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Mechanism and Preclinical Models of Neuropathic Pain: An Update

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Abstract: Neuropathic pain is a debilitating condition that is a product of nervous system damage or dysfunction. Since the drugs prescribed by the physician provide partial pain relief to the patients. Hence, current updates for its treatment are required. It is a global concern as neuropathic pain happens in many diseased conditions like cancer, trauma, surgery and diabetics, etc. Developed as well as developing countries are both trying to find suitable medicine. Understanding the mechanisms behind it can be crucial for the effective treatment and management of neuropathic pain. Central sensitization in the spinal cord and brain amplifies pain signals, increasing pain sensitivity even without tissue damage. Peripheral sensitization, at the injury site, sensitizes peripheral nerves, lowering pain thresholds. Recognizing and studying these sensitizations are vital for understanding and managing chronic neuropathic pain and improving patients' quality of life. The present manuscript encompasses a mechanism and model for neuropathic pain in animals with its advantages and disadvantages.

Keywords: Peripheral Sensitization; Central Sensitization; Neuropathic Pain; Pain Signals

1. Introduction

A painful condition that appears due to abnormalities in the somatosensory nervous system is called neuropathic pain (NP), which can be recognized by dysesthesias and paresthesias-like conditions [1]. Allodynia (pain in response to a stimulus that does not usually provoke pain) and hyperalgesia (an increased response to painful stimuli that do not occur normally) are identified as hallmarks of NP (**Figure 1**). The exact population of patients with NP is still unknown, but various published reports put an estimate between 100–560 million people globally [2]. NP is highly associated with patients with long-standing diabetes, stroke, cancer, AIDS, herpes virus infection, multiple sclerosis, and traumatic nerve injury. It is often associated with conditions like post-herniorrhaphy, syringomyelia, post-mastectomy, and Fabry neuropathy. Hence it is a state that occurs as a result of a multifactorial pathological condition, and now it becomes a global challenge for medical sciences [3].

Currently used medications like non-steroidal anti-inflammatory drugs (NSAIDs) and opiates have attained limited effectiveness or no response to treat NP [4]. Therefore, new treatment methods need to be investigated. It is a bit difficult to evaluate NP in humans. The probable reason may be the use of stimuli, which causes irreversible damage to the individual. In addition, it is also very difficult to select a large number of individuals for the reduction of subject variability. Henceforth, to broaden the underlying mechanisms and find novel treatments for NP, an ideal

animal model is needed, which must be validated as well as reproducible. Furthermore, it must enable researchers to evaluate various sensory deficits of NP.

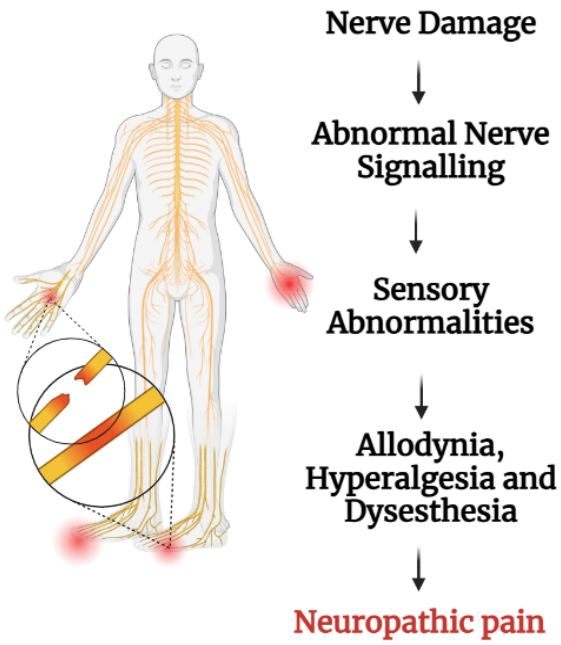

Figure 1. Development of NP.

Various animal models have been established that mimic conditions of NP in terms of mechanisms as well as symptoms, e.g., surgical models (chronic constriction injury (CCI), spinal nerve ligation, sciatic nerve transaction model, diabetes-induced NP models (streptozotocin and high-fat diet); post-herpetic neuralgia models; drug-induced NP models (anti-cancer drugs, anti-HIV drugs, formalin, pyridoxine, chronic alcohol); or disease-induced NP (cancer, HIV) [5]. Some animal models, like uremic-induced NP, inherited neuropathy, and optogenetic approach models, have also been developed. This review will exhaustively discuss different animal models with their limitations, advantages, disadvantages, and their development for preclinical evaluation.

NP affects millions of people on a global scale. The incidence rises with aging and is more prevalent in people over 50. According to a 2019 review, NP affects between 3% and 17% of the general population and is a serious health concern [6]. One of the researchers estimated it to be between 7% and 10%, increasing to around 20%–30% in people with diabetes [7]. However, the prevalence can vary depending on the population studied and the method used to diagnose NP. For example, a study in the United States found that the prevalence of NP was 7.1%, while a study in Europe found that the prevalence was 10.6%. Certain populations, such as those with diabetes, cancer, or HIV/AIDS, have a higher prevalence of NP [7].

NP can be due to consequences of nerve compression, trauma, infections, metabolic disorders, or diseases such as diabetes, multiple sclerosis, or cancer [8]. Compared to nociceptive pain, which is a typical reaction to tissue damage or inflammation, NP lasts longer than anticipated and is frequently described as a shooting, burning, or electric shock-like feeling. The underlying mechanisms of NP involve both peripheral and central nervous system alterations. Injury to peripheral nerves results in increased excitability, aberrant spontaneous firing, and enhanced sensitivity to stimuli [9, 10]. This abnormal activity may also cause inflammation and the production of chemical mediators (**Table 1**) that help to create and maintain pain signals inside the spinal cord and brain. Maladaptive plasticity results from the rewiring of neuronal networks that frequently occurs after nerve damage. The nervous system may become hypersensitive as a result of this, increasing pain signals and causing aberrant reactions to non-painful stimuli. This is a process known as central sensitization [10, 11]. The affected individuals' quality of life is significantly impacted by NP. It frequently causes physical and emotional pain, sleep problems, diminished mobility,

and difficulty going about everyday activities. Hallmarks of NP, i.e., allodynia, hyperalgesia, and dysesthesia, appear once the damage of nerves takes place due to abnormal signaling.

As the pain is subjective and manifests differently in each person, it also presents difficulties in terms of diagnosis. One form of homosynaptic facilitation in spinal cord neurons is windup, in which the action potential discharge elicited by a low-frequency (0.5 to 5 Hz) train of identical C-fiber strength stimuli gets larger on each successive stimulus [12]. Due to this, a repeated stimulation of nociceptors leads to a progressive increase in the response of the central nervous system to pain, which leads to a prolonged and exaggerated pain response. The first signs and symptoms commonly seen in NP include abnormal sensations (such as tingling or numbness) and heightened sensitivity to stimuli (hyperalgesia and allodynia) [13, 14].

1.1. Mechanism Involved in the Development of NP

NP arises from a complex interplay of various chemical mediators that contribute to its underlying mechanism. Glutamate, a neurotransmitter responsible for excitatory signaling, plays a crucial role in promoting heightened neuronal excitability and central sensitization. This heightened excitability amplifies the response to pain signals. Substance P, another key mediator, facilitates pain transmission and triggers inflammation, intensifying the perception of pain [15]. Calcitonin Gene-Related Peptide (CGRP) is implicated in enhancing pain sensitivity. It promotes peripheral sensitization, where pain-sensing nerves become more receptive to pain signals [15]. Additionally, CGRP contributes to neurogenic inflammation by releasing inflammatory substances. Prostaglandins, lipid molecules, also contribute to NP by promoting peripheral sensitization and inflammation. They augment pain signals and overall pain experience [16]. Nerve Growth Factor (NGF) plays a role in facilitating the sprouting of nerve fibers, leading to increased connectivity and sensitivity of pain-sensing neurons (nociceptors) [17]. NGF also contributes to neuroinflammation, further perpetuating NP. Neurotrophins, a group of growth factors, influence neuronal survival, plasticity, and pain processing in both the peripheral and central nervous systems. Dysregulation of neurotrophins can impact the development and persistence of NP. Cytokines, particularly proinflammatory cytokines such as TNF-α and IL-6, participate in neuroinflammation and peripheral sensitization. They contribute to the sensitization of pain pathways, enhancing pain perception and promoting a chronic pain state. Reactive Oxygen Species (ROS), reactive molecules, induce oxidative stress that can damage nerves [18]. This oxidative stress activates pain pathways, contributing to the generation and maintenance of NP. The intricate interplay and impact of these chemical mediators form a complex network of events that drive the development and persistence of NP. A comprehensive understanding of their roles provides valuable insights for identifying potential targets for therapeutic interventions aimed at easing neurogenic pain and enhancing the quality of life for individuals afflicted by this condition. The damaged neurons exhibit increased expression of sodium channels triggered by the lesion [19, 20]. Additionally, products released in the vicinity of spared fibers, such as nerve growth factor, induce the expression of channels and receptors on uninjured fibers. The spontaneous activity in C-nociceptors leads to secondary changes in central sensory processing, resulting in spinal cord hyperexcitability [4, 20]. The spinal cord's dorsal horn has several receptors and channels indicated in **Figure 2**. In the event of nerve injury or its dysfunction, neurochemicals will bind and subsequently perform the action to maintain the equilibrium like the inhibitory and excitatory neurotransmission state.

The output from the dorsal horn to higher centers in the brain is carried by spinal projection neurons along ascending pathways.

In order to get nociceptive information from the peripheral nervous system to the brain, it must first pass through the spinal cord. Primary afferent fibers, which carry sensory information from the periphery into the spinal cord's dorsal horn, connect with intrinsic dorsal horn neurons there. In order to perceive both non-noxious and noxious impulses, spinal projection neurons transmit this information to higher brain regions. The spinal cord's output during nociceptive transmission depends on a number of spinal mechanisms that can change the activity of dorsal horn neurons. Local N-methyl-d-aspartate receptor activation, local inhibitory and excitatory interneurons, and descending impacts from the brainstem are a few examples of these systems. Changes in these excitatory and inhibitory mechanisms that regulate spinal excitability can happen in response to inflammation or nerve injury. This central sensitization phenomenon frequently results in a dorsal neuron's enhanced response to incoming afferent signals and increased output to the brain [21].

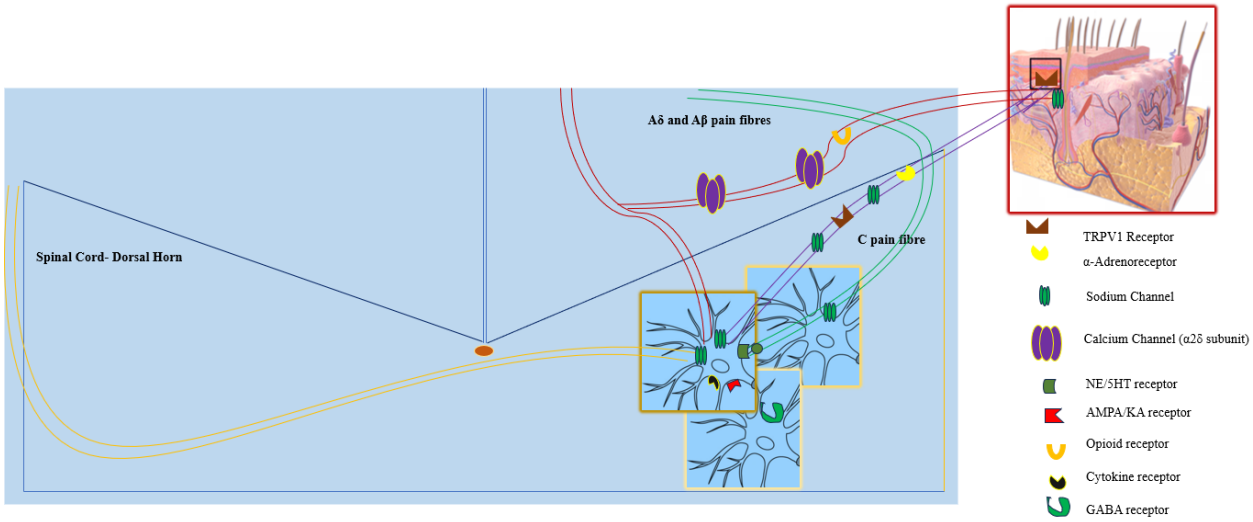

Figure 2. Receptor and channels available in the dorsal horn of spinal cord.

Table 1. Various chemical components involved in the mechanism of NP.

S.N.	Components	Mechanism	Reference
1	Glutamate	Acts as an excitatory neurotransmitter, contributing to neuronal hyperexcitability and central sensitization	[22]
2	Substance P	Mediate's pain transmission and inflammation	[23]
3	Calcitonin Gene-Related	Enhances pain sensitivity by promoting peripheral	[24]
4	Peptide (CGRP)	Sensitization and facilitating neurogenic inflammation	[24]
5	Prostaglandins	Contribute to peripheral sensitization and inflammation, amplifying pain signals	[25]
6	Bradykinin	Damaged tissue can activate nociceptors and cause pain	[24]
7	Nerve Growth Factor (NGF)	Facilitates sprouting of nerve fibers, enhances nociceptor sensitivity, and promotes neuroinflammation	[26, 27]
8	Neurotrophins	Alter neuronal survival, plasticity, and pain processing in the peripheral and central nervous systems.	[25, 28]
9	Cytokines	Proinflammatory cytokines, such as TNF-α and IL-6, contribute to neuroinflammation and peripheral sensitization.	[29, 30]
10	Reactive Oxygen Species (ROS)	Induce oxidative stress, leading to nerve damage and activation of pain pathways.	[31]
11	voltage-gated sodium channels (VGSCs)	involved in the generation of action potentials, which are the electrical signals that transmit pain signals	[32]
12	Gene expression	Nerve damage can change the expression of genes, which can lead to changes in the way that pain signals are processed and perceived. With damage to peripheral sensory fibers, a variety of changes in pain-related gene expression take place in dorsal root ganglion neurons. These changes, or plasticity, might underlie unique neuropathic pain-specific phenotype modifications—decreased unmyelinated-fiber functions but increased myelinated A-fiber functions. Another characteristic change is observed in allodynia, the functional change of tactile to nociceptive perception	[33]
13	TRPV1 (Transient Receptor Potential Vanilloid 1)	After nerve injury, TRPV1 is activated, which allows the influx of calcium ions into the nerve fibers, resulting in the generation and propagation of pain signals	[34]
14	Transient Receptor Potential Ankyrin 1 (TRPA1)	Increased sensitivity and expression from nerve injury leads to pain response (Chemical induced)	[35]
15	Sodium Voltage-Gated Channel α Subunit 1.3 (Nav1.3)	Hyperexcitability due to re-expression in the injured neuron as well as non-neuronal cells	[36]
16	Sodium Voltage-Gated Channel α Subunit 1.7 (Nav1.7)	Increased excitability and signals of pain in sensory neurons after nerve injury	[37]
17	Sodium Voltage-Gated Channel α Subunit 1.8 (Nav1.8)	Altered pain signaling and sensitivity due to upregulation and activation of threshold level	[38]
18	Sodium Voltage-Gated Channel α Subunit 1.9 (Nav1.9)	Hypersensitivity and amplification in pain signals due to increased activation and expression of the sensory neurons due to damage in the neuron	[39]
19	Calcium Voltage-Gated Channel α-2/δ Subunit 2.2 (Cav2.2)	Calcium influx leads release of level of neurotransmitters increases	[40, 41]
20	Calcium Voltage-Gated Channel α-1H (Cav3.2)	Hyperexcitability due to overexpression and enhanced activity	[42]
21	Potassium Voltage-Gated Channel Subfamily A Member 2 (Kv1.2)	Downregulation of potassium can reduce neuronal activity leads neuronal inhibition	[43]
22	Potassium Voltage-Gated Channel Subfamily A Member 2 (Kv1.4)	Increase the neuronal excitability which changes the expression and reduced potassium current	[41]

1.2. Animal Models of Neuropathic Pain (NP)

The various models of NP are not only important for the measurement of pain condition and treatment but also to understand the exact pathophysiology of that developed pain so that proper strategies can be adopted for its treatment. Most of the drugs available in the market are not giving accurate results. Hence, patients are getting partial or unsatisfactory relief from this pain. The following models of NP evaluation are discussed below with both prospects of selected models, which indicates their limitations with advantages (**Table 2**).

1.2.1. Surgical Models

CCI (Chronic constriction injury) induced NP

Procedure for development of model:

It is required to be performed under anesthesia (i.e., sodium pentobarbital 40 mg/kg/ketamine 60 mg/kg). In summary, rats are given ketamine (60 mg/kg i.p.) to induce profound anesthesia. The skin is sterilized with 0.5% chlorhexidine after the rat's lower back and thigh hair are shaved. An incision is made in the skin of the left thigh's lateral surface. As a result of this, the sciatic nerve is made visible (10 mm). Four ligatures (silk 4-0) are positioned around the nerve's proximal half of the trifurcation from the surrounding connective tissue. Such surgery brings the development of NO in a slow and progressive manner. The ligation should be made in such a way that each ligature should be at a distance of one millimeter. Up until a brief flick of the ipsilateral hind limb is noticed, the ligatures are loosely tied. Muscular and skin layers are promptly sutured with thread once the nerve is ligated, and a topical antibiotic is also required at the end [44] (**Table 2**).

Table 2. Models of NP in animals for clinical studies.

Sr. No.	Animal Model	Principle	Characterization/Advantages	Animal Used	Limitations	Symptoms (Emphasized)	Reference
Surgical Models							
1	Chronic constriction injury (CCI)	Four loose ligatures (Unilateral/bilateral) around sciatic nerve	a. The neuroanatomical (behavioral as well as anatomotical) changes over time in a short period. b. Mimics the post-traumatic peripheral painful neuropathy states in humans. c. It is a reliable and easily reproducible model. d. This is a suitable model for assessing cold allodynia when compared to partial sciatic nerve ligation (PNL) and spinal nerve ligation (SNL).	Rats, mice	a. Changes to heat and mechanical stimuli seen with unilateral CCI (CCI) are transient, lasting four weeks or less. b. Degree of allodynia produced is less as compared to other models. c. Variability of degree of damage from animal to animal due to variation in the snugness of ligations even by the same experimenter in different animals	Thermal and mechanical hypersensitivities	[45]
2	Spare nerve injury (SNI)	Ligation & transaction of tibial and common peroneal nerves leaving the sural nerve intact	a. Displays a distinct and separate anatomical distribution of injured nerves and intact sural nerves. b. Hypersensitivities are maintained for at least 6 months.	Rats, mice	a. Produces difficulty in performing behavioral tests as it causes degeneration of axons b. Hypersensitive area in the limb is difficult to test as it is the lateral part of the paw.	Cold and mechanical hyperalgesia/allodynia	[46]
3	Spinal nerve ligation (SNL)	Tight ligation of spinal nerves L5, L6 and L7	a. Experimental variability is less	Rats (L5, L6) Macaca fascicularis (L7)	a. Extensive muscle injury surgery can differ outcomes and make the pathomechanism more complex.	Mechanical allodynia	[47]
4	Partial sciatic nerve ligation (Seltzer model)	Ligation of the ipsilateral sciatic nerve at high thigh	Easy surgical procedure and consumes less time.	Rats, mice	a. Changes in the Dorsal Root Ganglion (DRG) are difficult to study	Mechanical and cold allodynia	[48]
5	Brachial plexus avulsion (BPA) model	Lesion made in brachial plexus	Valid model long-lasting mechanical and cold allodynia	Rats	Lesion made in brachial plexus may lead plasticity of CNS.		[49]
6	Sciatic Nerve Transection model	Complete transection of sciatic nerve at mid-thigh level	Suitable for simulating phantom limb pain	Rats	Ethical considerations are also the key issues in this model as animals demonstrate excessive autotomy in this model		[50]
7	Spinal Nerve Transection (SNT) model	Incision made along spinal nerve	May stimulate limb pain	Rats	Lacks the inflammatory component		[51]
8	Sciatic cryoneurolysis	Freezing of the sciatic nerve	Useful for postoperative and chronic pain and only for mononeuropathy	Rats, mice	Reduces weight loss and not validated by antineuropathic drug.	Thermal hyperalgesia and mechanical allodynia	[52]
Diabetic neuropathy models							
9	Streptozotocin induced rat model	Persistent hyperglycemia-induced injury to the nerves	a. Earlier development of diabetes followed by neuropathy (4–8 weeks) b. Reduce sensory and motor nerve conduction velocity.	Rats	a. Non-obese diabetic mice develop autoimmune T-cell-mediated insulin-dependent diabetes due to inheritable polygenic immunodeficiency b. Not validated by antineuropathic drug.	Thermal, cold and mechanical allodynia/hyperalgesia	[53]

Table 2. *Cont.*

Sr. No.	Animal Model	Principle	Characterization/Advantages	Animal Used	Limitations	Symptoms (Emphasized)	Reference
Diabetic neuropathy models							
10	Chinese hamster neuropathic model	Persistent hyperglycemia-induced injury to the nerves	a. No reduction on nerve fibres diameter b. Reduced conduction velocity of both motor and sensory components of hind lamb nerves (16%–22%)	Chinese hamster	a. Peripheral diabetic neuropathy (PDN) was less severe than human diabetic neuropathy. b. Needs further studies for validation.	Thermal, cold and mechanical allodynia/hyperalgesia	[54]
11	High-fat diet-fed female C57BL6/J mice model	Persistent hyperglycemia-induced injury to the nerves	a. It can be used as a model for obesity or prediabetic-related neuropathy.	Mice	a. Intradermal nerve fiber loss, and axonal atrophy was absent. b. Cannot be used for chronic diabetic neuropathy. c. Not validated by antineuropathic drugs		[55]
Post-herpetic neuralgia models							
12	Varicella Zoster virus model	Injection of viral infected cells in the footpad	a. Anxiety like pattern of ambulation (reduced entry into the central area of the open arena) that is positively correlated with mechanical hypersensitivity.	Rats, mice	a. Infection with all viral strains was associated with a dose-dependent mechanical hypersensitivity but not a thermal or cool hypersensitivity	Mechanical hypersensitivity	[56]
13	Non-viral model	Afferents with resiniferotoxin	a. Extensive ultrastructural damage of myelinated fibers in RTX-treated rats	Rats	a. The delayed tactile allodynia induced by RTX is likely attributable to damage to myelinated afferent fibers and their abnormal sprouting in lamina II of the spinal dorsal horn.	Thermal and mechanical sensitivity	[57]
Drug induced NP models							
14	Chemotherapeutic agents (Paclitaxel, oxaliplatin, vincristine, cisplatin)	Axon damage (axonopathy) leading to injury to nerves later spreads out centrally	Causes disruption of microtubule structure that interferes with axoplasmic transport to produce nerve damage.	Rats, mice, guinea pigs, rabbits	Chances of development of orthostatic hypotension, paralytic ileus and impotency	Numbness, distal dysparathesia,	[58]
15	Chronic alcohol consumption	Alcohol administration over a long time (approximately 70 days)	a. Impairment of axonal transport as well as cytoskeletal properties. b. Increased NFκβ activity, caspase 3 activity as well as PKC activity.	Rats	Augments the level of hormones like epinephrine, stress hormone (cortisol and adrenaline), as well as corticosterone	Allodynia, spontaneous burning pain, hyperalgesia	[59]
16	Anti-HIV drugs induced NP (Nucleoside reverse transcriptase inhibitors like zalcitabine)	Distal axonal degeneration causing inflammation and hence damage the nerves.	a. Significantly increased expression of phospho-p38 in microglia. b. Significant change in thigmotactic behavior	Mice, Rats, Rabbits	Reveal mechanisms of neuropathy but do not mimic HIV disease complexities.	Mechanical hypersensitivity	[60]
17	Formalin induced NP	ROS induced oxidative stress leading to tissue injury followed by nociceptive response	a. Biphasic nociceptive behavior is produced, i.e., Phase 1 and Phase 2 b. This model produces long-lasting thermal as well as mechanical hyperalgesia. c. A comparable pattern of activity in dorsal horn neurons is produced. d. NO is involved in the central mechanism of peripheral neuropathy.	Mice, Rats, Dogs	Time perception (critical ability of animals and humans in their daily activities) is distorted due to altered emotional states on formalin injection.	Thermal and mechanical hyperalgesia	[61]
18	Pyridoxine induced NP	Declined distal limb proprioception and sensory ataxia occurs depicting neuropathy	Pure degeneration of peripheral sensory neurons.	Rats, Mice	Associated with permanent sensory abnormalities	Hyperalgesia	[62]
Disease induced NP models							
19	Diabetes induced NP	Persistent hyperglycemia-induced oxidative stress caused nerve damage	a. Modifies axon–glia interactions at PNS nerves. b. Affects axonal energy metabolism c. Affects ion conductance particularly. d. Large myelinated motor nerve fiber dysfunction	Rats, Mice	Only mild neurophysiologic deficits are produced.	Mechanical allodynia and hyperalgesia	[63]
20	Cancer induced NP (Bone pain)	Multiple mechanisms like ectopic sprouting and sensitization of sensory nerve fibers due to cancer	a. Induction of neuroplastic changes in the spinal cord and supraspinally b. Cytokines and chemokines released cause nociceptive signaling and sensitization in bone	Rats, Mice	Tumor burden within spinal cord leads to hind leg paralysis that does not allow assessment of neuroceptive behaviors.	Hyperalgesia and allodynia	[64]
21	Inflammatory induced NP	Inflammatory mediators (TNF-α, Interleukins, nerve growth factor) induced spontaneous discharge in pain fibers results in greater sensitivity to peripheral stimulation	Abnormal spontaneous activity in approx. one third of A-fibers and one fifth of A-fibers and a smaller percentage of C-fiber sensory afferents beginning within 1–2 days after nerve injury	Rats, Mice	Not readily amenable sometimes	Allodynia (High regular and clock like discharge)Hyperalgesia	[65]
22	HIV induced neuropathy	Distal degeneration of axons leads to peripheral nerve damage	a. Multifocal inflammation due to hyperactive macrophage response. b. Monocytes as well as pro-inflammatory cytokinesinflux in DRGs and peripheral nerves causing neuronal injury.	Rats, mice, rabbits	Lack of evaluation for morphological abnormalities in the distal nerves and transient nature of the NP behavior	Hyperalgesia, allodynia	[66]
Other models							
23	Uremic peripheral neuropathy (Surgical method)	Compression of median nerves (especially in carpel tunnel) due to surgical induction of uremia	a. Decrease in nerve conduction velocity, b. Increase in amyloid disposition. c. Acute and chronic renal failure models can be used to evaluate uremic neuropathy.	Rats, mice	Variable results are produced due to extent of compensatory response produced by remaining normal tissue.	Hyperthyroidism	[67]

Table 2. *Cont.*

Sr. No.	Animal Model	Principle	Characterization/Advantages	Animal Used	Limitations	Symptoms (Emphasized)	Reference
Other models							
24	Inherited induced neuropathy (Charcot-Marie-Tooth neuropathy and tomaculous neuropathy)	Mutations in genes, i.e., PMP22, P0 and connexin 32	a. Muscle weakness of the lower limbs. b. Severe demyelinatio, decreased nerve conduction velocity, lower grip strength.	Transgenic mouse and rats	Variability as well as reproducibility issues sometimes.		[68]
25	Optogenetic approach	Visualization of signaling events and manipulation of cellular activities by light and molecular genetics	a. Powerful approach in assessment of LTMR (low threshold mechano receptors) derived pain. b. Ensures reproducibility of results. c. This model helps in selective stimulation or inhibition or silencing of subpopulations to elucidate complete neuronal circuitry. d. Targeting specificity of neurons along with its precise temporal control.	Rats, mice, rabbits	a. Difference in neuronal activity due to optogenetic stimulation and stimulus to skin in animals. b. Level, consistency, time course as well as specificity of opsin expression among animals. c. Animals may nonspecifically respond to light used.	Mechanical allodynia	[69]

To ensure that the ligature does not slide along the nerve, begin each ligature with a single loose loop. Then, grasp the two ends near the loop and tighten until the loop is just barely snug. Finish the knot by placing a second loop on top of the first to hold the loop in place. Lastly, trim the ligature's loose ends to about 1 mm. Minimal nerve constriction is necessary to avoid arresting the flow of blood to the brain; if a slight twitch is noticed, it should be stopped right away. Excessive ligature tightening can result in undesirable side effects such as axotomy and autotomy (self-mutilation), which can hinder the effectiveness of pain hypersensitivity testing.

Although many researchers have used CCI on the right sciatic nerve with comparable pain and behavioral effects, we would like to discuss unilateral constriction of the left sciatic nerve. Due to handedness, some researchers might find it simpler to operate on a particular side, while others have switched sides during experiments to account for bias.

Advantages

It is simple to perform and produces intense pain after 1–2 weeks of injury, which is similar to pain patterns that occur in humans [70].

Disadvantages

It causes focal ischemia & intraneural edema [71]. It produces significant alterations in sleep patterns that reduce the sleep efficiency of animals [72].

Precautions

Care must be taken to tie the ligatures to avoid twitches in surrounding muscles [44]. Anesthesia and antibiotics must be provided at the start and end of the experiment, respectively.

Spare nerve ligation-induced NP

Procedure for development of model

The lateral surface of the left thigh is incised using anesthesia (halothane 2%), and the biceps femoris muscle is divided into its proximal and distal parts to reveal the sciatic nerve with its three branches of the endpoints. The procedure includes an axotomy and ligation of the tibial and common peroneal nerves while leaving the sural nerve intact; additionally, the common peroneal and tibial nerves were tight-ligated using 5.0 silk and sectioned distal to the ligation, removing 24 mm of the distal nerve stump followed by muscle closure [47]. This model facilitates 15 or longer months and 30 days of hypersensitivity for rats and mice, respectively [73] (**Table 2**).

Advantages

This model provides additional mechanisms of NP as it facilitates the behavioral testing of the uninjured sural nerve territories (connected to the deserved areas). It allows the evaluation of the neurophysiological changes occurring in the intact sural afferent neurons after damage to adjacent nerves. The model is reproducible and easy to execute, and beginners can perform it with a high rate of success [74].

Disadvantages

Each stretching or touch should be avoided with the spared sural nerve. After surgery, rats and mice develop long-term hypersensitivity to mechanical stimulation but not to thermal stimulation [73].

Precautions

Avoid touching or stretching the sural nerve. Surgery should be performed under anesthesia. Lesions should be made carefully [74].

Spinal nerve ligation-induced NP

Procedure for development of model

In this model, left L5 & L6 account for the damaged sciatic nerve axons and L-4 for the undamaged sciatic nerve axons. Under sodium pentobarbital anesthesia (40 mg/kg), the left L5 and L6 spinal nerves are isolated and tightly ligated using a 3-0 silk thread. A similar kind of surgery is required to perform on the right side, except for ligation of the spinal nerves. A complete hemostasis also needs to be confirmed, and the wound is sutured (**Table 2**).

Advantages

It provides a limited analysis of the sciatic nerve of damaged/undamaged nerve fibers. This model also manifests symptoms of human patients of NP.

Disadvantages

The present model cannot be used when an experimental manipulation requires innervation by individual peripheral nerves. The development of the model using surgery is also very extensive.

Precautions

The procedure is complicated to perform and takes time to execute. It also needs full expertise to avoid L-4 nerve damage and in further processing of ligation [75].

Partial sciatic nerve ligation (PSNL) induced NP

Procedure for development of model

Under ether anesthesia, partial nerve injury is produced by exposing the ipsilateral (right) sciatic nerve, so that the dorsum of the nerve is loosed from surrounding connective tissues. The nerve is allowed to fix by pinching the epineurium with the help of forceps. Thereafter, 8-0 silicon-treated silk suture is inserted into the nerve with a 3/8 curved reversed cutting, and the wound is closed at the end (**Table 2**).

Advantages

This model has a simple operation and takes less time than the SNL model. It comprises high reliability and ease of surgical procedure. This partial nerve ligature model simulates nerve contusion rather than nerve compression. The pain illustrated may evolve into bilateral patterns [48].

Disadvantages

Difficult to avoid minor differences in the size of ligatured nerves [76].

Precautions

The dorsum of the nerve is carefully freed. Care must be given while pressing the nerve against underlying structures [48].

Brachial plexus avulsion (BPA) induced NP

Procedure for development of model

This surgery usually takes place using anesthesia by intraperitoneally administering 7% chloral hydrate solution in an amount of 0.6 mL/g body weight. The brachial plexus, extending from the sternum to the axillary point, is approached by a horizontal incision parallel to the clavicle. The central muscle of the pectoral is relocated, and the cerebral vein is left intact. The subclavian vessels are located and separated from the surrounding tissue from the lower trunk of the brachial plexus. The lower trunk is picked up with forceps in the avulsion category of rats and pushed out of the backbone by friction. The tissue layers are then brought together, and the skin is closed using 4-0 silk suture string [77].

Advantages

BPA is distinguished mainly by the rapid onset of pain. It produces the permanent development of neuropathy that can occur distant from the injury's location [78]. It is most reliable and produces long-lasting NP (up to 90 days).

Disadvantages

It is not associated with the change in heat thermal threshold.

Precautions

This model needs expertise due to anatomical complications of neurons [78].

Sciatic Nerve Transection induced NP

Procedure for development of model

It needs induction of anesthesia using methoxyflurane through the inhalation method, and then the left hindlimb is shaved. The sciatic nerve is exposed at the sciatic notch via a gluteal musculature-splitting incision and sharply transected, and further microsurgical epineural repair is performed with two or three nylon sutures (number: 9-0). The muscles are reapproximated and the wound is closed [79].

Advantages

The model induces intense anesthesia, i.e., pain in the region without any sensory input.

Disadvantages

Difficult to avoid minor differences in the size of the transection of nerves [76].

Precautions

Spontaneous degeneration of the nerve should be prevented using nerve stumps.

Spinal Nerve Transection (SNT) induced NP

Procedure for development of model

The rats are sedated with enflurane (2%–3%). The left transverse processes of the L6 vertebrae are removed by a dorsal skin incision, and the L4 and L5 spinal nerves are transected, and 1 mm segments of their distal ends are excised. Sutures are used to close the muscle and skin incisions with black silk 3-0 thread [80].

Advantage

Greater ease of operation and fewer mechanical irritations than ligation. It is a model that can mimic human phantom limb pain [80].

Disadvantage

Evaluation of pain is difficult to measure [81].

Precautions

Difficult to avoid minor differences in the size of the transection of nerves [76].

Sciatic cryoneurolysis (SCN) induced NP

Procedure for development of model

It is performed using halothane (2%–3%) and then the 3 cm incision is made posterior to the greater trochanter of the femur. During this, it is important to note that while surgery, the common sciatic nerve is required to be exposed, and it can be done by blunt dissection. The nerve is damaged by freezing with a 2 mm diameter cryoprobe cooled to −60 °C and nitric oxide as a coolant in the 30-s freeze cycle, 5-s thaw, and 30-s freeze cycles. Further, the wound is going to be closed, and the recovery of animals takes place.

Advantages

Cryoneurology-induced nerve injury can be reversible to provide a chance to investigate the effect of temporary nerve injury and healing. Compared to other peripheral injury models, such as CCI, spinal nerve ligation, and partial spinal nerve ligation, behavioral signs of touch-evoked allodynia last for around 15–21 days [52].

Disadvantages

Thermal hyperalgesia cannot be accessed here. This model results in transient weight loss that may vary the obtained results. Most of the time it is not a most acceptable model for researchers.

Precautions

The maximum freezing time of the nerve should last not more than 10 min [82].

Post-herpetic neuralgia model of NP

This is the model that is concerned with the Varicella Zoster virus model (Chinese hamster neuropathic model).

Procedure for development of model

In Dulbecco's modified Eagle's medium (DMEM), which is supplemented with 10% (v/v) foetal calf serum, 0.075% (w/v) $NaHCO_3$ penicillin-streptomycin (100 U/ml), and 2 mM l-glutamine, African green monkey kidney fibroblast cells (CV-1) are grown and maintained. In BHK-21 cells, viral stocks of the HSV-1 strain are created. When the cells had an 80% cytopathic impact, the VZV strain was extracted after being propagated on CV-1 cells. 107 plaque-forming units (pfu) of HSV-1-infected cells or VZV-infected cells are injected into the glabrous skin of the left footpad. The opposite-side hind paw is not immunized. Animals used as controls are given injections of CV-1 cells that haven't been infected (VZV) or are given HSV-1 that has been heat-inactivated (56 °C, 30 min). Animals received either a vehicle (sterile distilled water) or valaciclovir (50 mg/kg) by oral gavage twice daily for 6 (HSV-1) or 10 days starting on day 0 post-inoculation after receiving an injection of either VZV or HSV-1 as described above [83].

Advantages

VZV genetics mimicking neurological pain can be introduced to the animals, and respective treatment can be investigated [84].

Disadvantages

The mechanisms underlying NP using VZV infection are still elusive [85].

Precautions

Concentration of inoculum should be accurate, as it provides a dose-dependent NP that is developed.

1.2.2. Non-Viral Model of NP

To resolve the various limitations of NP, this model can be adopted. Here, the degradation of the capsaicin-sensitive afferent system as well as ultra-powerful TRPV1 agonists is the main point for the development of NP.

Procedure for development of model

Resiniferatoxin (RTX) is dissolved in Tween-80 (10%) and ethanol (10%) in isotonic sodium chloride solution and administrated at a single dose of RTX 50 mg/kg, i.p. After the intraperitoneal injections, the mice are housed in their respective cages [86].

Advantages

It induces cutaneous nerve degeneration that may be considered to assess the functional consequences of neuropathy that affects small-diameter sensory nerves. It provides a safe alternative for NP to develop in animals.

Disadvantages

In this model, large-diameter sensory nerves cannot be assessed, and the thermal responses are also found impaired after RTX administration [87].

Precautions

High doses of RTX may cause mechanical hypersensitivity. This model also produces degeneration or dysfunctions of large dorsal root ganglion neurons and their nerves [88].

1.2.3. Diabetic Neuropathy-Induced Models of NP

Streptozotocin (STZ) induced rat model:

Procedure for development of model

Streptozotocin is a chemotherapeutic agent and destroys islet cells that secrete insulin. It comprises glucose molecules, which are made available in the deoxy form linked to a highly reactive molecule of methyl nitrosourea responsible for the cytotoxic effects of STZ. The moiety of glucose guides to destroy the pancreatic β cells and causes insulin deficiency that leads to hyperglycemia. Excessively produced glucose converts into sorbitol, and it starts accumulating in and around the nerve, leading to neuroinflammation, which further attributes to damage of neurons [89]. Six adult Wistar rats weighing 250–300 grams (75–90 days old) are subjected to streptozotocin at the dose of 60 mg/kg i.p. for a single time. Diabetes develops within 3 days [90].

Advantages

It is a common method to perform and prevent the animal from surgical stress [89].

Disadvantages

It causes irreversible degradation of Langerhans islet cells.

Precautions

Altered divalent cation homeostasis must be avoided in this model [91].

1.2.4. Drug-Induced NP Models: Chemotherapeutic Agents (Paclitaxel, Oxaliplatin, Vincristine, Cisplatin):

Procedure for development of model

Paclitaxel-mediated sensory neuropathy is characterized by a burning sensation, mechanical allodynia, cold allodynia, and continuing recurrent distal burning discomfort [92]. Paclitaxel is required to be administered at the low dose of 80 mg/m^2 intravenously once every week. To develop NP condition, treatment of paclitaxel can be given for a certain duration within limited toxicity. To mimic this low-dose regimen, our studies involved i.p. injections of 2, 4, or 8 mg/kg paclitaxel every other day for a total of four injections, resulting in a cumulative human equivalent dose of 28.4–113.5 mg/m^2. It causes long-term mechanical allodynia in a similar manner to peripheral neuropathy

[93]. High doses of paclitaxel produce signs and symptoms of neuropathy within 24–72 h of administration, which include numbness, paresthesias, and burning pain.

Advantages

The advantage of this model is that it causes discomfort or pain without any systemic toxicity or loss of movement.

Disadvantages

Reproducibility is the problem [94].

1.2.5. Chronic Alcohol Consumption Induced NP

Development of model

Long-lasting alcohol intake has been reported to cause painful neuropathy in small fibers, it brings distal axonal degeneration. Alcohol intoxication causes pain amplification that outweighs its initial analgesic effect after a certain period and is known to develop NP syndrome withdrawal [95]. Alcohol-challenged rats develop neuropathy at the dose of 10 g/kg b.i.d. oral gavage of 35% v/v ethanol in double distilled water for 10 weeks. It has been reported that mechanical hyperalgesia and allodynia-like symptoms of NP appear after 10 weeks of alcohol intoxication [96].

Advantages

Ethanol is the most common cause of NP; hence, this model helps in developing an NP that mimics clinical symptoms of NP due to ethanol consumption.

Disadvantages

This model produces pain in areas where there is no sensory output [97].

1.2.6. Anti-HIV Drugs Induced NP (Nucleoside Reverse Transcriptase Inhibitors Like Zalcitabine, Indinavir, Didanosine, and Stavudine)

Procedure for development of model

Reverse transcriptase inhibitors (NRTIs) like the active components ddC (Zalcitabine), ddI (Didanosine), and d4T (Stavudine) are used in the effective treatment of HIV. However, they are known to induce NP-like conditions as well as known to induce sensory neurotoxicity [98]. In brief, general anesthesia (1%–2% isoflurane in O_2 and N_2O at a 1:1 ratio), male Wistar rats are administered d4T (Stavudine) treatment at a dose of 0.5 mL is injected i.v. via a tail vein. A second i.v. injection with the same volume and dose is given 4 days apart. Vehicle control animals receive equivalent volumes of sterile saline using the same administration protocol for d4T. Although patients are generally administered d4T orally, previous studies have demonstrated that both daily oral gavage and a single i.v. administration route produce similar nocifensive behavioral profiles in rats [99]. Later, both control and experimental groups were compared with treatment groups, and promising improvement in the treatment was recorded.

Advantages

This model shares several clinical features developed due to anti-HIV drug-induced NP, and these models may be beneficial for technical research and development to better manage AIDS survivors with potential medical strategies of care for HIV-related NP.

Disadvantages

It needs improvement in predictive validity of animal models of conditions associated with NP [60].

1.2.7. Pyridoxine-Induced NP

Procedure for development of model

Vitamin B6 is a coenzyme for many essential biological reactions. Pyridoxine was used for the treatment of conditions such as premenstrual or carpal tunnel syndromes and for secondary poisoning therapy of Gyromitra esculenta false morel mushroom [100]. Pyridoxine has been reported to cause sensory neuropathy abnormalities at its mega doses (800 mg/kg/mL) by affecting sensory nerve fibers of dorsal root ganglions (DRGs). Injured DRGs lead to the destruction of long myelinated fibers that results in cell death [62, 101]. Pyridoxine is dissolved in sodium chloride 0.9% solution at 50 °C and injected intraperitoneally (800 mg/kg/mL). The injections are carried out for 14 consecutive days. It is important to note that the fresh solution of pyridoxine solution is required to prepare immediately before each injection.

Advantages:

1) Pyridoxine has been shown to produce clear behavioral, electrophysiological, and anatomical deficits, without overt systemic morbidity as in cisplatin-induced neuropathy.

2) It is similar to chemotherapy-induced NP and metabolic neuropathy [102].

Disadvantages:

1) Greater inherent variability.

2) Longer time course.

Precautions for drug-induced neuropathy:

1) Factors like dose regimen, total duration of therapy, and total dose administered must be taken care of, as they may influence the development of peripheral neuropathy.

2) Dose calculations must be accurate, as overdose may develop nociceptive pain-like conditions [60].

1.2.8. Disease Induced NP Models

Diabetes induced neuropathy:

The debilitating symptom of diabetes and a leading cause of foot amputation is peripheral diabetic neuropathy (PDN). Improved vibration and thermal sensitivity rates, leading to sensory failure, include the clinical symptoms of neuropathy. Patients often encounter irregular symptoms like paresthesia, allodynia, hyperalgesia, and sudden soreness, coexisting with usual sensory deprivation [45]. A variety of animal models have been identified for diabetes, out of which β cell toxins, streptozotocin (STZ) and alloxan, are most commonly used for diabetes neuropathy [103]. The hyperalgesia and hyperresponsiveness in C-fibers are found in rats in subcutaneous STZ-induced diabetes over a period of around 2–3 weeks. Such models, however, also undergo other metabolic changes, such as ketoacidosis, modification of fat metabolism, and general physical fatigue (reduced development and bodily function, lethargy, polyuria, and diarrhea) alongside hyperglycemia [104].

Development of model:

Mostly STZ is used to develop a model of diabetic neuropathy. STZ is prepared in citrate buffer immediately before injection to prevent it from degrading. Inject an appropriate amount of the STZ solution IP into the mice so the final dosage is 50 mg/kg. One injection of STZ is given to each mouse for 5 days. In order to prevent sudden hypoglycemia 10% sucrose water can also be given to rats. After that, mice are tested for sufficient levels of hyperglycemia at 4 weeks post injection [105].

Advantages:

This model causes early appearance of neuropathy symptoms; for example, mechanical and thermal hyperalgesia and tactile allodynia have been documented to be maximal within 3 days [106].

Disadvantages:

This model is not validated by antineuropathic drugs [107].

Precaution:

1) Cages should be changed multiple times per week after STZ injection to provide dry bedding for polyuric animals [108].

STZ should be prepared in buffer just before the injection because it gets degraded easily [105].

Cancer induced neuropathic pain:

End-stage cancer pain is a serious medical issue, and disease causes remain unclear. Various models of animal cancer pain, like bone cancer pain, have been established [109]. These models have shown the different pharmacologic and neurochemical aspects of cancer pain, which suggests that inflammatory, neuropathic, and tumorigenic components are involved in pain pathogenesis. One of the most common cancer pains is bone cancer pain, which can be primary to breast, prostate, ovary, or lung cancer or metastatic.

Development of model: This model is based on osteolytic fibrosarcoma cells (NCTC2472) inoculated into C3H/HeJ femur mice that develop painful osteosarcoma. A surface incision of 1 centimeter is produced in the back leg to cut down the patellar ligament to expose the distal femoral condyles. The next step is the insertion of a 23-gauge needle to form a cavity for cell injection at the level of the intercondylar notch and an intramedullary femoral canal. 20 microliters of osteolytic murine sarcoma cells are being inserted into the bone cavity, NCTC2472 (about 2.5 × 106 cells). The cancer-induced degradation of the bone and osteoclastogenesis is reported in 5 days after injection of sarcoma, contributing to spontaneous (nocifensive action, spontaneous flattening) discomfort (spontaneous flinching) and evocative pain [110].

Advantages: It provides insights into the neurochemical and neurophysiologic mechanisms that underlie cancer pain.

Disadvantages: This model does not produce reproducibility.

Precautions: The number of variations should be reduced.

HIV-induced neuropathy:

Distal symmetrical polyneuropathy, which may involve up to 30% of AIDS patients, is marked by debilitating sensory malformations and is the most prominent manifestation of chronic pain in HIV-infected individuals [111]. HIV-1 tends to disrupt the nervous system by binding the outer envelope protein gp120 to CXCR4/CCR5 chemokine receptors, which are found in neurons and glial cells. Additional incidents of this type that aggravate axonal peripheral injury and neurotoxicity.

Development of model: Among rats, left sciatic nerve is removed without injury to the perineurium; HIV-induced neuropathy has been reproduced. The oxidized cellulose (oxycel) is used to supply protein specifically for the sciatic nerve and is filled with the HIV-1 receptor gp120 of either 20 or 400 ng. The oxycel that contains viral glycoprotein is wound loosely around the sciatic nerve, about 2–3 mm past trifurcation. Exposure to the epineural HIV-1 receptor protein gp 120 causes chronic, crippling peripheral neuropathy. After exposure to GP 120, allodynia and hyperalgesia appear 1–3 days later and last for a considerable amount of time [112].

Advantages: This model shares a large number of characteristics with clinical symptoms of NP.

Disadvantages: Mechanisms underlying NP due to HIV need further investigation.

Precautions: It requires strict observation of NP symptoms [113].

1.2.9. Uremic Peripheral Neuropathy

Uremic neuropathy is usually a progressive axonal neuropathy of the sensory-motor neurons. The uremic neuropathy may differ in clinical manifestations. In some cases, sensory effects can mainly appear early in the illness, with paraesthesia, discomfort, or sensation failure. A demyelinating phase considers the underlying pathology to be related to axonal degeneration and impairment. Numerous neurophysiological observations are connected to uremic neuropathy and are the gold standard in evaluation for nerve conduction research [67]. It has been reported that an increase in nerve conduction velocity in nerves is also an indicator of NP, which is considered a shred of evidence for developing NP.

Development of model

In this model, chronic uremia is induced by exposing the left kidney of rats through a flank incision under anesthesia (first operation). The adrenal gland and adherent fat are dissected free, and renal arteries are clamped. The cortical tissue is removed, leaving approximately 25 of the kidney intact. Bleeding is controlled by applying Histoacryl® to the cut surfaces. The animals are recovered completely within a few days of the operation. The right kidney is removed 7 days later in a similar operation. After that, in the rats with chronic uremia, in vivo recordings are made before the second operation and then at weekly intervals for 4 weeks. On these occasions, the weight and serum creatinine levels are measured. Four rats are examined at longer intervals for up to 30 weeks. The sciatic nerve is also dissected to carry out nerve conduction velocity (NCV).

In vitro recording

The technique has previously been described by Jefferys and Brismar [114]. Approximately 4 cm of the sciatic nerve is removed from the rat and immediately placed in oxygenated Ringer solution (composition: 147 mm NaC1, 5.9 mm KC1, 3.1 mm CaC1, 5.0 mm Tris Sigma buffer, pH 7.4 at 25 °C). The nerve is unsheathed and placed in the channel of the recording chamber. The response is then recorded with 4–5 electrodes placed at 4 mm intervals along the nerve. The chamber is positioned in a slot in a metal block maintained at 37 °C. The nerve is irrigated with oxygenated Ringer solution every 5 min. The velocity is estimated from the slope of the plot of the conduction distances against the latency to the initial phase of the responses [115].

Advantages

This model clearly defines changes in the sciatic nerve, i.e., NCV, that help in the assessment of NP.

Disadvantages

The margin of error is undetectable, and long-term survival does not seem possible with glomerular filtration rates below approximately 10% of normal, which is probably necessary for the development of neuropathy.

Precautions

Excessive bleeding while induction of uremia should be avoided [115].

1.2.10. Optogenetic Model: Investigating Neuropathic Allodynia and Ion Channels Involved in NP

It is well established that several ion channels, like Na^{2+} channel, Ca^{2+} channel, potassium channel, and BK channel also play a significant role in the induction of NP. Molecular genetics and light are combined for the first time in the field of optogenetics, which is a young and developing one. By expressing photosensitive proteins to see signaling events and change cell activity, this unusual combination is employed to regulate the activity of living cells.

Channels and NPs

Na^{2+} channel

There is a potential pathogenic role of sodium channels in the development of injury to the nervous system. In NP, an increased voltage-gated sodium channel causes hyperexcitability; due to this, there is a change in correlation of increased amplitude and negative shift that activates tetrodotoxin (TTX) that leads to primary sensory afferent neurons of the dorsal root ganglia (DRG) [116, 117].

Ca^{2+} channel

Clinical efficacy of calcium channel shown as a key target for NP & alteration of Ca^{2+} causing memory loss, hypertension & NP. α_1 subunit ($Ca_v2.3$ family), involved in the activation of the neuronal high-voltage channel, plays an important role in neurotransmitter release at central synapses. Voltage-dependent calcium channels (VDCCs) block ω-conopeptides (e.g., ziconotide) and reduce the release of neurotransmitters from synapses & develop pain mechanisms. It suggests that VDCCs are involved in pain transmission & pain-related phenomena [118].

Potassium channel

K^+ channels act as metabolic sensors, with cellular function & responsible for the metabolic activity of cytosolic ADP/ATP [118, 119]. Altered sensory function via hyperexcitability in injured axons & loss of DRG neurons. Reduced K^+ ATP increases excitability, amplifies excitatory neurotransmission, and causes cell death [119, 120].

BK channel

It is also known as a calcium-activated potassium channel. It expresses the large variety of neurons, controlling neurotransmitter release & maintain the superficial dorsal horn [121]. Opening BK channels leads to the reduction of depolarization-evoked action potential firing and neuronal hyperexcitability, thus modulating NP [122].

Pain among animals cannot be measured directly; thus, pain is interpreted by pain-like behaviors, e.g., withdrawal from a nociceptive stimulus (hyperalgesia and allodynia). Several behavioral models have been designed for the assessment of NP based on the type of injury (mechanical, heat, and cold), such as the Von Frey test, Randall-Selitto test, pinprick test, hot plate and tail flick test, Hargreaves test, cold plate test, acetone evaporation test, etc. (**Figure 3**).

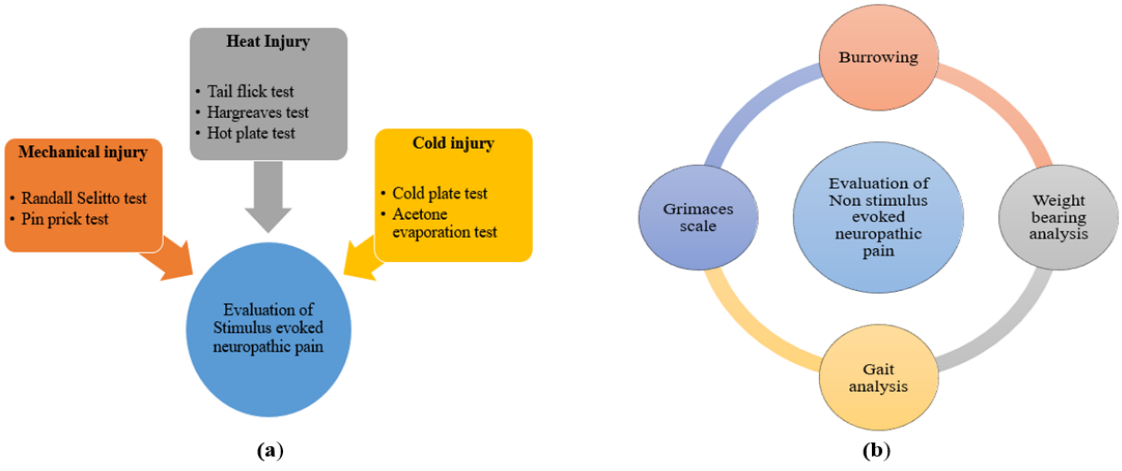

Figure 3. Method of pain evaluation (**a**) stimulus evoked (**b**) non stimulus evoked.

There are multiple factors responsible for the production of NP. Hence, different parameters are required to investigate the valid mechanism for which can mimic the pain in rats similar to human beings. **Figure 3a** explains the features of mechanical, chemical, heat, and cold injury causing pain. **Figure 3b**. Parameters for non-stimulus pain investigation in an animal model.

1.2.11. Tools and Techniques of Investigation of NP

Electronic von Frey test

This test is used to evaluate NP produced due to mechanical stimuli (allodynia and hyperalgesia). It operates under similar principles as Frey's manual, except that a single, unbending filament is applied progressively until a paw withdrawal is elicited. The intensity at which this response is produced is automatically recorded by the system and is defined as the threshold for paw withdrawal. The main advantage of the Von Frey electronic relative to the Von Frey manual is that a single filament is used to apply force on paws. Therefore, it gives a continuous-scale calculation of the paw withdrawal level, as the force is applied gradually and not in steps. Additionally, the experimental time is reduced dramatically, as few applications (usually 3–4) are needed to determine the pain withdrawal threshold [123]. Several systems, such as the Dynamic Plantar Aesthesiometer (Ugo Basile) and MouseMet or RatMet (TopCat Metrology), are commercially available that are particularly robust and user-friendly systems. The Dynamic Plantar Aesthesiometer (or Plantar Von Frey) houses rodents in an enclosure with a mesh screen floor, under which a movable touch-stimulator unit is placed. Under the direction of the researcher, the apparatus applies a von Frey (0.5 mm) filament to the plantar surface, increasing the force incrementally (0–50 g) until the paw withdrawal threshold is reached. The device automatically records the force at which paw withdrawal occurs, and the rate at which the force is applied can be changed. In addition, a programmable "hold" step with constant force application can also be incorporated into the experimental setup to determine the time to withdraw [124].

Randall-Selitto test

This is used to assess the reaction thresholds to mechanical pressure stimulation or paw pressure test, which has often been considered a measure of mechanical hyperalgesia. In this study, an elevated mechanical force is exerted on the paw or tail surface before vocalization or removal occurs. In practice, this test is useful for the assessment of nociceptive thresholds in rats rather than mice, as animals need to be heavily physically restrained with the tested paw held out, and mice rarely tolerate such kind of handling [125]. This method is used for measuring antinociceptive activity carried out by applying pressure on the hind paw and measuring the threshold of foot withdrawal using the Ugo Basile Analgesy-meter. The dorsal surface of the rat paw was kept under the domed-shaped plastic tip. The instrument linearly increases the mechanical force on the rat paw with the help of the tip, and this force is applied until the withdrawal of the paw is seen [126].

Pinprick test

Mechanical hyperalgesia is assessed by the pinprick test in which the surface of the injured hind paw is touched with the point of a bent gauge needle (at 90° to the syringe) at an intensity sufficient to produce a reflex withdrawal response. The paw withdrawal duration is recorded in seconds, and the normal quick reflex withdrawal response gives the value of 0.5 s [74, 127].

Tail flick test

This test is used to assess thermal hyperalgesia and allodynia, where heat stimulus is applied to the tail of the mice and rats, after which the time taken for the tail to flick is recorded. Heat stimulus can be radiant heat, where a beam of light is applied to the tail, or hot water, where the distal end of the tail of the rat is immersed in hot water at a temperature between 46 °C and 52 °C. However, it has been reported that the clinical translatability of the tail-flick test is unclear [128, 129].

Hot plate test

The thermal nociceptive threshold, as an index of thermal hyperalgesia, is assessed by Eddy's hot plate, maintained at a temperature of 52.5 ± 1.0 °C. The rats are placed on the hot plate, and withdrawal latency, concerning licking of the hind paw, will be recorded in seconds. The cut-off time of 15 s is maintained [127]. Nociceptive behaviors also comprise stamping, leaning posture, and jumping, but licking or hind paw withdrawal is considered the most prominent indicator of nociception [130].

Hargreaves test

This test is used to evaluate NP due to radiant or infrared heat stimulus as well as experiments involving pain sensitization or recovery of thermal pain response following neural injury and regeneration. This is carried out using a glass-bottom enclosure that is heated to minimize errors arising from heat sink effects. In this test, a radiant or infrared heat source is placed underneath the animal and applied to the plantar surface of the hind paw. Withdrawal latency, i.e., the time taken to withdraw from a heat stimulus, is recorded. The cut-off time of 10–12 s should be adjusted to avoid tissue damage in animals, which in addition provides sufficient time to detect allodynia. However, it needs animals to be acclimatized to the system so that ambulation can be reduced to accurately determine withdrawal latencies. Habituation time for rats and mice is 5 min and 30 min, respectively [131, 132].

Acetone evaporation test

This test is used to determine allodynia due to cold stimuli assessed by spraying 100 µL of acetone onto the surface of the rat paw (placed over a wire mesh), without touching the skin. The response of the rat to acetone is noted for 20 s and graded on a 4-point scale as defined by Flatters and Bennett (0, no response; 1, quick withdrawal, flick, or stamp of the paw; 2, prolonged withdrawal or repeated flicking; and 3, repeated flicking of the paw with licking of the paw). Acetone is applied three times to the hind paw, with a gap of 5 min between the acetone applications, and the individual scores noted at 20-s intervals are added to obtain a single score over a cumulative period of 1 min. The minimum score can be 0, and the maximum possible score can be 9 after observations [127]. The major drawback of this test is the consistent application of acetone due to low surface tension that makes it difficult to form uniform droplets with a syringe or pipette [133, 134].

Cold plate test

It is one of the simplest tests by which cold hyperalgesia is assessed in both mice and rats. Animals are placed on a cold metal plate kept at 2 °C, and the latency to the first lifting or shaking of the left hind paw is measured in seconds. A cutoff time of 150 s is imposed to prevent tissue damage. This model mimics hot plate tests, as several endpoints can be obtained [135]. It should be noted that some rat strains, instead of flinching and licking, simply avoid weight bearing or repositioning their position to minimize cool surface contact, and therefore all observations should be adopted following the specific animal models [136].

In humans, pain without any identifiable stimulus, also termed spontaneous pain, is a serious clinical problem. It is quite easy to evaluate this pain in humans by different questionnaires to describe the pain using a numeric pain scale (0–10) or verbal scale (no pain to worst pain). However, this becomes difficult in rodents. Therefore, new methods have been developed to assess spontaneous pain or nociceptive pain in rodents that include grimace scales and burrowing [137, 138].

Grimaces scale

To measure the intensity of pain, the facial expressions of mice can be used. Using the mouse Grimaces scale, five facial features are scored: nose bulge, cheek bulge, ear positioning, and orbital tightening. Orbital tightening is the narrowing of the orbital area and tightly closing or squeezing of the eyes. A nose bulge defines a bulge that is noticeable on the bridge of the nose, whereas a cheek bulge refers to the rounded projection of the cheek muscle compared to its typical appearance. Ear position denotes the ears being pulled back and apart from their standard position (may feature vertical ridges). Finally, whisker change describes the change in whisker position (maybe backward, forward, or clumped together). The more severe the severity of all these expressions, the more severe the pain is, and it is graded on a scale of 0-normal, 1-moderate, and 2-severe. This scale is highly accurate but needs several nociception to define response. A similar scale is also developed for rats that is used to evaluate pain based on scores of 0–2 depending on observed facial expressions [139].

Burrowing

Burrowing is a spontaneous, self-motivated behavior used to measure spontaneous or non-stimulus-evoked pain in mice and rats. A burrow filled with a substrate (food, pellets, sand, or marbles) is made from a long tube sealed at one end, secured, and lifted by screws on the other end to prevent non-burrowing behaviors from displacing the substrate inside. The burrows are placed in the rodent's cage for a pre-determined duration, and the amount of material displaced is weighed and recorded [136, 140].

2. Conclusions

A multitude of NP models have been created as a result of the identification of numerous mechanisms that eventually result in the development of NP. Because every model has unique characteristics, benefits, and limita-

tions, it is challenging to single out one as the best. The ease of use and high feasibility of surgical models make them popular. In order to produce better pain management based on each condition's unique pathogenesis, models such as drug-induced NP and disease-induced NP are helpful. Furthermore, new methods based on a variety of scientific disciplines have been developed recently; the optogenic method is based on light and molecular genetics. Despite the possibility that daily behavioral patterns in all models differ, a thorough understanding of the pathophysiology and distinguishable markers of NP is leading to the development of novel therapeutic interventions that are effective.

Author Contributions

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Conflict of interest

The authors declare no conflict of interest.

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