# Animal Models of Neuropathic Pain

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## 1 Introduction

In the recent history of neuromodulation period over half a century, the proof-based medical subspecialty is made. Its benefits are verified by improved pain relief, functional status, and health-related quality of life and low demand for healthcare resources. Neuromodulation is based on the innovative idea that paresthesia-inducing electrical stimulation could be analgesic. Its historic basis originates from Melzack and Wall's gate control theory of pain proposed in 1965. Neuromodulation has given us complete access to the systems of pain modulation and helped to understand the pathophysiology of pain. Neuropathic pain can be a consequence of an uncommon learning process which is associated with maladaptive plasticity of the central as well as peripheral nervous system. Various modifications of the peripheral nervous system have been defined in animal models of neuropathic pain, but their relation with human neuropathy symptoms are not fully understood. Mainly, neuropathic pain arises from injured myelinated fibers, abnormal activity in non-injured fibers, and also due to the more expression of

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calcium channels which results in more and more release of excitatory neurotransmitters and sympathetic propagation toward the spinal ganglia. Moreover, changes in the dorsal horn alter the activity of projections toward the brain stem and enhanced spinal hyperactivity. These effects are late, signifying the maintenance of spinal sensitization. These phenomena can convince the changes in the activity of thalamo-cortical networks through which independent processes are developed and maintain the pain. The change in the cortical body areas is the demonstration after nervous lesions, and these changes may relate with the emergence of pain.

Neuropathic pain can be produced by injury or disease of the somatosensory system. The clinical expressions of neuropathic pain vary including both stimulus-evoked and non-stimulus-evoked (spontaneous) symptoms. By pharmacological intervention, the beginning for allodynia and hyperalgesia in the several pain modalities can be modulated and measured in animals and humans. Animal models have been found valuable in studies and the treatment on neuropathic pain (Fig. 1).

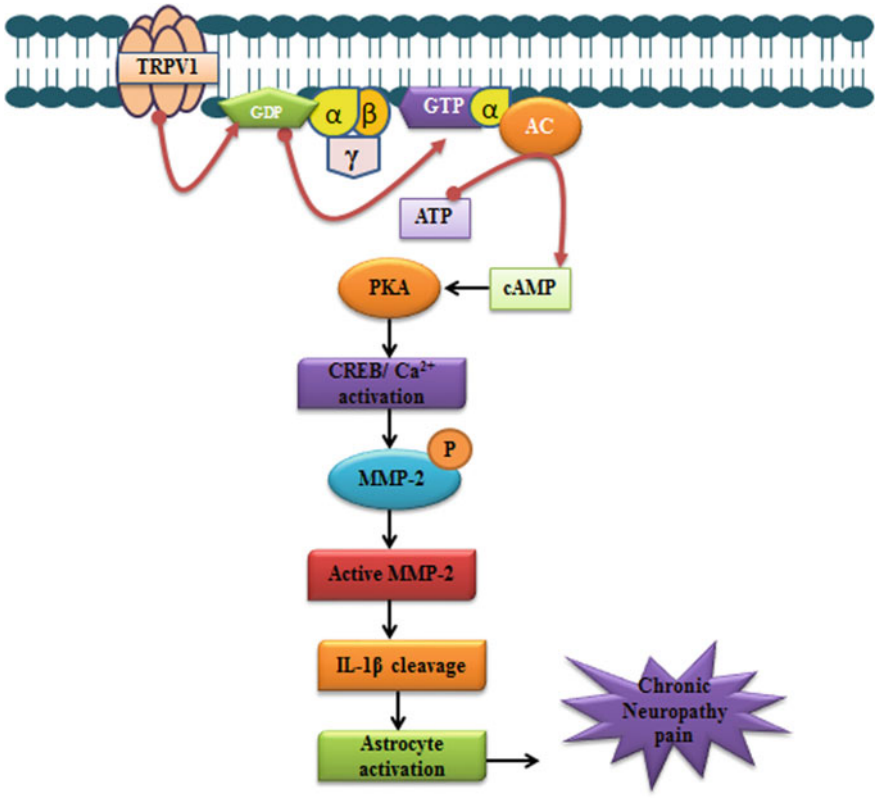


**Fig. 1** Proposed mechanism of MMP transcriptional regulation through TRPV1 signaling receptors involved in the formation of chronic neuropathic pain. TRPV1 modulates $Ca^{2+}$-dependent intracellular signaling pathways responsible to induce neuropathic pain. *TRPV* transient receptor potential cation channel, *PKA* protein kinase A, *cAMP* cyclic adenosine monophosphate, *CREB* cAMP response element-binding protein, *MMP* matrix metalloproteinase, IL-1β *Interleukin-1 beta*

## 2 Pathophysiology

Neuropathic pain arises through multiple and challenging pathophysiological mechanisms. In many peripheral and CNS diseases, neuropathic pain is a common problem. The peripheral nerve diseases that most usually cause distal symmetric peripheral neuropathies and focal neuropathies are related to trauma, as well as surgical interventions. Exemplary CNS diseases include multiple scleroses, spinal cord injury, and stroke causing neuropathic pain (Fig. 2).

Neuropathic pain has plethora of different mechanisms that extend from the periphery to the central nervous system where they involve the spinal cord, supraspinal, and descending modulation systems. Lesions exist anywhere within the central nervous system (CNS) or the peripheral nervous system (PNS) that can produce neuropathic pain. Nervous system lesions have played a main role to produce NP. Many types of pathological changes in peripheral mechanism such as ectopic discharges in lesioned fibers and their corresponding ganglia, alterations in the expression and regulation of intracellular $Ca^{2+}$ ion, sodium ion and modulatory

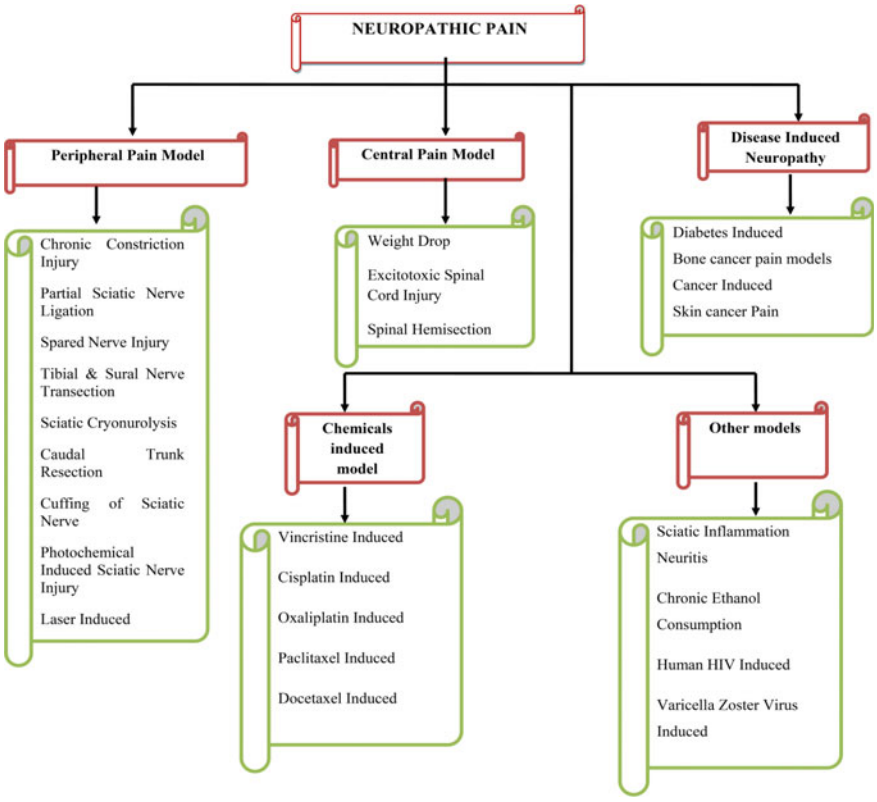


**Fig. 2** Classification of animal models of neuropathic pain

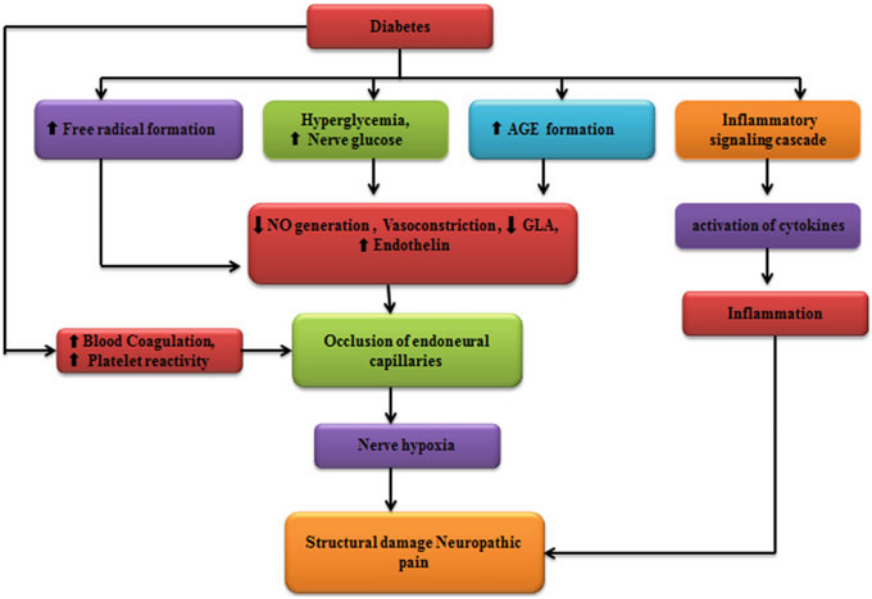


**Fig. 3** Possible mechanism for neuropathic pain induced by diabetes. *AGE* advanced glycation end product, GLA *gamma linolenic acid*

receptors on primary afferent terminals occur in peripheral axons and dorsal root ganglia after nerve lesions. Neuroimmune interactions resulting in altered production of inflammatory signaling molecules, sensory-sympathetic coupling, and other alterations in receptor signaling have also been described to play a role in the pathology of NP. On the other hand, in CNS neuropathic pain involves both spinal and supraspinal mechanisms. Hyperexcitability of second-order neuron, neuroinflammatory processes, formation of a glial scar-prevented axonal regeneration, selective neuronal loss, and failure of inhibitory mechanisms have been demonstrated after lesion in spinal mechanism in NP. But in supraspinal mechanism, there is alteration in nociceptive signals (Fig. 3).

## 3 Animal Models of Neuropathic Pain

Several models have been developed for different pain states, and the alteration of behavior has been interpreted as a response to external stimulus or expression of pain or discomfort. Animal models are a need in the study of neuropathic pain, and greatly of what we distinguish about pain comes from the studies in mice and rats. However, very few basic findings have been rendering so far from rodent models into effective pain therapy consequently; there is still a considerable need to discover novel treatment modalities. The evaluation of neuropathic pain in humans is

complex, because it is difficult to recruit the significant numbers of patients needed for a clinical trial. Thus, animal models provide advantage over humans to deveop and understand the mechanisms following neuropathic pain. The results in consistent sensory deficits (allodynia, hyperalgesia, and spontaneous pain) over a persistent period of time of animal models can be evaluated by sensory analysis. Animal models can be developed by establishing the degree of mechanical, chemical, and temperature induced allodynia and hyperalgesia, which help us to identify therapeutic potential of various pharmacotherapies (Table 1).

**Table 1** List of different animal models of neuropathic pain

| S. No | Name of model | Principle of injury | Species | References |
|---|---|---|---|---|
| 1. | Chronic constriction injury | Four loose ligatures around sciatic nerve | Female Sprague–Dawley rats | Bennett and Xie (1988) |
| 2. | Axotomy model | Complete sciatic nerve transection | Male Sprague–Dawley rats | Wall et al. (1979) |
| 3. | Partial sciatic nerve ligation | Tight ligation of one-third to half of sciatic nerve | Male Sabra rats | Seltzer et al. (1990) |
| 4. | Spared nerve injury | Axotomy of common peroneal and tibial nerves | Male Sprague–Dawley rats | Decosterd and Woolf (2000) |
| 5. | Tibial and sural nerve transection | Ligation of sural, common peroneal and tibial nerves | Wistar albino rats | Jain et al. (2009) |
| 6. | Sciatic cryoneurolysis | Freezing of the sciatic nerve | Wistar albino rats | De Leo et al. (1994) |
| 7. | Caudal trunk resection | Resection of caudal trunk between the S3 and S4 spinal nerves | Male Sprague–Dawley rats | Na et al. (1994) |
| 8. | Sciatic inflammatory neuritis | Applying Oxycel around the sciatic nerve | Male Sprague–Dawley rats | Eliav et al. (1999) |
| 9. | Cuffing of sciatic nerve-induced pain | Implanting a polyethylene cuff around the common branch of the sciatic nerve | Male Sprague–Dawley rats | Mosconi and Kruger (1996) |
| 10. | Photochemical-induced sciatic nerve injury | Thrombosis and occlusion in small vessels supplying sciatic nerve by photosensitizing dye and laser irradiation. | Male albino NMRI mice | Gazelius et al. (1996) |
| 11. | Laser-induced sciatic nerve injury | Epineurial vessels of nerve are irradiated with laser beam results in marked reduction in the blood flow to nerve | Sprague-Dawley rats | Meyer et al. (1985) |

(continued)

**Table 1** (continued)

| S. No | Name of model | Principle of injury | Species | References |
|---|---|---|---|---|
| 12. | Weight drop or contusive | Constant weight is dropped over the nerve to produce an injury | Male and female Long–Evans rats | Allen (1911) |
| 13. | Excitotoxic spinal cord injury | Intraspinal injections of excitatory amino acids | Male Sprague–Dawley rats | Brewer et al. (1999) |
| 14. | Spinal hemisection | Laterally hemisectioning spinal cord at T11–T12 laminae | Male Sprague–Dawley rats | Hains et al. (2002) |
| 15. | Vincristine-induced neuropathy | Direct injury of drugs to the nerves of peripheral nervous system | Male Sprague–Dawley rats | Pal (1999) |
| 16. | STZ-induced neuropathic pain | Persistent hyperglycemia-induced changes in the nerves by administering STZ | Mice | Freireich et al. (1966) |
| 17. | Chronic ethanol consumption-induced neuropathy | Administration of ethanol over extended period | Female Sprague–Dawley rats | Dina et al. (2007) |

## 3.1 Peripheral Pain Model

### 3.1.1 Chronic Constriction Injury (CCI)

Bennett and Xie established a model of peripheral mononeuropathy in rats by CCI to the sciatic nerve, which is most commonly working animal model of neuropathic pain. The success of the model was confirmed by identifying thermal and mechanical hyperalgesia. This constriction of the sciatic nerve is related to intra neural edema, focal ischemia, and Wallerian degeneration. The behavioral signs of spontaneous pain have been reported such as tingling, burning, electric-shock-like pain, dysaesthesia, mild-to-moderate autotomy, guarding, excessive licking, limping of ipsilateral hind paw, and avoidance of placing weight on the injury side (Jaggi et al. 2011). The CCI model produces unilateral peripheral mononeuropathy, and it has been observed that symptoms in this rat model correspond to causalgia or complex regional pain syndrome in patients (Bennett and Xie 1988).

**Procedure**

- Rats are anesthetized with sodium pentobarbital (40 mg/kg, intraperitoneal).
- The common sciatic nerve is exposed at the level of the middle of the thigh by blunt dissection through biceps femoris; 4 ligatures (4-0 chromic gut suture) are tied loosely around it with about 1-mm spacing.
- The incision is closed in layers. The success of the modeling is confirmed by detecting thermal and mechanical hyperalgesia (Di et al. 2014).

**Advantage**: Chronic constriction is the easiest model to develop neuropathic pain.
**Disadvantage**: There has been some degree of variation observed in the animals subjected to chronic constriction injury, which may complicate quantitative analyses.

### 3.1.2 Axotomy Model (Complete Sciatic Nerve Transection; Neuroma Model)

The model produces anesthesia dolorosa, i.e., pain in the area which lack any sensory input in that area. Autotomy (self-attack and mutilation of the denervated limb by injured animals) is observed in this model and often measured as assigns of neuropathic pain. Clinically, the more common systems of neuropathy comprise partial lesions to peripheral nerves.

**Procedure**

- Rat is anesthetized, and the common sciatic nerve is exposed.
- The connective tissue attached to the sciatic nerve is cleared off, and the sciatic nerve is tightly tied by nylon suture, proximal to its divergence into the tibial and the peroneal divisions, at two locations about 1-cm apart.
- The nerve is then completely transected between the pair of ligatures, and 5 mm of the nerve between the ligatures is removed to prevent the rejoining of nerves due to regeneration.
- The lesion to other adjacent saphenous nerve is also induced entire denervation of distal hind limb. Following the complete nerve transection, a neuroma develops at the proximal nerve stump consisting of regenerative nerves sprouting in all directions (Muthuraman et al. 2008).

**Advantage**: This model is useful to study the spontaneous pain in the area which lacks the sensory input in that area.
**Disadvantage**: A complete nerve transection or lesion is relatively uncommon in patients and is usually seen only after amputation such as phantom limb pain. Moreover, ethical considerations are also the key issues in this model as animals demonstrate excessive autotomy in this model.

### 3.1.3 Partial Sciatic Nerve Ligation (PSNL)

This model is developed by Seltzer et al. (1990) and is one of the more often employed models of neuropathy. The partial nerve injury models are relevant for understanding neuropathic pain injury, as the partial nerve injury is the main cause of pain disorders in humans.

**Procedure**

- PSNL is performed under intraperitoneal ketamine/xylazine (150 and 10 mg/kg, respectively) anesthesia.
- Briefly, the right sciatic nerve is exposed after the incision of skin and separation of the muscle.

- The sciatic nerve is freed of the adhering tissue gently for about 7 mm, and one ligature is made around approximately 1/3 and 1/2 diameter of the sciatic nerve.
- Great care is taken to tie the ligatures so that the diameter of the nerve is just barely constricted.

**Advantage**: This model is most relevant to clinical study because partial sciatic nerve injury is the main cause of pain disorders in human.
**Disadvantage**: The magnitude and duration of pain responses vary considerably depending on the suture material and strains

### 3.1.4 Spared Nerve Injury (SNI)

The 'spared nerve injury (SNI) model' of neuropathic pain was established by Decosterd and Woolf. Two versions of SNI injury of the sciatic nerve have been developed using the same surgical procedure, but with different combination of nerve transactions. In one variant, injury is given to the common peroneal and the sural nerves, sparing the tibial nerve (t) [SNIv(t)], while in the other versions, the tibial nerve is injured leaving the sural(s) and common peroneal (cp) nerves to intact (SNIv). Within 4 days of injury, mechanical and thermal hyperalgesia and allodynia are known to occur that persist for almost 24 weeks post-injury. The substantial changes seen in mechanical and thermal sensitivities closely mimic the clinical features of neuropathic pain.

**Procedure**

- Surgery is to be done under suitable anesthesia. Sciatic nerve is exposed in the thigh, and a section is made directly through biceps femoris muscle and its three terminal branches: the sural, common peroneal, and tibial nerves.
- Then, the peroneal and tibial nerves are tightly ligated with 5.0 silk thread.
- The surgical procedure is to be carried out cautiously to avoid any contact with or stretching of the intact nerve.

**Advantages**: This model closely mimics many of the features of clinical neuropathic pain. The changes in mechanical and thermal sensitivities in this model are robust, substantial, and persistent. The surgical procedure for creating this model is relatively easy as compared to previous models.
**Disadvantage**: The process of separation and ligation of the sural, common peroneal, and tibial nerves is tedious. So, it is difficult to achieve success in this model.

### 3.1.5 Tibial and Sural Nerve Transection

This is a new model of neuropathic pain developed by Lee et al. (2000). The majorities of these behavioral changes start from the third day of surgery, reach at peak in 1–2 weeks, and persist over a period of 1½ months. Clinically in this model, profound and stable neuropathic pain symptoms with mechanical allodynia, cold allodynia, chemical allodynia, mechanical hyperalgesia, and spontaneous pain have been recognized.

**Procedure**

- The rat is totally anesthetized with ketamine (60 mg/kg i.p.).
- The skin of its lateral surface of the left thigh is cut through the biceps femoris muscle to uncover the sciatic nerve and its trifurcation (the sural, common peroneal, and tibial nerves).
- After that, 2 mm sections of the tibial and sural nerve (distal to the trifurcation) are ligated and cut. The common peroneal nerve is left unligated.
- The muscle and the skin are closed in two layers.

**Advantage**: Tibial and sural nerve transection model system is clinically relevant, as it produces profound and stable neuropathic pain symptoms with mechanical, chemical and cold allodynia, mechanical hyperalgesia and spontaneous pain in experimental animals.
**Disadvantage**: Heat allodynia has not been demonstrated in this model of neuropathic pain.

### 3.1.6 Sciatic Cryoneurolysis

This model is an interesting animal model of peripheral neuropathy, as no transection or ligation is carried out. In its place, freezing of the sciatic nerve has been used to produce nerve injury (De Leo et al. 1994).

**Procedure**

- Surgical procedure is performed under inhalation of anesthesia using halothane in 100% $O_2$, induced at 4% and maintained at 2%.
- A segment (L 1.0 cm) of the common sciatic nerve proximal to its main trifurcation is shown by blunt dissection and suspended across forceps in the surgical opening.
- The nerve is lesioned in a 3-Obsec freeze, S-set thaw, 30-set freeze cycle using a 2-mm-diameter cryoprobe cooled to −60 °C with nitrous oxide as the refrigerant.
- The nerve is allowed to defrost and then returned to its normal position. With the help of surgical staples, the wound is closed and the animal recovered in room air.

**Advantage**: In this model, the nerve injury is produced by freezing the sciatic nerve, no need of transection or ligation.
**Disadvantage**: Autotomy not occurs immediately after the freeze lesion when the limb is dysfunctional.

### 3.1.7 Caudal Trunk Resection

In this model, left inferior caudal trunk of the rat is resected between the 3rd and 4th spinal nerves. There is the development of mechanical, cold, and warm allodynia in the tail durable up to several weeks, and signs of mechanical and thermal allodynia appear within a day after the nerve injury (Na et al. 1994).

**Procedure**

- Under sodium pentobarbital anesthesia (40 mg/kg, i.p.), the right superior caudal trunk is exposed and transected at the level between the 1st and 2nd spinal nerves that innervated the rat tail.
- To avoid the probable reunite of the proximal and distal ends of the severed trunk, a portion of the trunk, approximately 2 mm inside, is removed from the proximal end.
- This surgery eliminated the 1st spinal nerve innervation to the tail via the right superior caudal trunk.
- This model is easy to apply the mechanical stimulation with von-Frey hairs and to localize the sensitive area in the tail.

**Advantage**: Behavioral tests have to be performed on the tail, instead of the hind paw. Testing on the tail is easier and more consistent.
**Disadvantage**: The proximal and distal ends of the severed trunk may get reunite and can influence the outcomes.

### 3.1.8 Sciatic Inflammatory Neuritis (SIN)

The SIN model is an established model of neuropathic pain and is preferred by various research groups to impose damage to nerves. Within 3 h of injection, this method has been shown to produce mechanical allodynia, but no apparent thermal hyperalgesia. The development of mirror allodynia is the characteristic trait of this type of neuropathy. Clinical studies report that the major cause of 50% of human neuropathies is inflammation or infection rather than trauma. Moreover, the traumatic nerve injuries are also accompanied by various inflammatory events (Said and Hontebeyrie 1992).

**Procedure**

- The surgical procedure is to be carried out under sodium pentobarbital anesthesia (45 mg/kg, i.p., supplemented as necessary). The central sciatic nerves are exposed at the mid-thigh level by blunt dissection through the biceps femoris and separated from adjacent tissue.
- The nerve is enclosed on one side, in a band (approx. 3 mm wide and 25 mm long) of sterile hemostatic oxidized cellulose.
- The Oxycel is applied by passing curved forceps beneath the nerve (cautiously avoiding stretching of nerve), then grasping one end of the band and pulling it under the nerve.
- The grasped end is gently folded over the nerve, the other end is folded over in the opposite direction, and the excess is to be trimmed away.
- The Oxycel is proposed to act as a sponge. It is wrapped around the nerve with a loose knot and does not pose any harm or injury to nerve constriction.

**Advantage**: This model has shown to produce mechanical allodynia within 3 h of injection.
**Disadvantage**: This model cannot induce apparent thermal hyperalgesia.

### 3.1.9 Cuffing of Sciatic Nerve-Induced Pain

In this model, neuropathic pain is induced by surgically implanting a polyethylene cuff (2 mm in length, inner diameter 0.7 mm) around the common branch of the sciatic nerve of rats. Sciatic nerve cuffs are made from thick-walled polyethylene tubing. Internal diameters of 0.026″ and 0.028″ are formed by elongating commercially available 0.023″ tubing with reaming bits. Tubing is cut into either short (<0.5 mm) segments or longer segments of 2–5 mm in length (Mosconi and Kruger 1996).

**Procedure**

- Sciatic nerves are exposed in the thigh using a Zeiss surgical microscope and clear from the connective tissue by dissection with least contribution of the epineurial vasculature.
- Cuffs are applied to the nerve, and after a brief blanching, the epineurial vessels filled and flow remained intact through closure of the incision.
- Two to four cuffs were put at intervals approximately 0.5 mm, and sciatic nerve was enclosed at 3–6 mm.
- Wounds are closed in two layers, and the animals are permitted to recover before being returned to cages in the vivarium (Mosconi and Kruger 1996).

**Advantage**: The neuropathic pain model is characterized by heat hyperalgesia lasting for 3 weeks and mechanical allodynia lasting for 2 months.
**Disadvantage**: This model is difficult to establish.

### 3.1.10 Photochemical-Induced Sciatic Nerve Injury

The aluminum foil is placed under the nerve to isolate the nearby tissue and to reflect light. It has been proved that the nerve injury is not induced by the heat generated by the laser, but is a result of the photochemical reaction forming thrombosis and occlusion in small vessels supplying the nerve (Kupers et al. 1998).

**Procedure**

- Under isoflurane anesthesia, the left sciatic nerve is exposed at the mid-high level and dissected free from surrounding tissue.
- A strip of aluminum foil is placed under the nerve to reflect the laser beam. Immediately after injection of erythrosin B (32.5 mg/kg in saline, iv) into the tail vein, the part of the sciatic nerve just proximal to the trifurcation is irradiated with a laser with an output power of 100 mW at 532 nm.
- The laser beam is focused into a collimated fiber delivery system. A multimode fiber with a diameter of 200/220 m is used.

- The intensity of the laser light at the tip of the probe was approximately 70 mW. In order to determine the optimal exposure time, different groups received laser irradiation of 2-, 5- or 10-min duration.
- After the surgery, the wound is closed with clips.

**Advantage**: In this model, highly reproducible mechanical, heat, and cold allodynia and signs of spontaneous pain have been documented.
**Disadvantage**: In this model, the concomitant requirement of photosensitive dyes is essential.

### 3.1.11 Laser-Induced Sciatic Nerve Injury

In this model, the segment of the sciatic nerve just distal to the gluteus muscle is marked with epineurial sutures, and epineurial vessels of nerve are irradiated with laser beam of 1 mm diameter for 30 s (a diode-pumped solid-state laser operating at 532 nm with an output power of 100 mW). Laser irradiation results in marked reduction in the blood flow to nerve (Chiang et al. 2005).

**Procedure**

- Anesthetise the animals by using 1.5% isoflurane with a mixture of 50% oxygen and 50% nitric oxide by open-mask system at 1.5 l/min and the animal could be placed on a homeothermic blanket.
- A rectal thermal probe connected to a computer ensured that animal's body temperature remained at 37 °C for the duration of the surgery.
- The rat is placed in a prone position, and the left sciatic nerve is exposed at the level of mid-thigh.
- A thin plastic film is introduced under the nerve, on top of which a strip of aluminum foil (width 5 mm) is placed for reflection of the low-energy laser beam.
- A Q-switched diode pumped a solid-state laser of the L-series with an output power of 5 mW.
- The laser light, with a wavelength of 532 nm, is focused into a transmitter fiber with a diameter of 125 mm of a PF 418 probe.
- The sciatic nerve is exposed to laser irradiation for 20 min immediately after administration of erythrosin B (32.5 mg/kg, i.v.).
- Complete hemostasis is achieved before wound closure. Each surgery carried out for less than 60 min.

**Advantage**: On postoperative day 2, animals develop characteristic sign of neuropathic pain including spontaneous pain behaviors such as tingling, burning, thermal hyperalgesia, and mechanical allodynia. These phenomena peak during 7–21 days after surgery and last for 3–6 weeks.
**Disadvantage**: In this model, nerve injury is produced by laser, which is a time-consuming procedure for surgery (60 min).

## 3.2 Central Pain Models

### 3.2.1 Weight Drop or Contusive

Injury reproducibility is a significant characteristic of experimental models of spinal cord injuries (SCIs). In this model, the spinal cord at lower thoracic–lumbar level is exposed and a constant weight is dropped over the nerve to produce an injury, which is characterized by severe paraplegia and complete segmental necrosis. Clinically, the hypersensitivity to light mechanical stimulation of the skin has been noted to develop within 1 day of injury and parallels the allodynia experienced by patients rapidly following spinal injury (Allen 1911).

**Procedure**

- Spinal cord contusion or transection (TX) surgeries are performed under pentobarbital (50–70 mg/kg) anesthesia and prophylactic administration of gentamycin sulfate (1 mg/kg).
- The thoracic area of the rat is properly shaved, and prodine and alcohol are applied to the skin.
- During surgery, body temperature is maintained at 37 °C using either a Harvard homeothermic feedback-controlled heating pad or heated gel packs.
- Mid-to-low thoracic regions are incised. Laminectomy of the caudal portion of T9 and all of T10 exposed the spinal cord.
- For the TX group, the spinal cord is severed with microscissors and gentle aspiration. The ends of the cord distracted and the cavity are then carefully explored with a glass probe to cut any residual fibers.
- After determining that the TX is complete, the cavity is packed with gel foam. For contusion injuries, at position T8 and T11/12 spinous processes, spinal clamps are attached, a transducer is placed over the transverse process of T9, and the impact rod is centered above T10.
- The rod contacted with dura is determined by the completion of a circuit that activated a tone. The surgical site is sutured in layers, and an antibacterial spray is applied immediately after surgery.

**Advantage**: Clinically, the hypersensitivity to light mechanical stimulation of the skin has been noted to develop within 1 day of injury and parallels the allodynia experienced by patients rapidly following spinal injury.
**Disadvantage**: The surgical procedure used in this model is very complicated to achieve nerve injury.

### 3.2.2 Excitotoxic Spinal Cord Injury

This model simulate the elevations of excitatory amino acids (EAAs), a well-documented neurochemical change following spinal cord injury (SCI). The continuous pathological symptoms associated with metabotropic receptor agonist quisqualic acid QUIS injections closely resemble the cascade of events,

pathogenesis of ischemic, traumatic SCI, and cavities in the clinical condition of post-traumatic syringomyelia (Brewer et al. 1999).

**Procedure**

- Male Sprague–Dawley rats weighing 250–275 g are anesthetized with a mixture of ketamine, acepromazine, and rhombus (0.65 cc/kg, sc).
- Laminectomy-alone animals are also used as a control for the effects of surgery.
- Animals are placed in stereotaxic apparatus, and the spinal column is immobilized with a vertebral clamp.
- One intraspinal injection is made by laminectomy between spinal segments T12–L2 and the dura incised longitudinally and reflected bilaterally.
- A small hole for unilateral injections is made in the pia mater. QUIS, [125 mM], is injected unilaterally at three rostrocaudal levels (0.5 mm between tracks).
- Injections are made at a depth of 1000 mm below the surface between the dorsal root entry zone and dorsal vein.
- These parameters placed injections in the center of the gray matter between spinal laminae IV–VI.
- The total volume of QUIS or saline injected is 1.2 ml (0.4 ml per injection, delivered over a time interval of 60 s).
- A Hamilton microliter syringe with a glass micropipette extension (tip diameter 5–10 mm) is used for intraspinal injections. The syringe is positioned in a microinjector unit (Kopf 5000) attached to a micromanipulator.
- After injections, muscles are closed in layers and the skin is closed with wound clips (Brewer et al. 1999).

**Advantage**: This model has been shown to be a valuable model for studying the central mechanism of neuropathic pain.
**Disadvantage**: Dose dependently QUIS causes the toxicity of the neurons.

### 3.2.3 Spinal Hemisection

In this model, neuropathic pain like thermal and mechanical allodynia is established extensively not only in limbs but also in tail. Moreover, allodynia lasts for a long time following spinal cord hemisection (Kim et al. 2003).

**Procedure**

- Animals (175–200 g) are deeply anesthetized by intraperitoneal injection of sodium pentobarbital (40 mg/ kg), and the surgical field is shaved and prepared with Betadine.
- A longitudinal incision is made exposing several segments, and the left side of the spinal cord was laterally hemisected at T13 by the following procedure: Following the palpation of the dorsal surface to locate the rostral borders of the sacrum and dorsal spinous processes of the lower thoracic and lumbar vertebrae, the T11–T12 laminae are determined by locating the rib which attaches to the rostral end of the T13 vertebrae and counting two vertebral segments rostrally.

- The T13 spinal cord is laterally hemisected with a No. 11 scalpel blade without damage to the posterior spinal vessel or branches.
- Muscle and fascia are sutured and skin-closed with autoclips, and animals are allowed to recover on a 36.5 °C heating pad. Postoperative treatments included saline (1.0 cc sc) for rehydration and penicillin-G (0.35 ml/kg; i.m.) as a prophylactic antibiotic. Following the surgery, animals are maintained under the same preoperative conditions and the general health of the animals is carefully monitored.

**Advantage**: The numbers and types of effected fibers can be controlled at regular basis in each animal. The injured and intact sides are completely separated.
**Disadvantage**: The surgical procedure is difficult to achieve nerve injury.

## 3.3 Anticancer Agents-Induced Neuropathy Models

### 3.3.1 Vincristine-Induced Neuropathy

Vincristine is a widely employed chemotherapeutic agent for the treatment of several malignancies and primary brain tumors. It has been reported that clinical use of vincristine leads to the development of neurotoxicity of peripheral nerve fibers resulting in sensory-motor neuropathy. Among the various chemotherapeutic agents, vincristine produces consistent neurotoxicity in all the patients even at the therapeutic doses. Painful paresthesias are seen as the foremost symptom in major population of patients with this dose-dependent neuropathy.

**Procedure**

- Inject vincristine (0.1 mg/kg prepared with distilled water—1.0 mg/ml vincristine sulfate) intraperitoneally (0.1 mg/kg, diluted to 1 ml in saline) to rats in two 5-day cycles with 2 days break between cycles.
- In totality, 10 vincristine injections are to be given to rats on days 0–4 and 7–11 (Flatters and Bennett 2004).

**Advantage**: The use of vincristine has been associated with neurotoxicity of peripheral nerve fibers leading to sensory-motor neuropathy.
**Disadvantage**: It is difficult to develop vincristine-induced animal model of neuropathy.

### 3.3.2 Cisplatin-Induced Neuropathy

Cisplatin induces peripheral sensory axonal neuropathy disturbing large and small diameter sensory fibers. It generally causes clinical symptoms with a classic glove and stocking fashion after a cumulative dose of 300 mg/mm, and neuropathy may persist for long time (Markman 2003).

**Procedure**

- Cisplatin is administered intraperitoneally (i.p.) once a week at a dose of 5 mg/kg for 6 (36 days) weeks (cumulative dose: 30 mg/kg i.p.).
- Cisplatin is diluted in normal saline (0.9% NaCl) and delivered in a volume of 10 ml/kg bodyweight and given as i.p. injection.
- Injection is always performed after completion of mechanical and cold withdrawal testing.

**Advantage**: This model usually causes clinical signs and symptoms with a typical glove and stocking fashion after a cumulative dose of 300 mg/mm, and neuropathy may persist for years
**Disadvantage**: It has been very difficult to develop cisplatin-induced animal model of peripheral neuropathy, because of the development of nephrotoxicity before the development of neurotoxicity.

### 3.3.3 Oxaliplatin-Induced Neuropathy

Oxaliplatin is a key drug in the treatment of advanced metastatic colorectal cancer, but it causes acute peripheral neuropathy and chronic neuropathy. The elimination of $Ca^{2+}$ and oxalate chelate by oxaliplatin is one of the reasons for the neuropathy, and there is little behavioral evidence (Sakurai et al. 2009).

**Procedure**

- Oxaliplatin (1, 2 and 4 mg/kg) or sodium oxalate (1.3 mg/kg) is administered i. p. twice a week for 4 weeks (on days 1, 2, 8, 9, 15, 16, 22 and 23).

**Advantage**: Oxaliplatin-induced neuropathy model is used to study both peripheral and chronic neuropathy.
**Disadvantage**: This method is slight difficult to set up.

### 3.3.4 Paclitaxel-Induced Neuropathy

Conventionally, the mechanisms underlying the pathogenesis of taxane-induced peripheral neuropathy (TIPN) include disruption of microtubule-based axonal transport, macrophage activation, and microglial activation in peripheral nerve as well as inside the spinal cord (Argyriou et al. 2008). As a result of the problematic signal transduction, there is evidence of a 'dying back' process initially affect the distal nerve endings followed by Schwann cells and neuronal bodies, or disturbed axonal transport changes in the affected neurons (Peters et al. 2007).

**Procedure**

- In this experimental model of neuropathy, low dose of paclitaxel (1 or 2 mg/kg i.p) has been reported to evoke pain syndrome without inducing systemic toxicity or motor impairment in rodents.

- Paclitaxel administration on four alternate days (days 0, 2, 4, and 6, with cumulative dose of 4 or 8 mg/kg) has been noted to produce peripheral neuropathy characterized by cold allodynia, long-lasting tactile (mechanical) allodynia, and endoneuronal edema of the sciatic nerve.

**Advantage**: TIPN model for neuropathic pain not affects the structure of internodal myelin in peripheral nerves.
**Disadvantage**: Sensory symptoms commonly start symmetrically in the feet, but after sometimes, it appears simultaneously in both hands and feet. With long-term dosing, the painful symptoms rise in severity and may include loss of vibratory sensation.

### 3.3.5 Docetaxelinduced Peripheral Neuropathy

Docetaxel-treated rats have been shown to exhibit reduced tail nerve conduction velocity, thermal threshold changes, and degeneration of skin nerves in the food pad.

**Procedure**

- Docetaxel-induced neuropathy model has been developed in which weekly i.v. injection of docetaxel (5; 10 or 12.5 mg/kg) for 4 weeks induces neuropathy in rats (Jaggi et al. 2011).

## 3.4 Disease-Induced Neuropathy Pain

### 3.4.1 Diabetes-Induced Neuropathy (STZ-Induced Neuropathic Pain)

Peripheral diabetic neuropathy (PDN) is a shocking complication of diabetes and a leading cause of foot amputation. Animals develop other metabolic derangements along with hyperglycemia including ketoacidosis, alterations in lipid metabolism, and general physical debility. Some of these symptoms complicate the interpretation of data in studies of nociception. It has been reported that general debility rather than peripheral neuropathy may underlie the altered measures of nociception in rats with STZ-induced diabetes. STZ has also been employed to induce diabetic neuropathy in mice (Jaggi et al. 2011) (Table 2).

**Procedure**

- Rats are usually made diabetic with a single dose of STZ (40–60 mg/kg, depending on bodyweight after an overnight fast to reduce competition between glucose and STZ for uptake into β-cells.
- In mice, a total dose of 180–200 mg/kgSTZ, given across 1 or 2 injections, can be used, with the higher dose reflecting correction for surface area and a degree of trial and error.

**Table 2** Diabetic neuropathy

| S. No | Anesthesia Dose | Route | Principle of injury | Species | References |
|---|---|---|---|---|---|
| 1. | STZ (180 mg/kg) | i.p. | Persistent hyperglycemia-induced changes in the nerves | Mice | Freireich et al. (1966) |
| 2. | STZ (40 mg/kg) | i.p. | Persistent hyperglycemia-induced changes in the nerves | Male Sprague–Dawley rats | Calcutt (2004) |

- High doses of STZ can initially produce a severe hyperinsulinemia as insulin is released from degenerating β-cells, followed by marked insulinopenia.
- Both of these phenomena can cause early death in a substantial proportion of a cohort of mice unless mitigated by first sugar, then insulin, replacement.
- To counter high cohort mortalities in STZ-injected mice, lower doses of STZ given daily for anywhere from 2 to 5 days may be used to induce diabetes.
- Such multiple low-dose regimens produce a less aggressive hyperinsulinemia and subsequent insulinopenia that allow animals to survive for months.
- Nevertheless, these regimens may also produce a mild and inconveniently slowly developing neuropathy, with some animals reverting to normoglycemia over time.

**Advantage**: Low cost, quick induction of diabetes, conviction of duration of diabetes, and a broad background literature.
**Disadvantage**: Uncontrollable degree of B cell death and subsequent insulinopenia that can lead to between-study variability in the neuropathy phenotype.

One of the caveats that occasionally raised for this model has direct STZ toxicity to organs and is a particular concern for nephropathy studies. However, it is not a factor in the onset of neuropathy.

## 3.5 Other Models

### 3.5.1 Post-herpetic Neuralgia Model (Varicella Zoster Virus-Induced Neuropathy)

VZV is an infectious human alpha herpesvirus that causes chickenpox and is neurotropic, but this virus becomes dormant in neurons of the dorsal root ganglia (DRG) and trigeminal ganglia (TG). It may undergo reactivation, after a specific latent period, and induces herpes zoster (shingles) characterized by painful vesicular rash that appear along the sensory dermatomes. Post-herpetic neuralgia (PHN) is the major complication of zoster that triggers severe pain in the affected dermatome which persists for almost 3–4 months. Further, ganglionic replication is caused by processes such as focal hemorrhage, demyelination, degeneration of axons, necrosis of sensory fibers, and supporting cells. The afferent nerve fibers that innervate the

damaged skin show impaired function and degeneration of posterior nerve along with sensory roots, further extending the damage to adjacent regions of spinal cord and brain stem in the central system. This damage may affect the sensory and ganglionic nerves triggering abnormal afferent pain signals that add to the nociceptive signals originating from the lesions themselves (Kennedy et al. 2013).

**Procedure**

- VZV (strain Dumas) is propagated on fibroblast (primary human embryonic lung) cells and harvested when cells exhibit 80% cytopathic effect (cpe) (equivalent to $10^4$–$10^5$ plaque forming units).
- Inject 50-μl viral inoculums subcutaneously into the mid-plantar glabrous footpad of the left hind limb.

### 3.5.2 Human Immunodeficiency Virus (HIV)-Induced Neuropathy

HIV-1 interacts with the nervous system through binding of gp120 (external envelope protein) to CXCR4/CCR5 (chemokine receptors) that is present in neurons and glial cells resulting in peripheral axonal damage and neurotoxicity. HIV-associated sensory neuropathy is a major complication of HIV infection and highly active antiretroviral therapy (HAART), and clinical data also reveal that 50% of HIV patients are suffering from such neuropathies. It has been evidenced that HIV sensory neuropathy is triggered by pro-inflammatory molecules such as MAP kinase, tumor necrosis factor-α (TNF-α), stromal cell-derived factor 1-α (SDF1-α), and C-X-C chemokine receptor type 4 (CXCR4). However, the exact mechanistic aspect of painful HIV sensory neuropathy is not clear.

**Procedure**

- After anaesthetizing the rat with 1–2% isoflurane in 1% $O_2$ and 1% $N_20$, expose the left sciatic nerve to gp120 by placing a 5 mm × 2 mm piece of gel foam soaked in saline containing 200 ng HIV-1 gp120-MN in direct contact with the nerve, to form a pool of protein solution around the nerve that is left in place for 30 min.
- Then, the oxidized regenerated cellulose, previously soaked until saturation in the same saline-gp120 solution, is wrapped loosely around the sciatic nerve 2–3 mm proximal to the trifurcation so as not to cause any nerve constriction and left in situ.
- The nerve is gently manipulated back into place and skin incisions closed with 4/0 silk sutures.

**Table 3** Alcohol induced hyperalgesia

| S. No | Dose | Route | Principle of injury | Species | References |
|---|---|---|---|---|---|
| **1** | 6.5% ethanol | Oral | Administration of ethanol over longer period (12 weeks) | Female Sprague–Dawley rats | Dina et al. (2007) |
| **2** | Ethanol (6.5%) | Oral | Administration of ethanol for 3 weeks | Male Sprague–Dawley rats | Ferrari and Levine (2010) |

### 3.5.3 Chronic Ethanol Consumption/Withdrawal-Induced Neuropathy

The alcohol diet (6.5% ethanol v/v in Lieber-DeCarli formula) induced hyperalgesia with more quick onset and severity in females. Continuing alcohol consumption has been reported to induce small-fiber painful neuropathy characterized by distal axonal degeneration, i.e., dying back neuropathy. Although early use of alcohol leads to some extent of analgesia, yet over a period of time induction of pain outweighs analgesia and results in a neuropathic pain with sign and symptoms that have been described as like tearing flesh off the bones (Jaggi et al. 2011) (Table 3).

**Procedure**

All experimental rats are fed an ethanol-containing Lieber-DeCarli liquid diet (ED, 6.5% ethanol) daily over a period of 12 weeks (Dina et al. 2007).

## 4 Conclusion

Various animal models have been established and described for neuropathic pain that show predictive value for both humans and animals; still the exact pathophysiological mechanisms for the development, maintenance, and progression of the neuropathic pain syndrome are not yet clearly revealed. Although the most widely employed models still work on the principle of nerve ligation, other models featuring transaction of nerve branches of peripheral nerves are also advantageous and more frequently used currently. Models involving spinal hemisection and excitotoxin-induced SCI are preferred for conceptualizing central pain mechanisms. Furthermore, other models that make the use of chemotherapeutic agents, HIV, ethanol, and others have also been employed for the better understanding of pathogenetic mechanisms and treatment strategies for pain amelioration.

**Ethical Statement**

All institutional guidelines, national guidelines, state and local laws and regulations with professional standards for the care and use of laboratory animals should be followed. Studies involving animals must state that the institutional animal ethical committee has approved the protocol. For authors using experimental animals, a statement should be made that the animals' care is in accordance with institutional

guidelines and animals used have been treated humanely and with regard for the alleviation of suffering. Researchers should treat animals as sentient and must consider their proper care and use and the avoidance or minimization of discomfort, distress, or pain as imperatives. Animal experiments should be designed only after due consideration of animal health. It should be ensured that all researchers who are using animals have received instruction in research methods and in the care, maintenance, and handling of the species being used. All the surgical procedures should be performed under appropriate anesthesia and follow only those procedures which avoid infection and minimize pain during and after surgery.

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