhibitory neurons gets decreased. Moreover, the regulation of pain via endogenous mechanisms of a body system is impaired throughout the descending pain pathway. Various ion channels (e.g., TRPV1, TRPA1, Nav1.6, etc.), secondary messenger pathways (e.g., PI3K/Akt, CREB, mTOR, ERK 1/2), cytokines (e.g., IL6, IL1B, TNFα, NFKβ, etc.) and other mediators (VEGF, oxidative stress, histamine, serotonin, etc.) are involved in the development and maintenance of central sensitization [50]. One of the key receptor systems that closely regulates this central neuron-based phenomenon is NMDA (Figure 1.8). The NMDA receptor-mediated long-term potentiation (LTP) has been observed in the pain pathways across the neurons residing in the spinal dorsal horn [106].

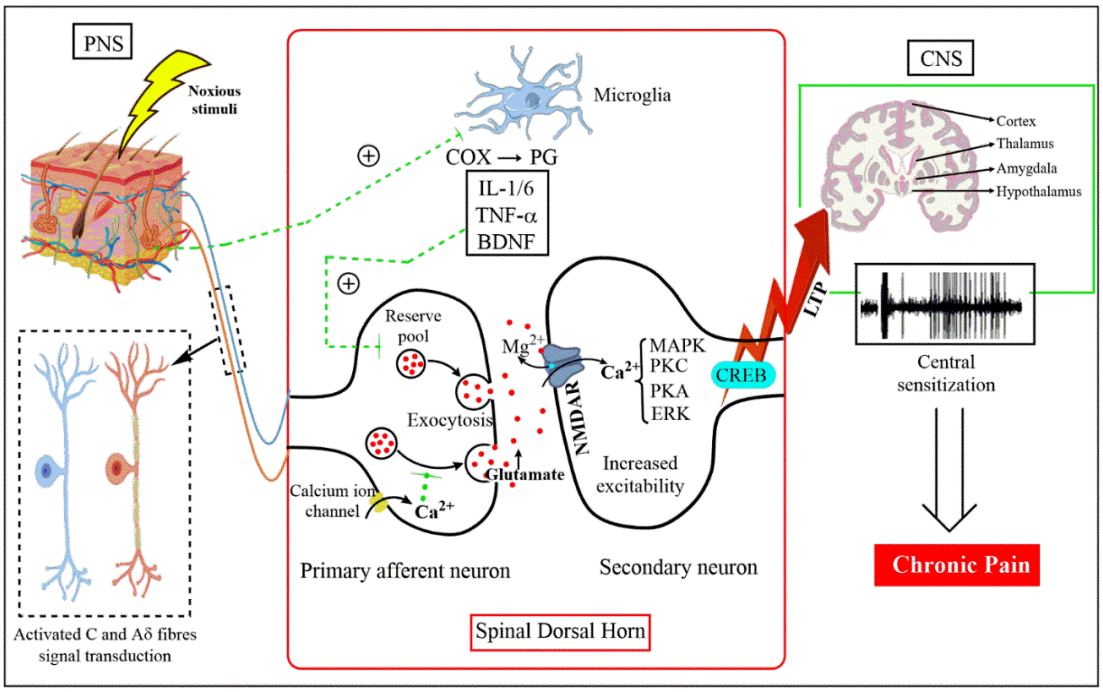

Fig.1.8 Role of NMDA receptor system in the neurobiology of chronic pain. Intense or persistent injury by noxious stimuli causes depolarization of C and Aδ fibers. Excitation of these fibers leads to exocytosis of a reserve pool of excitatory neurotransmitter, glutamate, into the synaptic cleft. The excess release of glutamate in the synaptic cleft increases the activity of NMDAR in the secondary neurons. Glutamate binds to the glutamate binding site of NMDAR, releasing the magnesium ion block and allowing calcium ion influx into the cell. Hence, raising the intracellular calcium ion concentration in the neuron. A high concentration of calcium ions results in the activation of different secondary messengers such MAPK, PKA, PKC, and ERK, activating different cascades of calcium-dependent signaling pathways. This series of events facilitates the transmission of pain signals to the brain by increasing the excitability of output neurons. The prolonged damage to peripheral nerves activates the resident macrophage cells, microglia, releasing a host of cytokines and producing prostaglandins, further contributing to central excitation. Reprinted with permission by American Chemical Society from own source reference [53].

The potentiated function of neurons in the central nociceptive pathways owing to the membrane hyperexcitability, highly efficient synaptic transmission, reduced inhibition results from profound plasticity in the somatosensory nervous system. This state characteristically portrays central sensitization that arises from nerve injury or inflammation [108]. The most investigated form of the aforementioned profound

plasticity is the NMDA-mediated LTP evident in higher CNS regions and the central synapses responsible for perception and transmission of sensory signals [106]. Investigating the spinal dorsal horn mechanism that accounts for LTP has been difficult due to the sheer complexity of the spinal neural network and the difficulty in accurate slicing. However, whole-cell and intracellular patch-clamp studies have revealed that both increased synaptic activity and increased postsynaptic depolarization lead to LTP [109]. Development of LTP in the spinal dorsal horn requires activation of the NMDA receptor that results in high Ca^{2+} influx. While the release of substance P and calcitonin gene-related peptide (CGRP) after nerve injury is believed to potentiate the current modulated by NMDA receptors in the neurons. Substance P binds to the neurokinin-1 receptor to induce prolonged membrane depolarization. This prolonged membrane depolarization removes the Mg^{2+} block from the NMDA receptors, causing an influx of Ca^{2+} ions [106,108]. CGRP in particular potentiates the activity of substance P and activates protein kinase A (PKA) and protein kinase C (PKC) by binding to postsynaptic CGRP1 receptors [110]. CGRP also causes the enhanced release of brain-derived neurotrophic factor (BDNF) that binds to its high-affinity tropomyosin receptor kinase B (trkB) receptors that result in the activation of extracellular signal-regulated kinase (ERK) and PKC pathways and enhancing C-fiber responses mediated by NMDA receptor [111]. Activation of PKC, PKA, ERK, and calcium/calmodulin-dependent protein kinase II (CaMKII) causes a.) phosphorylation that decreases the threshold and activation kinetics of NMDA and AMPA receptors resulting in increased signal transmission; b.) ERK phosphorylation decreases K^{+} currents leading to enhanced neuronal excitability; c.) ERK, PKC, and CaMKII cause the trafficking of NR1-containing AMPA to the membrane; and d.) Activation of cyclic adenosine

monophosphate (cAMP) response element-binding protein (CREB) pathway to enhance gene expression that results in strengthened synapses [108]. PKA or PKC phosphorylation of NR1 results in an increased response of NMDA receptors to glutamate, thereby causing hyperexcitability leading to the central sensitization and chronic pain. Moreover, COX and prostaglandin pathways are involved in the peripheral as well as central sensitization [112]. Bradykinin, an inflammatory peptide was observed to activate NMDA receptor activity in the spinal dorsal horn and by thus promoting hypersensitivity [113]. The mechanism behind this phenomenon was the activation of bradykinin B2 receptors followed by the activation of phospholipase C_β and phospholipase A_2 (PLA_2). The PLA_2 stimulates the arachidonic acid which further gets converted to the PGE2 by the action of COX and prostaglandins [113]. Finally, the PGE2 activates PKA and PKC that phosphorylates the NMDAR, regulates NMDAR trafficking to the membrane, and alters their kinetic properties.

1.11.3 NR2B assembly sub-unit of NMDA critically mediates chronic pain

NR2B subunit is very essential for making the NMDA receptors functional after getting assembled into a working system. This subunit is highly distinct from other subunits of NMDARs as it consists of a larger intracellular c-terminal tail. The NR2B subunit of the NMDA receptor system is highly expressed across the descending and ascending pain pathways and several animal models of chronic pain have demonstrated a strong reproducibility to this pipeline. A recent study has reported that the NR2B subunit phosphorylation regulates synaptic plasticity under migraine condition [114]. Furthermore, the animal model of peripheral nerve injury, cancer pain, and chemotherapy-induced neuropathic pain have also suggested the critical involvement

of this subunit in the progression of chronic pain [95,115,116]. Moreover, peripheral nerve injury decreases the expression of GluN1 (NR1) and increases the expression of GluN2B (NR2B) in the DRG [117]. During chronic pain condition the hyperactivity of presynaptic NMDA receptors at primary afferent neurons results in the elevated release of glutamate (excitatory neurotransmitter) into the spinal dorsal horn [118]. In the DRG, presynaptic NMDA receptors are more resilient to Mg^{2+} block and suppression of action potential as compared to the postsynaptic NMDA receptors [118]. The NR2B causes the depolarization and excitatory post-synaptic potential after activation due to the binding of the pre-synaptic glutamate. When the threshold is attained due to temporal and spatial summation the Mg^{2+} get dissociated from NR2B. Postsynaptic elevation of Ca^{2+} influx causes calcium-calmodulin binding that initiates several downstream pathways. This ultimately results in the sustained excitatory post-synaptic potential which causes LTP via 1) CamKII>PKA>cAMP>CREB and 2) MAPK pathways [119]. Widespread expression of the NR2B subunit has been observed in parts of the cerebral cortex responsible for pain behavior including the ACC and the insular cortex [120]. The majority of NMDA receptor-mediated currents in the ACC are due to receptors having NR2A and/or NR2B subunits. This observation was further refined by a study that reported the decrease in NMDA receptor-mediated LTP in the ACC by the administration of NR2B antagonists. It is noteworthy that even though the LTP was decreased, it was not completely attenuated [121]. LTP in the insular cortex also depends on the NMDA receptors containing both NR2A and/or NR2B subunits. This was confirmed by the observation that NMDA antagonists prevented the development of LTP in IC due to tetanic stimulation in rats [122]. Suppression of NR2B subunit and its downstream pathway (CREB) in spinal dorsal horn accounts for the attenuation of

circadian pain in nerve-injured rats [97]. Moreover, spinal NR2B tyrosine phosphorylation is reported to contribute to the central sensitization and synaptic plasticity in neuropathic pain and chronic migraine [94]. Thus, it is evident that the NR2B subunit plays an important role in NMDAR mediated central sensitization and maintenance of chronic pain.

1.11.4 KIF17 as a major mediator for the trafficking of NR2B subunit

Kinesins are ATP-guided microtubule selective molecular motors that are responsible for the trafficking of cargo to the synaptic membrane. They utilize the chemical energy and convert it to mechanical energy and move along the microtubule tracks. kinesins are further categorized into 14 families which are divided into subfamilies. Various type of kinesins plays role in transporting various cargos, especially to the dendritic endings. A member of the kinesin-2 family is KIF17, a plus (+) end-directed protein found in neuronal cells that translocate and insert NR2B subunit of NMDARs into the synaptic membrane [104]. KIF17 is a homodimeric motor protein with a paired head domain that attaches to the microtubule, and it also consists of a coiled-stalk and cargo binding tail domain [123]. In absence of cargo, KIF17 remains in the auto-inhibited state in cytoplasm by making conformational changes to the central hinge. The KIF17 is known to transport the NR2B subunit of the NMDA receptor system selectively from the cell body to dendritic endings [124] (fig.3). The binding of almost all kinesins to their respective cargos is facilitated by various kinesin-associated scaffolding or adapter proteins. A study in c. *elegans* has revealed the interaction of KIF17 with NR2B subunit [105]. The NR2B subunit is synthesized and packed into vesicles to the cytoplasm which leads to the activation of cytosolic motor protein KIF17. 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 (Figure 1.9) [105]. Finally, the cargo is carried by KIF17 using ATP driven motor from the cell soma to the synaptic terminal along the microtubule [102,104]. The expression of KIF17 and NR2B is found to be associated with chronic pain. A study has suggested the involvement of KIF17 mediated NR2B trafficking in bone cancer pain [125]. Authors have observed a significant increase in mLIN10, NR2B, and KIF17 expression in the spinal cord of the bone cancer pain rat model. Afterward, it has been found that the peripheral nerve injury in rats leads to the increased expression of the -

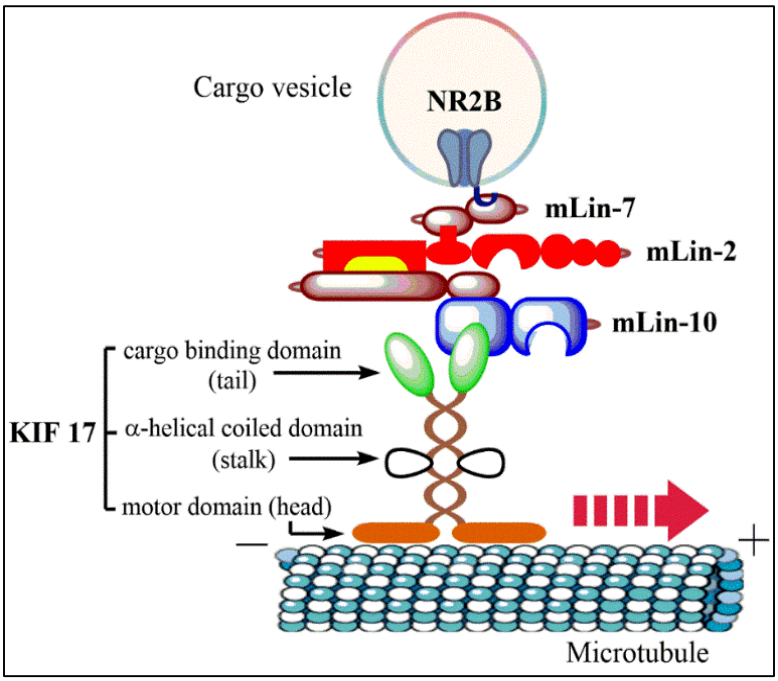

Fig. 1.9 Schematic representation of KIF17 and cargo (NR2B) binding. Kinesin-2 family member, KIF17 consists of head, stalk, and tail. The head is the motor domain that binds to the microtubule and the tail is the cargo binding domain. The tail domain binds to cargo vesicles via adaptor/scaffolding proteins- mLin10, mLin2, and mLin7 Reprinted with permission by American Chemical Society from own source reference [53].

NR2B in DRG of rats [126]. This line of evidence suggests the involvement of the KIF17-NMDA pathway in the development and maintenance of chronic pain. Moreover, when this pathway was targeted using various pharmacological and genetic

approaches, a significant attenuation in pain hypersensitivity was observed [127,128]. Interestingly the expression of KIF17 and NR2B genes is simultaneously up and down-regulated. A study has shown that knockdown or the functional blockade of KIF17 impairs the expression of NR2B. Whereas, upregulating the NR2B expressions by NMDA receptors using D(-)-2-amino-5-phosphonopentanoic acid resulted in increased levels of KIF17 [104]. A possible explanation for this could be that the transcription factor for the KIF17 and NR2B subunit is the same i.e. nuclear respiratory factor-

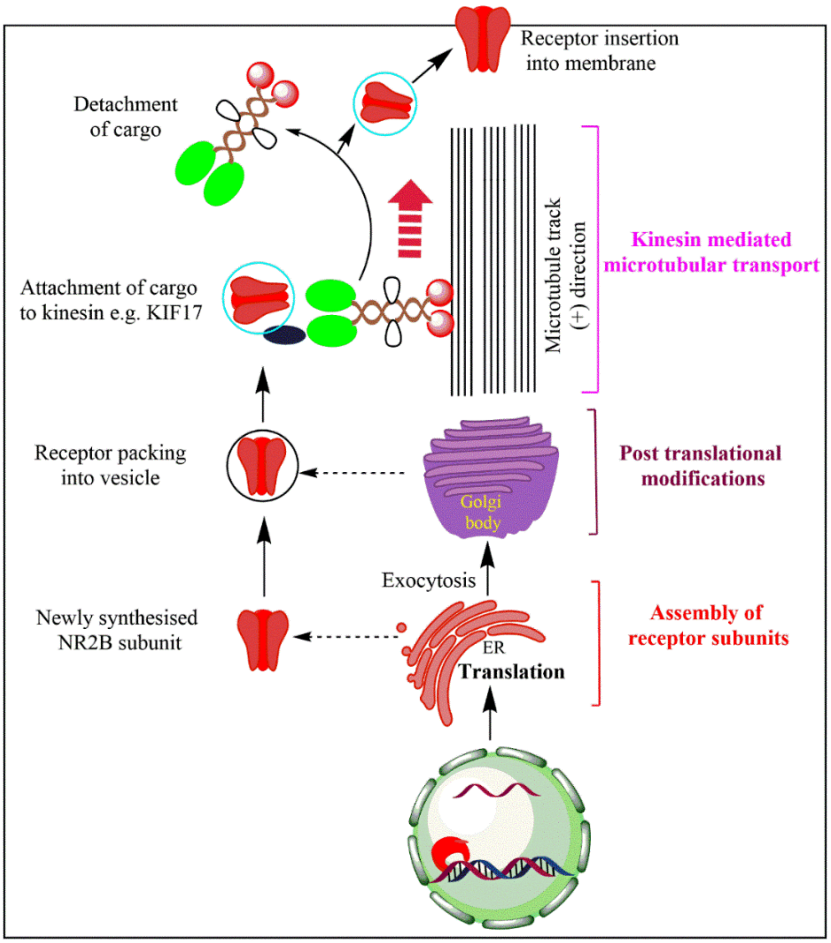

Fig. 1.10 Kinesin mediated transport of NMDA receptor. Kinesins plays an essential role in NMDA transportation and regulation across the neurons. KIF17 is popularly known for facilitating NMDA trafficking. This motor protein carries NR2B subunit-containing vesicles to the cellular membrane via microtubular anterograde transport. Synthesized NR2B subunit is packed into vesicles by the Golgi bodies and released into the cytoplasm. NR2B vesicles bind to KIF17 by various scaffolding proteins and is

transported to the dendritic synaptic terminal via microtubular anterograde transport. Near the synaptic terminal, CaMKII phosphorylates KIF17 to release the NR2B vesicle. Finally, the NMDA receptor insertion into the synaptic membrane occurs making it functional. Reprinted with permission by American Chemical Society from own source reference [53]

-(NRF1) [129]. Although, the NRF1 is not a lone player that participate in transcription of the KIF17 gene but it gives an important insight into the close regulation of neuronal molecular motors with glutamatergic transmission at the transcriptional level.

The loading of cargo to the KIF17 is also regulated by Septin 9 that belongs to the family of G-proteins. Septin 9 acts as a scaffolding and diffusion barrier thus regulating the localization of cytoplasmic proteins. This protein targets the KIF17-mLin10 interaction by modulating the conformation of KIF binding site residues. However, it is not known that Septin 9 interacts with KIF17 directly or indirectly (with the help of adapter proteins). This unloading of cargo by Septin 9 interfere with NR2B subunit translocation and insertion to the membrane [124]. The release of KIF17 cargo is also regulated by calcium-calmodulin-dependent protein kinase II (CaMKII) which phosphorylates the KIF17 at ser-1029 site and regulates its interactions with mLin10 [130]. This series of the event make cargo to get released from phosphorylated kinesin motor. Hence, at the pre-synaptic end CaMKII causes phosphorylation of KIF17 in order to disassemble the NR2B vesicle. Interestingly CaMKII dependent phosphorylation regulated both NR2B and KIF17 and indicating that both proteins share common switches for the synthesis and trafficking across the dendrites.

The anterograde trafficking of NR2B mediated by KIF17 is one of the key regulators of NMDAR formation at dendritic synaptic terminals. Upregulation of KIF17 is found to decrease the pain threshold and promote the pathophysiology of neuropathic pain [131]. Similarly, blocking the activity of CaMKII KN93 to prevent

the synthesis of KIF17 also resulted in the reduction of bone cancer mediated pain hypersensitivity [128]. Recent investigations have also indicated the KIF-based anterograde transport of NR2B as a therapeutic target to achieve anti-nociceptive response in pre-clinical studies [132,133]. In spared nerve injury model (SNI), development of sustained pain, hypersensitivity was found to be correlated with the elevated KIF17 expressions [132]. Equivalent results have been obtained when CREB phosphorylation was inhibited as it decreased the transcription of KIF17 in neurons which ultimately led to the impaired NMDA trafficking [132]. Thus, in a nutshell, KIF17 and NR2B are closely regulated by a common mechanism across the neuronal cells and could be utilized as therapeutic target against variety of disorders.

1.12 Aurora kinase and its inhibitor tozasertib/VX680

Aurora kinase is a serine-threonine class of enzymes belonging to the phosphotransferase group that maintains cellular processes including proliferation, mitosis, and activation of several intracellular signaling. There are three types of aurora kinase enzymes namely A, B, and C that are identified in mammals. The structure of the human aurora kinase enzyme consists of an N-terminal domain (39-129 residues), serine/threonine-protein kinase domain (250-300 residue), and a C-terminal domain (15-20 residues). In past decade many pharmaceutical companies and academic institutions have reported the development of aurora kinase inhibitors against variety of pathophysiological disorders. Inhibition of aurora kinase B was found to be associated with the side effects including febrile neutropenia, stomatitis, gastrointestinal toxicity, hypertension, and fatigue. Furthermore, the use of specific aurora kinase A inhibitors could avoid the aurora kinase B mediated neutropenia. Adjunctive agents such as granulocyte stimulating factors could also alleviate the

neutropenia associated with aurora kinase B inhibitors. Recently it has been shown that aurora kinase B plays a critical role in spinal microgliosis during the neuropathic pain condition in nerve-injured rats. Aurora kinase-mediated distribution and regulation of kinesins is reported in several studies which suggest its potential as a target to modulate the kinesin activity. Although, there is no previous work done suggesting the role of aurora kinase mediated regulation on kinesin in pathophysiology of chronic pain. Thus, we have selected a pan aurora kinase inhibitor, tozasertib, or VX-680 to use a multifarious approach for targeting chronic pain. The drug was developed by Vertex (Cambridge) and named VX-680 whereas later it was named M-0457 by Merck (New Jersey). This was the first aurora kinase inhibitor compound to enter clinical trials and at nanomolar concentration, with inhibitory constant values 0.6, 18, and 4.6 nM it inhibits A, B, and C isoforms of aurora kinase respectively.

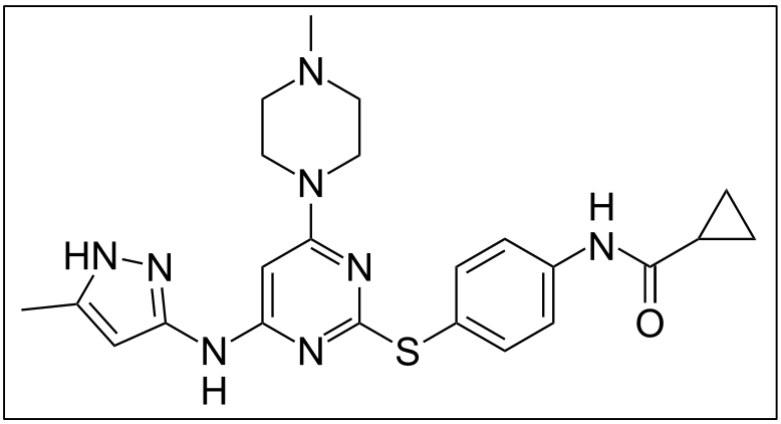

Figure 1.11 The 2D chemical structure of tozasertib

Tozasertib is an anticancer drug undergone clinical trials for lymphoblastic leukemia, myelogenous leukemia, chronic myelogenous leukemia, myelodysplastic syndromes, and colorectal cancer. I*n-vitro* studies have suggested that tozasertib can regulate cell death via caspase-3, receptor-interacting serine/threonine-protein kinase 1 (RIPK-1) and poly (ADP-ribose) polymerase, mast cell responsiveness via NFkB

signaling, epigenetic functioning via histone deacetylases, and other downstream cellular processes via cyclin B, ERK, and cdc25c which is an M-phase inducer phosphatase 3 enzyme. Further, the neuroprotective effect of tozasertib is also known to be mediated through the DLK/JIP3/MA2K7/JNK signaling pathway. Therefore, we rationalized using tozasertib as a potential candidate to test against aurora kinase mediated kinesin regulation during chronic pain conditions.