



Review Article

Animal Models and Behavioral Assessment for Chronic Pain Diseases

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Abstract

Chronic pain diseases (CPDs) affect approximately one in five adults worldwide, imposing a substantial socioeconomic burden and remaining a major unmet clinical need in pain medicine. Animal models of chronic pain are indispensable pre-clinical tools for dissecting the pathogenic mechanisms underlying diverse CPDs, screening novel analgesic candidates, and bridging fundamental laboratory discoveries and clinical pain management. An ideal experimental animal model should faithfully recapitulate the core clinical manifestations, pathological alterations, and persistent pain phenotypes observed in human CPD patients, while also enabling reproducible, standardized, and multidimensional behavioral assessment of pain hypersensitivity and spontaneous pain. Nevertheless, no single existing model can fully reproduce the full spectrum of clinical features of human CPDs, given the heterogeneous etiologies and complex biopsychosocial components of chronic pain. Therefore, investigators have to rationally select appropriate animal paradigms according to concrete scientific hypotheses and specific pain subtypes. This review systematically summarizes the detailed establishment workflows, typical pain-related behavioral phenotypes, major merits and inherent limitations, practical selection principles, as well as standardized behavioral evaluation approaches of widely-adopted animal models covering neuropathic pain, inflammatory pain, cancer pain, visceral pain and musculoskeletal pain. It is expected to offer practical reference information for pain researchers to reasonably choose and properly apply chronic-pain animal models in pre-clinical pain investigations, and ultimately facilitate the translational success of therapeutic approaches including pharmaceutical agents and interventional therapeutic techniques.

Keywords

Chronic Pain, Animal Model, Behavioral Assessment, Neuropathic Pain, Inflammatory Pain

1. Introduction

Chronic pain diseases (CPDs) refer to chronic disorders characterized by chronic pain (persistent or recurrent for more than 3 months) as the main clinical manifestation. The 11th Revision of the International Classification of Diseases (ICD-11) of the WHO classifies chronic pain into seven categories [1]. Compared with acute pain, chronic pain loses the warning function of tissue damage and is classified as a chronic disease [2, 3].

An ideal animal pain model should maximally simulate the clinical characteristics of human CPDs, especially reflecting the element of pain duration. Because the pain behavioral manifestations of animals need to persist for weeks or even months to simulate the clinical characteristics of human CPDs [4]. However, no single animal pain model can fully simulate

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all clinical manifestations of CPDs at present. Therefore, researchers need to select appropriate models based on specific scientific questions and the types of CPDs to be simulated, and interpret the research results cautiously.

Currently, commonly used animal models of chronic pain in pain research mainly cover neuropathic pain, inflammatory pain, cancer pain, visceral pain, and musculoskeletal pain. This paper systematically reviews the construction methods, pain behavioral characteristics, main advantages and limitations, key points for model selection, and pain behavioral assessment methods of commonly used animal models in the field of pain research, aiming to provide a reference for researchers in this field to select animal models.

2. Neuropathic Pain Models

2.1. Peripheral Nerve Injury Models

Peripheral neuropathic pain is chronic pain caused by local damage or lesions of the somatosensory nervous system, with typical phenotypes including both spontaneous pain (such as burning, stabbing, and electric shock-like pain) and stimulus-evoked pain (such as allodynia and hyperalgesia). Currently, in the research of peripheral neuropathic pain, models constructed based on peripheral nerve injury are widely used, including chronic constriction injury (CCI), spared nerve injury (SNI), spinal nerve ligation (SNL), and chronic constriction injury to infraorbital nerve (CCI-ION) models.

2.1.1. CCI Model

The CCI model was first established by Bennett and Xie in 1988 by loosely ligating the sciatic nerve to simulate chronic pain caused by peripheral nerve compression [5]. Model animals mostly exhibit typical pain behavioral phenotypes such as spontaneous pain, mechanical allodynia, and thermal hyperalgesia.

Modeling Procedure:

- 1) *Experimental Preparation:* Adult rats/mice are used and adaptively fed for 7 days. Prepare 4-0 chromic catgut, sterile surgical instruments, isoflurane for anesthesia, normal saline, antibiotics, etc.
- 2) *Anesthesia and Skin Preparation:* Inhaled isoflurane general anesthesia (induction 5%, maintenance 2%-3%). Rats are fixed in prone position, hair from the right buttock to the back of the thigh is removed, and the area is disinfected repeatedly with 75% ethanol and iodophor for 3 times.
- 3) *Nerve Exposure:* A longitudinal incision (1.5-2 cm) is made along the buttock to the back of the thigh, the biceps femoris is bluntly dissected to expose the sciatic nerve trunk (from the inferior border of the piriformis muscle to the nerve branch), and the surgical area is moistened with normal saline to prevent nerve dry injury.
- 4) *Nerve Ligation:* The sciatic nerve trunk is loosely ligated

3-4 times at 1 mm intervals with saline-soaked catgut, and the force is controlled to slightly indent the nerve surface to ensure tightness - clear visualization of veins in the epineurium without obvious congestion indicates appropriate ligation; obvious local venous congestion indicates over-ligation.

- 5) *Postoperative Treatment:* The surgical area is rinsed with saline, muscles and skin are sutured in layers, erythromycin ointment is applied to the wound, penicillin (50,000 U/kg) is injected intraperitoneally, and animals are returned to the feeding cage after full recovery.

Precautions:

- 1) *Ligation Force:* Ligation force is critical to model success; over-ligation may cause motor nerve injury and limb paralysis, while under-ligation may lead to model failure due to ineffective nerve compression. Novices are recommended to gain experience through pre-experiments to ensure consistent ligation force in the same batch of experiments.
- 2) *Nerve Protection:* Blunt dissection is used throughout nerve separation to avoid nerve damage with sharp instruments; the surgical area is always kept moist with normal saline to prevent irreversible nerve injury due to dehydration.
- 3) *Postoperative Observation:* Observe the diet, activity, and wound healing of rats for 3 consecutive days; animals with bleeding, infection, or hind limb paralysis should be excluded from the model.
- 4) *Experimental Conditions:* Rats or mice of the same strain and similar weight should be selected for the same batch of experiments, and environmental conditions should be controlled: 12-hour light/dark cycle per day, room temperature maintained at 22-25 °C, to minimize interference from environmental factors.

Advantages and Limitations:

Advantages: The CCI model well reproduces a series of typical pain phenotypes caused by chronic nerve compression in sciatica and other diseases induced by various clinical conditions; mechanical allodynia, thermal hyperalgesia, and spontaneous pain appear on the 6th day after surgery, and symptoms persist for weeks to months.

Limitations: Ligation tightness is difficult to control, leading to variations in nerve compression degree and pain behavior among different individuals and laboratories.

2.1.2. SNI Model

The SNI model, a neuropathic pain model proposed by De Coster and Woolf in 2000 [6], induces neuropathic pain by injuring part of the branches of the sciatic nerve. Construction of the classic SNI model requires exposing the three major branches of the sciatic nerve (tibial, common peroneal, sural nerves), ligating and cutting two branches, and preserving one intact. Two common methods are available: one is preserving the sural nerve and cutting the common peroneal and tibial nerves; the other is preserving the tibial nerve and cutting the

common peroneal and sural nerves. A simplified method uses 6-0 sutures to ligate the common peroneal and sural nerves together, cutting the nerve 2-4 mm distal to the ligation line, avoiding traction on the tibial nerve, significantly shortening the operation time [7].

Modeling Procedure:

- 1) *Experimental Preparation:* Same as the CCI model.
- 2) *Anesthesia and Skin Preparation:* Same as the CCI model.
- 3) *Nerve Exposure:* A 1.5–2 cm longitudinal incision is made along the buttock to the back of the thigh, the biceps femoris is bluntly dissected to expose the main trunk and three major branches of the sciatic nerve: tibial, common peroneal, and sural nerves. The nerve is kept moist with saline continuously to prevent drying.
- 4) *Nerve Ligation:* A glass needle is used to lift the tibial and common peroneal nerves, which are then ligated separately with 6-0 nylon sutures (appropriate force to slightly thin the nerve diameter). The nerve is cut 2 mm distal to the ligation site, and 1–2 mm of the nerve is removed to prevent regeneration. The sural nerve is kept undisturbed as much as possible.
- 5) *Postoperative Treatment:* Same as the CCI model.

Precautions:

The sciatic nerve branches are easily confused at the trifurcation; they can be distinguished by functional localization: the tibial nerve innervates plantar muscles, the common peroneal nerve innervates dorsal extensor muscles of the foot, and the sural nerve innervates lateral foot sensation.

Advantages and Limitations:

Advantages: SNL is regarded as the gold-standard model for simulating post-traumatic neuralgia and chronic post-surgical neuralgia. It induces significant mechanical allodynia with almost no thermal hyperalgesia and has little impact on motor function, so it is mainly used for analgesic drug screening and mechanism research.

Limitations: This selective branch injury is rare in clinical practice; the model is relatively "artificial" with a single pain phenotype, mainly characterized by intense mechanical allodynia without obvious thermal hyperalgesia, inconsistent with diverse clinical manifestations.

2.1.3. SNL Model

First proposed by Kim and Chung in 1992 [8], the SNL model remains a classic animal model of neuropathic pain. Selective ligation of the lumbar spinal nerve (L5) causes axonal injury and peripheral nerve inflammation, inducing long-term mechanical allodynia, thermal hyperalgesia, spontaneous pain, and other abnormal pain sensations, simulating the pathological characteristics of peripheral neuropathic pain caused by clinical peripheral nerve injuries (such as intervertebral disc herniation, nerve entrapment, post-surgical neuropathy). Some scholars ligate both L5 and L6 spinal nerves to make the model closer to peripheral neuropathic pain characteristics, enhancing phenotypes such as reduced mechanical pain

threshold and more severe spontaneous pain.

Modeling Procedure:

- 1) *Experimental Preparation:* Same as the CCI model.
- 2) *Anesthesia:* Same as the CCI model.
- 3) *Surgical Area Preparation:* Rats are fixed prone on the operating table, and hair on the lumbar (L4-L6) back is shaved.
- 4) *Surgical Incision:* A 1.5-2 cm longitudinal incision is made along the midline of the rat's back from the upper edge of the iliac crest to the cephalic side, cutting through the skin and subcutaneous fat in sequence; the erector spinae muscles on both sides are bluntly dissected with hemostats (no sharp dissection to avoid bleeding), exposing the L5 transverse process (the large L5 transverse process is an important anatomical marker).
- 5) *Spinal Nerve Exposure:* Microforceps are used to gently remove connective tissue on the surface of the L5 transverse process, and a small bone rongeur is used to carefully bite off part of the L5 transverse process (only bone is removed to avoid damaging the underlying nerve), exposing the L5 spinal nerve (single nerve bundle, diameter 0.5-1 mm) running below the transverse process.
- 6) *Nerve Ligation:* A glass needle is used to lift the L5 spinal nerve, and a 6-0 nylon suture is placed 5 mm distal to the spinal ganglion for tight ligation. The ligation force is to narrow the nerve diameter by 1/3-1/2, ensuring axonal compression without immediate rupture (over-ligation easily leads to complete nerve transection and shortened pain phenotype duration; under-ligation results in model failure).
- 7) *Postoperative Treatment:* Same as the CCI model.

Precautions:

- 1) *Accurate Localization of L5 Spinal Nerve:* The rat L5 transverse process is 0.5 cm from the iliac crest; do not bite too deeply when removing the L5 transverse process to avoid damaging the underlying spinal ganglion or spinal cord (cerebrospinal fluid overflow indicates dural injury, and further operation should be stopped).
- 2) *Avoid Excessive Nerve Injury:* Soak the ligation suture in normal saline first to avoid dry irritation to the nerve; ligate slowly with microforceps without causing congestion of blood vessels on the nerve surface (over-ligation causes acute nerve necrosis and unstable postoperative pain phenotypes).
- 3) *Bleeding Control:* The lumbar erector spinae muscles are rich in blood supply and prone to small vessel bleeding during blunt dissection; sterile gauze compression is generally used for hemostasis (hemostat use is prohibited to avoid tissue damage); electrocoagulation (low power) is used for hemostasis if blood vessels accompanying the spinal nerve are injured.
- 4) *Postoperative Pain Management:* Buprenorphine (0.1 mg/kg) can be injected intraperitoneally once 24 hours after surgery; prolonged administration is avoided to

prevent affecting the pain phenotype of the model.

- 5) *Observe Motor Dysfunction*: After unilateral L5 spinal nerve ligation, mild foot drop is observed in the operated hind limb of the animal, but the animal can walk and eat; models with severe paralysis (inability to stand) should be excluded.

Advantages and Limitations:

Advantages: Simulates the clinical manifestations of radicular pain caused by lumbar disc herniation or spinal stenosis compressing nerve roots; mechanical allodynia and spontaneous pain appear early and are severe.

Limitations: High surgical difficulty, large trauma, and high postoperative mortality.

2.1.4. CCI-ION Model

The CCI-ION model, a modified facial neuropathic pain model based on the classic CCI model, was established by Vos et al. in 1994 [9]. Its core mechanism is to induce axonal degeneration, myelin injury, and peripheral nerve inflammation through controllable constriction injury of the infraorbital nerve, thereby simulating the core symptoms of clinical trigeminal neuralgia (especially the second branch of trigeminal neuralgia) and post-traumatic facial neuropathy.

Modeling Procedure:

- 1) *Experimental Preparation*: Same as the CCI model.
- 2) *Anesthesia*: Intraperitoneal injection of 2% pentobarbital sodium at 50 mg/kg.
- 3) *Surgical Area Preparation*: Rats are fixed supine on the operating table, head turned to the contralateral side (exposing the operated facial area), hair is shaved along the nasal side to the infraorbital region, and disinfected alternately with iodophor and 75% alcohol for 3 times.
- 4) *Incision and Tissue Dissection*: A 1.0–1.5 cm longitudinal incision is made along the right lateral nasal margin of the rat (below the line connecting the nasal ala and medial orbital angle), cutting through the skin, subcutaneous fat, and levator labii superioris muscle. Microforceps are used for blunt dissection to expose the infraorbital foramen (Anatomical landmark: located approximately 0.5 cm below the medial aspect of the orbit, a small oval-shaped foramen about 0.8 mm in size, through which the infraorbital nerve exits).
- 5) *Nerve Exposure*: A glass dissector is used to gently lift connective tissue around the infraorbital foramen and slowly separate the infraorbital nerve exiting the foramen (milky white nerve bundle, single or double branches, running in subcutaneous tissue toward the nasal tip and upper lip); expose ~5–8 mm of the nerve, keeping it moist with normal saline continuously to avoid dry injury.
- 6) *Nerve Ligation*: 5-0 chromic catgut or 6-0 nylon suture is selected to loosely ligate the nerve 2–3 mm distal to the infraorbital foramen. The ligation force is to slightly indent the nerve diameter without obvious congestion of blood vessels on the nerve surface, ensuring incomplete

blockage of nerve blood flow but chronic compression of axons.

- 7) *Postoperative Treatment*: Same as the CCI model.

Precautions:

- 1) *Accurate Localization of Infraorbital Foramen*: The infraorbital foramen is the only anatomical marker for exposing the infraorbital nerve; in rats, it is located at the intersection of 0.5 cm downward from the medial orbital angle and 0.3 cm lateral to the nasal ala.
- 2) *Avoid Excessive Nerve Injury*: Ligation force is critical; over-ligation causes acute ischemic necrosis of the nerve and short pain phenotype duration; under-ligation results in model failure with no pain phenotype.
- 3) *Bleeding Control*: Same as the SNL model.

Advantages and Limitations:

Advantages: Specifically used as a model for facial pain (especially trigeminal neuralgia), it well simulates facial mechanical allodynia—the most prominent clinical manifestation of trigeminal neuralgia patients, i.e., tactile allodynia induced by facial touch.

Limitations: Small surgical space (especially in mice), high difficulty in exposing and ligating the infraorbital nerve.

2.1.5. Peripheral Nerve Injury Model Selection Guide

The SNI model is mainly used for drug screening and pain mechanism research due to its stable phenotype and high pain intensity; the SNL model is suitable for radicular pain research; the CCI model is suitable for studying chronic pain caused by nerve entrapment; the CCI-ION model is targeted at research on trigeminal neuralgia and other orofacial pain.

2.2. Postherpetic Neuralgia (PHN) Models

Postherpetic neuralgia (PHN) is a common clinical chronic neuropathic pain syndrome; pain lasting more than one month after herpes zoster rash healing is clinically defined as PHN. Its core pathogenic factor is varicella-zoster virus (VZV) infection. After primary infection, the virus induces varicella, and after varicella healing, the virus remains latent in the dorsal root ganglia; when the body's immune function declines, the latent virus is reactivated and proliferates, causing herpes zoster. The clinical symptoms of PHN are complex and diverse, usually limited to the skin area of the initial herpes outbreak, most commonly a unilateral zosteriform area on the trunk. Patients may present with persistent pain (burning, sharp, stabbing, or deep pain), allodynia (pain induced by slight skin touch), paresthesia (itching, numbness, or sensory loss), and emotional disorders (insomnia, depression, etc.) due to long-term severe pain. This section introduces three commonly used PHN animal models: virus-induced models (including VZV inoculation model and HSV-1 alternative model) and resiniferatoxin (RTX)-induced model.

2.2.1. VZV Model

First established by Fleetwood-Walker's team in 1999 [10], this model was optimized in 2004 by adjusting VZV-infected cell types and inoculation methods, prolonging chronic pain duration to 60-100 days, closer to the chronic clinical course of PHN [11].

Modeling Procedure:

- 1) *Experimental Preparation:* Male SD rats/mice are selected and adaptively fed for 7 days. Cell-associated active human VZV strains (such as VZV1587, VZV1755) or GFP-labeled strains for observation are used; virus titer is adjusted to 2×10^5 PFU, with GFP-positive cells accounting for >70%.
- 2) *Baseline Detection:* Mechanical paw withdrawal threshold and thermal hyperalgesia threshold of both hind paws of rats are measured one week before virus inoculation and recorded as normal baselines for comparison after pain abnormalities occur.
- 3) *Inoculation Operation:* 100 μ l of VZV-infected cell suspension is injected subcutaneously into the unilateral hind paw pad or periorbital facial area; control groups are injected with equal volume of uninfected cell suspension or UV-inactivated VZV solution respectively.
- 4) *Model Verification:* Pain behavioral tests are performed weekly after surgery; the model is successfully constructed if rats develop allodynia 3 days after inoculation, persisting for more than 60 days, and VZV early regulatory proteins (such as IE62, IE63) are detectable in the dorsal root ganglia.

Precautions:

- 1) *Virus-related:* Cell-associated active VZV must be used; UV-inactivated virus cannot induce pain abnormalities in rats. Pre-experiments are required to verify virus activity; virus leakage must be avoided, and all operations must be performed in a BSL-2 laboratory.
- 2) *Inoculation Operation:* The hind paw pad is preferred as the inoculation site; facial or periorbital areas can be selected for constructing facial postherpetic neuralgia models. Rats of the same batch, same side, and same injection dose are used to minimize errors caused by individual differences.
- 3) *Postoperative Observation:* In addition to pain testing, record food intake, mental state, and skin condition at the inoculation site; female rats have longer pain duration and higher sensitivity, so male rats of the same batch, same weight, and same gender are generally used unless experimental requirements specify otherwise.
- 4) *Experimental Control:* Blank control group (blank cell suspension) and negative control group (inactivated virus) are set to exclude the effects of simple tissue injury and inflammation.

Advantages and Limitations:

Advantages: Uses VZV, the real pathogen of human herpes zoster, to directly study viral latency, reactivation, and inter-

action with the host nervous system. After successful modeling, animals exhibit long-term mechanical allodynia and spontaneous pain, consistent with the core clinical manifestations of PHN.

Limitations: VZV is highly human-specific and cannot complete a full infection cycle in rodents; restricted viral replication and spread lead to unstable model success rate. High cost, requiring live virus operation (BSL-2 laboratory), complex virus culture and titration, and long modeling cycle. Large variability in pain phenotypes.

2.2.2. HSV-1 Model

Takasaki et al. first used herpes simplex virus type 1 (HSV-1) as an alternative pathogen to establish a PHN pain model [12]. Subcutaneous inoculation of HSV-1 establishes latent infection in rodents, which can be reactivated under specific conditions, causing similar neuropathological changes and pain behaviors. Detailed discussion of VZV and HSV-1 inoculation models refers to the latest reviews [13].

Modeling Procedure:

- 1) *Experimental Animals:* 8-week-old male C57 mice are selected; this strain is sensitive to HSV-1 with stable genetic background and good reproducibility.
- 2) *Virus Preparation:* Wild-type HSV-1 strain (KOS strain) is amplified in Vero cells or human embryonic kidney 293T cells, purified by high-speed centrifugation and filtration, and virus titer is determined as 1×10^6 PFU/ml by plaque assay; virus solution is aliquoted and stored at -80°C to avoid repeated freeze-thaw cycles.
- 3) *Virus Inoculation:* The skin on the left hind paw pad or tibia of mice is disinfected with alcohol cotton balls (subcutaneous inoculation causes minimal trauma and does not require anesthesia); 10 μ L of HSV-1 virus solution is injected subcutaneously with a micro-syringe.

Precautions:

- 1) *Virus Control:* Strictly control virus dose and concentration; excessively high dose causes severe systemic infection or death in mice, while excessively low dose fails to induce stable pain phenotypes. Pre-experiments are required to determine the appropriate dose, usually 1×10^5 – 1×10^6 PFU/ml.
- 2) *Consistent Virus Strain:* The same batch and strain of virus must be used for modeling; different virulence of strains affects modeling success rate and pain abnormality duration, reducing experimental reproducibility.
- 3) *Experimental Operation:* Areas close to peripheral nerves such as the tibia are preferred, where subcutaneous tissue is thin and virus easily invades peripheral nerve endings, improving modeling success rate. Avoid deep needle insertion to injure muscle layer or shallow insertion causing virus solution overflow.
- 4) *Safety Protection:* HSV-1 can infect humans; gloves and masks must be worn during operation, and all procedures are performed in a BSL-2 laboratory. After experiments,

virus-contaminated items are autoclaved, and animal excrement and carcasses are disposed of as medical waste.

Advantages and Limitations:

Advantages: HSV-1 is susceptible to rodents, establishes latency, and reliably induces long-term stable mechanical allodynia and thermal hyperalgesia.

Limitations: HSV-1 is not the pathogenic virus of PHN; biological characteristics (including neurotropism and reactivation) differ from VZV, leading to etiological differences.

2.2.3. Resiniferatoxin (RTX)-induced Model

Although virus-induced models are most etiologically similar to human PHN, they are complex to operate with limited success rates. Therefore, Pan et al. developed a neurotoxin-based modeling method [14]. RTX, a natural capsaicin analog and potent TRPV1 receptor agonist extracted from Euphorbia plants, selectively injures unmyelinated C fibers and myelinated A δ fibers in sensory neurons, inducing pain phenotypes of PHN patients such as paresthesia, mechanical allodynia, and spontaneous pain. It has advantages of short modeling cycle, stable pain phenotype (persisting for more than 4 weeks), and no virus operation, becoming an important alternative model for PHN mechanism research and analgesic drug screening. The key of the RTX-induced model is "targeted delivery": local subcutaneous injection, perineural injection, or intrathecal injection precisely targets peripheral nociceptive sensory neurons; subcutaneous injection in the sciatic nerve innervation area is the mainstream protocol due to simple operation and minimal trauma.

Modeling Procedure:

- 1) *Experimental Animals:* Adult C57BL/6 mice (20–25 g) or SD rats (220–280 g) are preferred. C57BL/6 mice have stable TRPV1 receptor expression, consistent sensitivity to RTX, and good reproducibility of pain phenotypes, suitable for mechanism research.
- 2) *Core Reagent:* RTX (purity $\geq 98\%$, Sigma-Aldrich Cat. No. R8756) is dissolved in anhydrous ethanol to 10 mmol/L stock solution, aliquoted, and stored at $-20\text{ }^{\circ}\text{C}$ protected from light; diluted to working concentration with normal saline before use (mice: 10–20 $\mu\text{g/ml}$; rats: 50–100 $\mu\text{g/ml}$), with final ethanol concentration $\leq 5\%$ to avoid solvent toxicity.
- 3) *Surgical Area Preparation:* Animals are fixed in prone position and anesthetized with 1% pentobarbital sodium (50 mg/kg). Hair on the operated hind limb is shaved, disinfected alternately with iodophor and 75% alcohol for 3 times, exposing the injection area (posterior thigh to plantar, sciatic nerve innervation area).
- 4) *Subcutaneous Injection:* Diluted RTX solution (15 $\mu\text{g/ml}$) is drawn with a micro-syringe and slowly injected subcutaneously into the operated hind paw plantar or posterior thigh (1 cm from sciatic nerve branch) of mice; injection volume is 10 μl (single-site injection, dose 0.15 μg per mouse); injection speed is controlled at 5 $\mu\text{l}/10\text{ s}$ to avoid local irritation from rapid drug diffusion.

5) *Perineural Injection:* One sciatic nerve is surgically exposed (method same as CCI model for rats), and 50 μl of RTX solution (80 $\mu\text{g/ml}$) is slowly injected under the epineurium with a micro-syringe; this protocol has stronger targeting and reduces systemic toxicity.

6) *Intrathecal Injection:* Mice receive 5 μl of RTX via L5–L6 interspace intrathecal injection; high technical difficulty to avoid spinal cord injury.

Precautions:

- 1) *Reagent Handling:* RTX is sensitive to light and heat; all operations are protected from light, prepared fresh and used within 30 min to avoid degradation; strictly control solvent ratio, final ethanol concentration $\leq 5\%$ to prevent local skin irritation or neurotoxicity.
- 2) *Dose Individualization:* Mice: 0.1–0.2 μg per mouse (subcutaneous injection); $<0.1\text{ }\mu\text{g}$ fails to deplete TRPV1 neurons; $>0.2\text{ }\mu\text{g}$ causes systemic toxicity. Rats: 3–5 μg per rat (perineural injection).
- 3) *Pre-experiment:* Different mouse strains have varying sensitivity to RTX; BALB/c mice are more sensitive than C57BL/6 mice. Three dose gradients (low, medium, high) are designed for first-time use to determine the optimal dose.
- 4) *Systemic Toxicity:* Excessive RTX causes nausea, vomiting, and hypotension in animals (TRPV1 receptor activation in gastrointestinal tract and smooth muscle). Closely observe respiratory status and body weight changes during modeling; intraperitoneal injection of normal saline (10 ml/kg) for fluid replacement and dexamethasone (0.5 mg/kg) for anti-inflammation are administered promptly if body weight decreases by $>10\%$ or dyspnea occurs.

Advantages and Limitations:

Advantages: Precisely simulates the core pathology of PHN; the RTX model directly and repeatedly reproduces sensory nerve fiber loss; single systemic or local administration induces mechanical and thermal allodynia behavioral phenotypes lasting for weeks to months.

Limitations: Does not reflect viral infection process, cannot be used to study virological mechanisms in PHN pathogenesis; transient analgesia due to neuronal apoptosis in the early stage of administration, inconsistent with early allodynia in clinical PHN.

2.2.4. PHN Model Selection Guide

The VZV model is the best choice for studying pathogenesis and developing antiviral therapeutic drugs but is the most difficult; the HSV-1 model is a compromise, commonly used in research on herpesvirus infection pathogenesis and herpetic neuralgia; the RTX model targets the consequences of nerve injury, often used for screening therapeutic analgesic drugs.

2.3. Diabetic Neuropathic Pain Models

Diabetic peripheral neuropathy (DPN) is a common complication of diabetes, caused by peripheral nerve injury due to long-term hyperglycemia. Pain symptoms are usually symmetrically distributed, progressing from distal to proximal limbs, forming "stocking" or "glove"-like acral pain. In addition to sensory abnormalities, some patients may be accompanied by motor nerve involvement symptoms such as muscle weakness and atrophy. This section introduces four types of diabetic animal models: spontaneous, high-fat diet-induced, chemical drug-induced, and modified composite models.

2.3.1. Spontaneous Models

1) db/db Mouse Model

The db/db genetically engineered mouse is recognized as one of the best type 2 diabetes model mice [15]. A point mutation in the leptin receptor gene causes abnormal hypothalamic signal transduction, leading to hyperphagia, energy metabolism disorder, obesity, and subsequent typical clinical phenotypes such as hyperglycemia, hyperinsulinemia, and insulin resistance, simulating the natural progression of human type 2 diabetes and complicated DPN [16]. Mechanical allodynia and thermal hyperalgesia are its typical phenotypes.

2) ob/ob Mouse Model

The ob/ob genetically engineered mouse is a diabetes model constructed by knocking out the leptin gene [16, 17]. Due to lack of leptin, an appetite-regulating hormone, animals exhibit obvious hyperphagia and extreme obesity. These model mice develop severe insulin resistance and impaired glucose tolerance in the early stage, with fluctuating blood glucose with age. Their pain behavior may show stage changes: hyperalgesia in the early stage and hypoalgesia in the late stage, similar to the clinical phenotype of human diabetic neuropathic pain.

Advantages and Limitations:

Advantages: Classic gold-standard models for simulating the development of human type 2 diabetes and its complications (such as DPN); the evolution of neuropathy and pain phenotypes is basically consistent with the course of human DPN.

Limitations: High cost of genetically engineered animals; stable neuropathic pain phenotypes usually appear after 12-24 weeks of feeding.

2.3.2. High-fat Diet-induced Model

A metabolic syndrome model established by long-term feeding of high-fat and high-sugar food, mainly used for mechanism research of type 2 diabetes and complications [18]. This modeling method simulates the process of obesity, insulin resistance, and progressive hyperglycemia in humans caused by poor dietary habits.

Modeling Procedure:

Most studies use C57BL/6J mice or Wistar rats as experimental animals, fed with high-calorie diets containing 45%-60% fat for 12-24 weeks. High-fat and high-sugar diet induces

metabolic disorders such as weight gain, obesity, insulin resistance, and impaired glucose tolerance.

Advantages and Limitations:

Advantages: Highly similar to the main cause of human type 2 diabetes (poor dietary habits) in etiology. Induces mild hyperglycemia and insulin resistance, suitable for studying neuropathy in the pre-diabetic or early stage.

Limitations: High-fat diet alone usually only induces mild hyperglycemia; neuropathy and pain behavior are weak and variable, requiring a long time (>6 months) to manifest.

2.3.3. Chemical Drug-induced Model

Intraperitoneal injection of streptozotocin (STZ) in animals is a common method for constructing type 1 diabetes models [19]. STZ, a glucose analog, is highly toxic to pancreatic β -cells, massively destroying β -cells through DNA alkylation, leading to insufficient insulin secretion and elevated blood glucose levels. The standard modeling process is intraperitoneal or tail vein injection of STZ in male SD rats or C57BL/6J mice around 8 weeks old.

Modeling Procedure:

Type 1 Diabetic Neuropathy: Single high-dose injection (60-65 mg/kg, intraperitoneal or intravenous) or multiple low-dose injections (e.g., 20-30 mg·kg⁻¹·d⁻¹ for 5 consecutive days). Single high-dose injection rapidly induces severe hyperglycemia, while multiple low-dose regimen simulates the progressive process of autoimmune diabetes.

Type 2 Diabetic Neuropathy: Commonly used dose is 35 mg/kg, inducing moderate and relatively stable blood glucose, a balanced choice for establishing an ideal type 2 diabetic neuropathic pain model. Animals develop persistent hyperglycemia (>16.6 mM) within 1 week after STZ injection.

Advantages and Limitations:

Advantages: The STZ model is the most commonly used and economical DPN model in history. It rapidly induces stable hyperglycemia (within 1-2 weeks) after injection, and pain behaviors such as mechanical allodynia and thermal hyperalgesia appear within weeks; short experimental cycle, good reproducibility, low cost, often used for analgesic drug screening.

Limitations: The mouse type 1 diabetes model induced by STZ alone shows absolute insulin deficiency, different from the pathophysiology of type 2 diabetes dominated by insulin resistance; acute hyperglycemia induced in a short time cannot fully reflect the chronic course; low-dose insulin is usually administered to prevent ketoacidosis, which may introduce new variables affecting experimental results.

2.3.4. Modified Composite Model

The modified composite model is the most commonly used diabetic neuropathy model, combining the dual advantages of diet-induced and chemical-induced models, successfully simulating the natural course of clinical type 2 diabetes [20].

Modeling Procedure:

A typical composite model procedure includes two steps:

first, experimental animals (mostly C57BL/6 mice or SD rats) are fed with high-fat and high-sugar diet for 8-16 weeks to form obesity and insulin resistance; then low-dose STZ (30 mg/kg) is injected once or multiple times to induce mild β -cell failure, further elevating blood glucose to a relatively stable level.

Advantages and Limitations:

Advantages: The model produces stable moderate to severe hyperglycemia, significant insulin resistance, early and clear neuropathic pain manifestations, and neuropathological changes. It avoids the mild phenotype of the simple high-fat diet model and the acute toxicity of the simple STZ model, and is currently recognized as an ideal type 2 diabetes model.

Limitations: More complex operation, staged modeling, higher time and technical requirements than single models.

2.3.5. Diabetic Neuropathic Pain Model Selection Guide

Spontaneous models and modified composite models are currently the closest to the occurrence and development of human type 2 diabetes DPN, suitable for mechanism research and long-term intervention experiments; the STZ model is suitable for drug screening research due to its simplicity, low cost, and good reproducibility; the high-fat diet-induced model is suitable for research on pre-diabetic neuropathy and pain behavior [21].

2.4. Post-spinal Cord Injury Pain (PSCIP) Models

Post-spinal cord injury pain (PSCIP) is a common complication after spinal cord injury, with typical characteristics of neuropathic pain. Clinically, it presents as severe spontaneous burning, stabbing, or tearing pain, accompanied by allodynia and hyperalgesia; most patients have persistent moderate to severe pain. This section introduces four animal models commonly used to study neuropathic pain after spinal cord injury: Allen's strike method, spinal cord hemisection method, spinal cord wedge-shaped block hemisection method, and excitotoxic drug injection method.

2.4.1. Allen's Strike Method

This method simulates the mechanical injury process of spinal cord injury through precisely controlled impact force. First created by Allen in 1911 [22], Koozekanani et al. [23] standardized and improved the model in 1976 using dogs as experimental subjects and high-speed photography to record the movement of the impactor in Allen's method. Anderson [24] pointed out in 1982 that the traditional Allen's method cannot independently adjust spinal cord compression volume and initial compression velocity, so a pneumatic impact device was designed to achieve independent control of these two parameters, with accurately repeatable impact velocity and spinal cord compression volume after testing.

Modeling Procedure:

Adult rats weighing 200-250 g are selected; the T13-L1 lamina is surgically removed to expose the spinal cord, which is accurately struck at the L1 segment with an Allen impactor at a force of 400 g·cm. Common behavioral tests (Tarlov score and BMS score) and neuroelectrophysiological tests (soma-tosensory evoked potentials, motor evoked potentials) are used to determine model success; successfully modeled animals immediately show signs of spinal cord injury such as lower limb paralysis and urinary retention.

Advantages and Limitations:

Advantages: Highly simulates the mechanical external force factors of traumatic spinal cord injury; repeatable injury degree by controlling impact force and position, suitable for mechanism research and drug screening of neuropathic pain after spinal cord injury.

Limitations: Injury degree may vary among individuals leading to large result variability; high surgical difficulty, requiring special instruments and equipment.

2.4.2. Spinal Cord Hemisection Method

The spinal cord hemisection model is established based on the pathological characteristics of clinical Brown-Séquard syndrome. Arvanian et al. [25] first confirmed that signal transmission from surviving axons to motor neurons in the chronic stage of incomplete spinal cord injury is delayed and degenerates, and axonal demyelination is observed using a rat thoracic spinal cord unilateral hemisection model, providing a key basis for applying this model to chronic pathological research of spinal cord injury.

Modeling Procedure:

Rats or mice can be used; one side of the spinal cord is completely cut with a surgical blade at the T10 segment. This method ensures unilateral injury, suitable for comparing functional differences between the injured and healthy sides.

Advantages and Limitations:

Advantages: Simple operation, good reproducibility, suitable for comparative study of bilateral asymmetric pain phenotypes.

Limitations: Compared with complex injuries in human spinal cord injury, the dynamic changes of the model in chronic pain progression are limited, not fully matching the progressive characteristics of human diseases.

2.4.3. Spinal Cord Wedge-shaped Block Hemisection Method

Modeling Procedure:

An improvement based on the hemisection method, suitable for evaluating the effect of graft therapy on spinal cord injury [26]. Wedge-shaped resection of spinal cord tissue forms neat injury edges, better controlling injury range, facilitating histological comparison and regeneration research, with good injury reproducibility.

Advantages and Limitations:

Advantages: Wedge-shaped resection of spinal cord tissue better controls injury site and degree, suitable for studying local neural circuit remodeling and spatiotemporal dynamic changes of pain.

Limitations: Less stable simulation of long-term pain phenotypes compared with other models.

2.4.4. Excitotoxic Drug Injection Method

Yeziarski et al. [27] first established a model by injecting quisqualic acid, an AMPA receptor agonist of glutamate, into the rat spinal cord based on elevated excitatory amino acids as a typical neurochemical change after spinal cord injury. It achieves gradient loss of neurons in specific areas of the spinal cord gray matter, and injured animals exhibit spontaneous pain and mechanical allodynia. The pathological spinal cord injury process is highly similar to ischemic spinal cord injury, also simulating the pathological manifestations of post-traumatic syringomyelia. Subsequent studies based on this original method also use NMDA as an excitotoxic drug for injection modeling, focusing on central pain research after spinal cord injury [28].

Modeling Procedure:

Rats or mice can be used; after laminectomy, excitatory amino acids (such as glutamate, NMDA receptor agonist, or alginate) are microinjected into the spinal cord dorsal horn/pericentral canal. Pathological injury is mainly caused by neuronal death without destroying spinal cord structural integrity, simulating excitotoxic spinal cord injury. This model is suitable for studying nerve cell transplantation and specific neuronal death mechanisms.

Advantages and Limitations:

Advantages: Specific induction of neuronal hyperexcitation and death by injecting excitotoxic drugs; simple operation, suitable for exploring the effect of excitotoxicity on chronic pain and screening analgesic drugs.

Limitations: Drug exudation causes non-specific injury, not suitable for pathological research of spinal cord trauma.

2.4.5. PSCIP Model Selection Guide

The Allen's strike method is most appropriate when focusing on clinical relevance; spinal cord hemisection or wedge-shaped block hemisection is preferred for mechanism research requiring reproducible and precise injury; drug injection method is selected for in-depth study of excitotoxicity or neuronal-specific injury.

2.5. Central Post-stroke Pain (CPSP) Models

Central post-stroke pain (CPSP) is central neuropathic pain caused by ischemic or hemorrhagic cerebrovascular lesions damaging the central somatosensory pathways. Pain symptoms usually occur 3-6 months after stroke, mainly manifested as persistent or paroxysmal hemiplegic pain in the contralateral upper and lower limbs, trunk, and face of the lesion; pain phenotypes include spontaneous pain (burning, electric shock-

like pain, etc.) and evoked pain (allodynia and hyperalgesia). Currently, researchers have established various CPSP animal models, among which chemical injury and vascular occlusion are the two most commonly used modeling methods.

2.5.1. Chemical Injury Method - Type IV Collagenase Model

Modeling Procedure:

Neurotoxic substances are injected into specific brain regions related to pain processing to destroy the regions, simulating the clinical manifestations of CPSP. Specific brain regions mainly include the ventral posterolateral nucleus of the thalamus, spinothalamic tract, and other key parts of the central pain pathway. Type IV collagenase causes microhemorrhage and inflammatory response by destroying the extracellular matrix, resulting in focal injury [29, 30]. Adult rats, mice, and rhesus monkeys are the most commonly used experimental animals; modeled animals exhibit persistent (more than 14 days) mechanical allodynia and thermal hyperalgesia phenotypes.

Advantages and Limitations:

Advantages: Accurate lesion localization, specifically targeting pain-processing brain regions; good model reproducibility, small individual differences; long disease course, suitable for chronic pain mechanism research and long-term observation of drug efficacy; small surgical wound, low mortality.

Limitations: A hemorrhagic stroke model, different from the pathological mechanism of ischemic stroke; inconsistent control of injection technical parameters (injection speed, volume, concentration) may lead to model instability.

2.5.2. Vascular Occlusion Method

First proposed by Tamura et al. [31] in 1981, this model is constructed by temporarily occluding the middle cerebral artery (MCAO), causing ischemic injury in a wide range of brain regions including the sensory cortex, thalamus, and basal ganglia. The MCAO model is a classic model for studying the mechanism and sequelae of ischemic stroke, widely used in CPSP mechanism and treatment research. Modeling methods include suture occlusion and electrocoagulation.

Modeling Procedure:

1) Suture Occlusion MCAO Model

A cerebral ischemia model established by ligating the origin of the middle cerebral artery to cause temporary blood flow interruption. The scope and severity of ischemic injury are controlled by adjusting the insertion depth and occlusion duration of the suture. Blood flow is usually occluded for a certain time (such as 60-90 min) and then the suture is withdrawn for reperfusion, simulating the blood flow reperfusion process after clinical ischemic stroke [32, 33]. The allodynia induced by this model is less typical than that of the collagenase model, often masked by obvious motor dysfunction symptoms.

2) Electrocoagulation MCAO Model

Permanent vascular occlusion is achieved by electrocoagulating the middle cerebral artery after craniotomy. The advantage of this method is stable and reliable occlusion without spontaneous recanalization. There are relatively few pain behavioral studies on the electrocoagulation MCAO model; it is known that the neurological deficit is obvious, but pain behavior may be mild and transient.

Advantages and Limitations:

Advantages: Simulates the pathological process of ischemic stroke. No craniotomy required, controllable ischemia time.

Limitations: Large variation in lesion scope, significant individual differences among animals.

2.5.3. CPSP Model Selection Guide

The chemical injury model is used to explore whether a specific brain region plays a role in CPSP. The vascular occlusion method is selected to study the analgesic efficacy of drugs or other treatments on CPSP. However, the pain phenotypes and duration of these two CPSP animal models are somewhat different from human diseases.

3. Inflammatory Pain Models

Inflammatory pain is a pain state caused by tissue injury or release of inflammatory mediators, the most common clinical pain type, with clinical manifestations including spontaneous pain, allodynia, and hyperalgesia. The pathological basis of inflammatory pain is peripheral sensory nerve sensitization caused by local inflammatory response. Such pain is common in clinical diseases such as arthritis, myofasciitis, and post-operative pain. This section introduces five commonly used inflammatory pain models: complete Freund's adjuvant, formalin, capsaicin, carrageenan, and bee venom models. Although some inflammatory pain models are acute pain models, they are helpful for studying the mechanism of acute pain chronicity and are introduced here together.

3.1. Complete Freund's Adjuvant (CFA) Model

Modeling Procedure:

0.1 ml of complete Freund's adjuvant (CFA, emulsifier of heat-inactivated *Mycobacterium tuberculosis*) is injected subcutaneously into the plantar or tibial area, inducing a strong immune response and inflammatory reaction; local inflammatory manifestations (redness, swelling, heat, etc.) appear 2–4 h after injection. Spontaneous pain, mechanical and thermal hyperalgesia phenotypes in model animals generally persist for more than 3 weeks. The CFA model is particularly suitable for studying the pathogenesis of chronic inflammatory pain and observing long-term analgesic effects [34].

Advantages and Limitations:

Advantages: The inflammatory and hyperalgesic state of the CFA model lasts for weeks, suitable for long-term drug efficacy observation with small test result variability; its inflammatory characteristics are similar to the pathological

manifestations of human rheumatoid arthritis.

Limitations: Large tissue damage causing severe structural damage, inconsistent with mild clinical inflammation; high-dose CFA may induce systemic immune response.

3.2. Formalin Model

Modeling Procedure:

The formalin model is an acute inflammatory pain model induced by plantar injection of 0.05–0.1 ml of formalin (2.5%–5%), famous for its obvious biphasic pain response. First proposed by Dubuisson and Dennis [35] in 1977, it is one of the classic models for studying the mechanism from acute to chronic pain. The pain behavior of the model shows a biphasic response: the first phase occurs 10 min after injection, mainly induced by direct stimulation of peripheral C fibers; the second phase appears 15–60 min after injection, mainly caused by nerve sensitization and inflammation. A short pain-free period (about 5–15 min) occurs between the two phases.

Advantages and Limitations:

Advantages: Typical biphasic response of neurogenic/inflammatory pain. Simple experimental operation without special instruments; the entire behavioral observation only takes 1–2 h and is easy to repeat.

Limitations: Short pain duration, generally not suitable for chronic pain research; obvious tissue damage; formalin itself has tissue-fixing effect, which may affect pathological observation; certain pain behavior differences among different animal individuals; less clear clinical relevance than the CFA model.

3.3. Capsaicin Model

Modeling Procedure:

The capsaicin model is an acute pain model induced by topical application of capsaicin to activate TRPV1 [36]. Capsaicin, a TRPV1 receptor agonist, specifically activates TRPV1 on primary sensory neurons. Capsaicin (usually 1%–5% concentration) is injected around the plantar or joints of rats or mice; low-concentration plantar injection induces transient thermal hyperalgesia, while high-concentration or repeated administration causes long-term mechanical allodynia. Other administration routes include intradermal, intrathecal, or peripheral nerve capsaicin application.

Advantages and Limitations:

Advantages: Clear mechanism, specific activation of TRPV1 receptor. Pain duration adjustable by dose. Can simulate neurogenic inflammation process.

Limitations: Short pain duration; large variability in capsaicin sensitivity among different animals.

3.4. Carrageenan Model

Modeling Procedure:

0.1 ml of 0.5% carrageenan (a sulfated polysaccharide) is injected subcutaneously into the plantar of rats or mice. Local

swelling occurs minutes after injection, spontaneous pain behavior peaks at 2–4 h, followed by thermal hyperalgesia lasting for 24–48 h, suitable for studying the stepwise activation process of the inflammatory cascade [37]. Compared with CFA, carrageenan-induced inflammation is self-limiting and does not cause long-term tissue damage.

Advantages and Limitations:

Advantages: Controllable inflammatory process; inflammation and hyperalgesia resolve spontaneously.

Limitations: Short pain phenotype duration, not suitable for chronic pain research; mild inflammatory response; insignificant thermal hyperalgesia phenotype.

3.5. Bee Venom Model

Modeling Procedure:

0.2 mg/50 µl of bee venom is injected subcutaneously into the plantar of rats or mice; experimental animals immediately exhibit paw withdrawal, paw licking, and other spontaneous pain behaviors after injection, and mechanical and thermal hyperalgesia appear within 1–2 h after injection, persisting for 72–96 h. This model has both acute and chronic pain characteristics, producing persistent spontaneous pain and primary hyperalgesia, as well as secondary hyperalgesia away from the injection site, suggesting the involvement of central sensitization mechanisms [38, 39].

Advantages and Limitations:

Advantages: Spontaneous pain is its prominent phenotype, exhibiting both primary and secondary hyperalgesia. Highly similar to clinical manifestations of insect stings.

Limitations: Bee venom contains multiple active components, difficult to analyze the mechanism; activity may vary among different sources and batches of bee venom; less commonly used than traditional models.

3.6. Inflammatory Pain Model Selection Guide

Selection of an appropriate inflammatory pain model should consider research purpose, experimental conditions, and clinical disease characteristics to be simulated. For research on chronic inflammatory pain mechanisms and long-term drug treatment, the CFA model is preferred for its good stability, long duration, and ability to well simulate the pathological characteristics of chronic inflammatory diseases. For acute pain mechanism research and rapid drug screening, the formalin and carrageenan models are more suitable, both with simple operation and short time, suitable for preliminary screening. For TRPV1 receptor and related pathway research, the capsaicin model is the inevitable choice due to its specific activation of the receptor and clear mechanism. For spontaneous pain and central sensitization mechanism research, the bee venom model has unique advantages, providing persistent spontaneous pain behavior difficult to observe in other models.

4. Bone Cancer Pain Models

Bone cancer pain is a type of chronic cancer pain caused by primary or metastatic bone tumors, characterized by persistent dull pain and movement-induced allodynia. The etiology of bone cancer pain is complex, mainly manifested as peripheral and central sensitization induced by various cytokines and inflammatory mediators in the tumor microenvironment, and pathological fractures caused by bone destruction. Bone cancer pain has both nociceptive and neuropathic pain phenotypes.

Modeling Procedure:

The most commonly used bone cancer pain model is the rat tibial injection of breast cancer cells method [40], based on the classic model established by Medhurst et al [41].

- 1) Animal and Cell Selection: Female SD or Wistar rats are commonly used; Walker 256 breast cancer cells or MRMT-1 rat breast cancer cells are suspended in PBS, adjusted to a cell concentration of 1×10^7 cells/ml for standby.
- 2) Intratibial Inoculation Surgery: A longitudinal incision is made ~0.5 cm below the tibial plateau to fully expose the tibial bone surface; a 22 G syringe needle is vertically drilled into the bone surface until entering the bone marrow cavity. Replace with a 10 µl micro-syringe, inject 3–10 µl of pre-mixed tumor cell suspension (containing 1×10^5 – 3×10^5 tumor cells) into the bone marrow cavity, keep the needle for 1–2 min before pulling out to avoid tumor cell overflow, seal the bone hole with bone wax, and then suture the surgical incision.
- 3) Model Verification: X-ray imaging is used to observe the degree of tibial bone destruction; HE staining is used for histological observation of bone tissue pathological changes; typical pathological changes are usually visible 7–14 days after inoculation.

Advantages and Limitations:

Advantages: Can reproduce the progressive bone destruction process, similar to the imaging characteristics of clinical bone metastasis cancer patients; model animals show a progressive aggravation process from persistent dull pain to severe movement-induced pain, similar to the pain evolution of clinical bone cancer pain patients.

Limitations: High technical requirements for operation; tibial drilling and intramedullary injection require fine operation; large individual differences in pain behavior and bone destruction degree among animals.

5. Visceral Pain Models

Visceral pain refers to pain originating from visceral tissues. Compared with somatic pain, it is significantly different in nature, localization, and conduction pathways. Visceral pain is poorly localized, manifested as distending pain, colic, and discomfort, radiating to distant body surface areas, often accompanied by strong emotional changes and autonomic nerve re-

sponses. Visceral pain is a common symptom of various functional gastrointestinal diseases (such as irritable bowel syndrome) and urinary system diseases (such as interstitial cystitis, bladder pain syndrome). Its pathogenesis is complex, involving peripheral sensitization, central sensitization, neuroinflammation, intestinal flora imbalance, and other pathophysiological processes, becoming a key and difficult point in current pain research. Currently, commonly used methods for constructing visceral pain animal models mainly include intraperitoneal cyclophosphamide injection, rectal formalin perfusion, colorectal distension, visceral nerve electrical stimulation, and neonatal colorectal stimulation.

5.1. Intraperitoneal Cyclophosphamide Injection Model

The intraperitoneal cyclophosphamide injection model is a classic animal model for studying pain manifestations such as overactive bladder and chemical cystitis. By intraperitoneally injecting cyclophosphamide into experimental animals, its metabolite acrolein enters the bladder through the kidneys, directly stimulating the bladder epithelium, inducing bladder inflammation and hyperalgesia, thus well simulating the typical symptoms of clinical interstitial cystitis and bladder pain syndrome [42].

Modeling Procedure:

Rats or mice are usually used as experimental animals; cyclophosphamide is administered intraperitoneally at a dose of 75 mg/kg, once every 3 days for 3 consecutive times.

Pain Behavioral Manifestations:

Intraperitoneal cyclophosphamide injection induces obvious bladder-related pain behaviors in animals; visceral pain degree can be evaluated by measuring urodynamic indicators such as urination frequency, bladder capacity, urination pressure threshold, and pain behavior score. Reflex bladder activity: increased urination frequency with decreased urine volume per time. Pain-related behaviors: typical manifestations include abdominal licking, hind limb scratching of the lower abdomen, and body curling.

Advantages and Limitations:

Advantages: Can reproduce the core clinical symptoms of clinical chemical cystitis; simple modeling method; good reproducibility.

Limitations: May cause systemic toxic reactions; mainly applicable to bladder-related visceral pain research, relatively limited application scope.

5.2. Rectal Formalin Injection Model

The rectal formalin injection model is an animal model that induces rectal inflammatory pain by injecting formalin solution into the rectum, mainly used to study persistent visceral pain related to inflammatory bowel disease [43]. This model can simulate common intestinal inflammation and visceral hypersensitivity in patients with clinical inflammatory bowel

disease.

Modeling Procedure:

Rats are generally selected; low-concentration formalin solution (such as 1%-5%) is injected into the rectum as a chemical stimulant, directly damaging the rectal mucosa, activating the intestinal immune system, inducing local inflammatory response; formalin stimulation sensitizes rectal primary afferent nerve endings, improving their sensitivity to mechanical and chemical stimuli, thus producing visceral hyperalgesia.

Pain Behavioral Manifestations:

Rectal formalin injection induces typical visceral pain-related behaviors in animals, divided into two phases. Acute phase (10 min after injection): severe pain reactions such as abdominal contraction, arched back, and hind limb extension. Sustained phase (10-60 min after injection): pain manifestations are reduced but persistent, i.e., abdominal licking and body curling.

Advantages and Limitations:

Advantages: Can induce stable and reproducible visceral hypersensitivity; well simulates the phenotypic characteristics of inflammatory bowel disease-related visceral pain; pain-related behaviors are easy to observe and quantify.

Limitations: Formalin concentration and dose should not be too high to avoid excessive tissue damage; not suitable for non-inflammatory visceral pain research.

5.3. Colorectal Distension Model

A visceral pain model based on mechanical distension of the colorectum, commonly used to study irritable bowel syndrome (IBS). This model directly uses mechanical stimulation to activate intestinal mechanoreceptors and nociceptors, simulating the hypersensitivity of IBS patients to intestinal distension [44].

Modeling Procedure:

Rats are often used as experimental animals; a balloon catheter is inserted into the colorectum through the anus, and gas or liquid is injected into the balloon to cause mechanical distension of the colorectum. Distension pressure is graded: sub-threshold pressure (<20 mm Hg), physiological pressure (20-45 mm Hg), and noxious pressure (>45 mm Hg) to evaluate visceral sensitivity at different levels. Visceral hypersensitivity induced by this model can persist for weeks.

Pain Behavioral Manifestations:

Pain behavior assessment mainly relies on quantitative scoring of abdominal withdrawal reflex (AWR) and visceromotor response (VMR). AWR score: graded 0-4 according to the degree of abdominal muscle contraction during balloon distension. VMR: electromyographic detection of abdominal muscle electrical activity during colorectal distension. Behavioral responses: including arched back, body stiffness, etc. Autonomic responses: elevated blood pressure or increased heart rate.

Advantages and Limitations:

Advantages: Precisely controllable stimulation intensity

(pressure, duration) and position; no obvious tissue damage or inflammation; can evaluate different levels of visceral sensitivity from physiological to pathological range; suitable for studying viscerosomatic convergence and viscerosomatic responses.

Limitations: Requires special equipment and surgical skills; animals need to be anesthetized during the experiment (at least for catheter fixation); large individual differences requiring large sample size; mainly reflects mechanical visceral pain, limited simulation of chemical stimulation-induced visceral pain.

5.4. Visceral Nerve Electrical Stimulation Model

A visceral pain model that directly electrically stimulates visceral nerves, with the advantage of precisely controllable electrical stimulation parameters and direct activation of visceral afferent nerve fibers, well simulating acute or chronic visceral pain mediated by visceral nerves, thus very suitable for studying the neural mechanism of visceral pain and neuromodulation therapy [45].

Modeling Procedure:

Rats are often selected as experimental objects; nerves innervating a certain visceral organ (such as greater splanchnic nerve, splenic nerve, pelvic nerve, etc.) are surgically exposed, and electrodes are placed at the exposed site for electrical stimulation. Electrical stimulation parameters (frequency, intensity, pulse width, stimulation time) are set according to specific experimental purposes. This model induces excitation of spinal and supraspinal central neurons by directly activating visceral afferent fibers (especially A δ and C fibers) through electrical stimulation, simulating the excited state of the visceral pain conduction pathway.

Pain Behavioral Manifestations:

Pain behaviors induced by visceral nerve electrical stimulation vary with stimulation intensity and site. Mild stimulation induces autonomic responses such as blood pressure/heart rate changes. Moderate intensity stimulation induces visible pain behaviors such as abdominal muscle contraction and body twisting. High-intensity stimulation leads to strong escape behaviors, squealing, and other acute pain manifestations.

Advantages and Limitations:

Advantages: Precisely controllable stimulation parameters for quantitative research; good reproducibility, self-control before and after stimulation.

Limitations: Requires complex surgery and professional equipment; interspecies differences in neural anatomy and physiology; electrical stimulation may activate both afferent and efferent fibers, complicating result interpretation; inconsistent with the pathological process of clinical visceral pain diseases.

5.5. Neonatal Colorectal Stimulation Model

An animal model that induces persistent visceral hypersensitivity in adulthood by applying colorectal stimulation in the neonatal period, simulating the clinical association between early life trauma and subsequent functional bowel diseases such as irritable bowel syndrome [46].

Modeling Procedure:

Usually starting from postnatal day 8-21 in rats, colorectal distension (with a small-diameter balloon or mechanical probe) is performed daily for 5-10 min. Neonatal colorectal stimulation excites nociceptive pathways in the spinal dorsal horn and brainstem, altering central nervous system developmental plasticity, causing persistent neural remodeling and pain processing dysfunction. Adult rats have increased sensitivity to visceral stimuli, exhibiting long-term visceral hypersensitivity even without obvious persistent tissue inflammation or injury.

Pain Behavioral Manifestations:

Behavioral manifestations of the neonatal colorectal stimulation model are mainly evaluated in adulthood (weeks after stimulation ends). Reduced basal pain threshold: significantly reduced thresholds of visceral reflex and abdominal withdrawal reflex. Anxiety-like behavior: anxiety-like manifestations in the elevated plus maze test. Depression-like performance: despair behavior in the forced swimming test.

Advantages and Limitations:

Advantages: Simulates the developmental origin hypothesis of functional bowel diseases; reflects the long-term impact of early life events on adult pain sensitivity; no persistent tissue inflammation or injury in adulthood, closer to the pathological characteristics of IBS.

Limitations: Long modeling cycle; stimulation protocols may vary among different laboratories; complex mechanism involving multiple aspects of developmental neurobiology; ethical controversy.

5.6. Visceral Pain Model Selection Guide

The cyclophosphamide model is suitable for bladder pain syndrome research; the formalin model is suitable for studying inflammatory bowel disease-related pain; the colorectal distension model or neonatal stimulation model is suitable for visceral hypersensitivity mechanism research; the visceral nerve electrical stimulation model is suitable for studying visceral pain neural pathways.

6. Lumbar Disc Herniation Animal Models

Currently, commonly used animal models simulating this disease in pain research include: chronic compression of dorsal root ganglia (CCD), autologous nucleus pulposus transplantation, and annulus fibrosus puncture models.

6.1. CCD Model

Modeling Procedure:

Proposed by Hu and Xing in 1998 [47], the CCD model is an animal model for simulating sciatica caused by lumbar disc herniation. The key operation is implanting a hard object into the rat L5 intervertebral foramen to achieve continuous compression of the DRG.

1) Intervertebral Foramen Exposure

Adult SD or Wistar rats are selected; after anesthesia, a longitudinal incision (2-3 cm long) is made along the lumbar midline, cutting through the skin and subcutaneous fascia, and muscles are dissected along the paraspinal area at L4-L6 level to fully expose the lamina, facet joints, and intervertebral foramina between L4 and L5. After stripping connective tissue around the intervertebral foramen, the DRG is exposed.

2) Compression Steel Rod Implantation

An L-shaped thin steel rod (usually stainless steel) is selected; its diameter is critical to model success. According to the target DRG size, the commonly used diameter is 0.5-0.8 mm. Its tip is vertically aligned and carefully inserted into the intervertebral foramen. Along the nerve root course, the steel rod is slowly and gently advanced 4 mm, with its tip just below or lateral to the DRG, forming stable compression. The long arm of the steel rod is bent and firmly fixed to surrounding muscles and tissues to prevent postoperative slippage. Postoperative management is the same as the CCI model.

Pain Behavioral Manifestations:

This model can simulate radiating pain symptoms caused by human disc herniation; postoperative animals develop mechanical allodynia (appearing 1-3 days after modeling, lasting 2-4 weeks), thermal hyperalgesia, and spontaneous pain behaviors (repeated paw licking, paw lifting, and weight avoidance) and motor dysfunction (abnormal gait and asymmetric limb use).

The pain behavioral response of CCD model animals is dose-dependent on compression intensity: mild pressure causes paresthesia, severe pressure leads to motor dysfunction (limb paralysis, muscle weakness). Long-term compression causes chronic hyperalgesia in animals; even after decompression, hyperalgesia may persist long-term, similar to chronic hyperalgesia in the chronic process of human disc herniation.

Advantages and Limitations:

Advantages: Injury mechanism similar to the pathogenesis of clinical radicular pain, good reproducibility, stable spontaneous pain and evoked pain phenotypes.

Limitations: High surgical difficulty and technical requirements, requiring microsurgical skills; compression degree differences may cause individual variations; mainly simulates mechanical compression pathological process without involving chemical factors.

6.2. Autologous Nucleus Pulposus Transplantation Model

Modeling Procedure:

This model transplants the animal's own caudal vertebra nucleus pulposus tissue around the intervertebral foramen and nerve roots to simulate chemical inflammation caused by herniated nucleus pulposus stimulating nerve roots [48]. Adult SD rats are generally used; after anesthesia, 1-2 distal caudal vertebrae are excised under sterile conditions, nucleus pulposus tissue is removed and placed in normal saline for standby. A midline incision is made on the back, paraspinal muscles are bluntly dissected to expose the L4-L5 or L5-L6 segment lamina, facet joints, etc., and the collected caudal vertebra nucleus pulposus tissue (2 caudal vertebrae nucleus pulposus volume) is transplanted around the exposed nerve roots or intervertebral foramen, making the nucleus pulposus tissue contact the nerve roots.

Pain Behavioral Manifestations:

The pain characteristics of this model are mainly persistent mechanical hyperalgesia and thermal hyperalgesia. In addition, model animals exhibit motor dysfunction and abnormal posture, such as lameness of the affected limb, weight avoidance, and abnormal walking posture.

Advantages and Limitations:

Advantages: Highly consistent with human chemical radiculitis caused by disc herniation, extremely similar to the pathological changes after herniated nucleus pulposus contacts nerve roots in humans.

Limitations: Cannot simulate the mechanical compression factor of disc herniation; difficult to control the amount and position of transplanted nucleus pulposus, easily causing large individual differences; short pain duration of the model, only 2-4 weeks, cannot well simulate the chronic protracted process of disc herniation.

6.3. Annulus Fibrosus Puncture Model

Modeling Procedure:

Artificially causing annulus fibrosus damage using the poor self-healing property of the annulus fibrosus, inducing intervertebral disc degeneration, leading to nucleus pulposus protrusion from the defect, similar to the process of human disc herniation [49].

Rats, rabbits, or mice can be used; the lumbar spine is mostly selected as the puncture site. After anesthesia, animals are fixed in prone position; a posterolateral approach is often used for annulus fibrosus puncture. Under X-ray fluoroscopy guidance, a puncture needle of appropriate size (such as 21-27 G) is inserted into the intervertebral disc, and the depth and angle of the puncture needle are adjusted to make the needle insert into the center of the intervertebral disc parallel to the endplate, with a depth of 2.0-3.0 mm. The needle is kept in the disc for 5-30 s before slowly withdrawing.

Pain Behavioral Manifestations:

This model can induce progressive mechanical allodynia, usually starting 3 days after surgery, stabilizing within 2-4 weeks, and persisting for more than 8 weeks; persistent thermal hyperalgesia, thermal paw withdrawal latency reduced

from normal 10-12 s to 6-8 s, and this thermal hyperalgesia state persists for a long time; spontaneous pain behaviors such as frequent paw licking, paw lifting, weight avoidance of the affected limb, as well as motor dysfunction and abnormal posture.

Advantages and Limitations:

Advantages: Simulates the natural history of human disc herniation; the degree of intervertebral disc degeneration can be controlled by changing the selection of puncture needles (different diameters), puncture depth, and combination with inflammatory factors.

Limitations: Mainly simulates intervertebral disc degeneration caused by annulus fibrosus injury, rarely directly compressing nerve roots; slow pathological process, not suitable for acute research.

6.4. Lumbar Disc Herniation Model Selection Guide

The CCD model is suitable when focusing on the effect of mechanical compression on nerve roots, mainly used to explore the pathophysiological principles of nerve compression or compare the effects of different decompression methods; the autologous nucleus pulposus transplantation animal model can be considered when focusing on the chemical mechanism of pain in disc herniation; the annulus fibrosus puncture model is an ideal choice if the research focuses on the process and mechanism of intervertebral disc degeneration itself, or needs to simulate the long-term process of chronic disc herniation, such research usually focuses on strategies to delay intervertebral disc degeneration or treatment of chronic pain [50].

7. Summary of Animal Model of CPDs

In conclusion, there is no perfect animal model of chronic pain diseases at present; researchers should select the most appropriate model from existing ones according to specific scientific questions. When constructing new models, the following conditions should be met as much as possible: able to simulate the core symptoms of clinical CPDs; good reproducibility and stability; objective and quantifiable pain behavioral indicators; similar mechanism to human CPDs; applicable to evaluating the efficacy of drugs and other treatments.

Currently, no animal model can fully mimic all clinical features of human chronic pain. Selection should be based on pain type, research purpose, surgical difficulty, modeling cycle, and translational value. Future model development should focus on better recapitulating the emotional and cognitive dimensions of pain, reducing species differences, improving standardization, and combining genetic engineering and in vitro organoid models to bridge preclinical and clinical research gaps.

8. Pain Behavioral Assessment Methods

Pain behavioral assessment is a technical method to evaluate the evolution of various pain behavioral phenotypes in CPD model animals before and after modeling. According to different assessment methods, it can be divided into two categories: reflexive behavioral testing and non-reflexive complex behavioral testing. Reflexive behavioral testing mainly assesses simple spinal reflexes induced by external stimuli (such as paw withdrawal reflex, paw licking reflex), also known as evoked pain testing. Evoked pain testing is further divided into mechanical stimulation, thermal stimulation, and cold stimulation testing according to the nature of stimulation.

Non-reflexive and complex behavioral testing assess complex cognitive-behavioral behaviors requiring the functional areas of the cerebral cortex and hypothalamus, including: spontaneous pain testing, conditioned place preference test, spontaneous activity observation, swimming test, etc. Modern pain research increasingly emphasizes the combined use of multiple methods to comprehensively capture various pain phenotypes and improve the reliability of test results.

8.1. Spontaneous Pain Behavioral Assessment Methods

Spontaneous pain refers to pain experience without obvious external stimulation, one of the most common symptoms in clinical chronic pain patients. Model animals exhibit various spontaneous pain behaviors, including spontaneous protective behaviors (such as licking, scratching, paw lifting), abnormal posture (affected limb avoidance, uneven weight bearing), activity changes (decreased activity, abnormal gait), and facial expression changes.

8.1.1. Spontaneous Protective Behavior Score

This method specifically assesses animal self-protective behaviors such as paw lifting, paw licking, scratching, and limb shaking, usually evaluated by recording the frequency or duration of such behaviors. There is no "original" relevant literature; Bennett and Xie described the key behavioral indicator of "spontaneous paw lifting/licking behavior" in detail in their 1988 paper on the chronic constriction injury model of the sciatic nerve [5].

8.1.2. Postural Abnormality Behavior Analysis

Assesses postural protection and weight bearing changes caused by pain in animals. Video recording and analysis of animal standing posture are used to quantify weight bearing asymmetry and posture score. For example, animals under visceral pain often assume an "abdominal contraction" posture. A dynamic weight bearing analysis system (such as Incapacitance Tester) is used to measure bilateral limb weight distribution, objectively quantifying the degree of spontaneous pain in animals [51].

8.1.3. Animal Pain Facial Expression Scale

A recently developed method to quantify spontaneous pain by analyzing changes in animal facial expressions, with indicators including orbital tightening, nasal whisker tightening, ear position, cheek bulge, etc., graded 0-2; higher total score indicates more severe pain [51].

8.1.4. Conditioned Place Preference Test

A complex behavioral test for assessing the affective-motivational component of pain, detecting whether animals can learn to associate an environmental location with pain relief or analgesic drug access and form a preference for that location. It assesses both the negative affective component of pain and the pleasurable feeling after pain relief, more objectively and truly reflecting pain perception [52]. For example, in neuropathic pain models, after administration of an effective analgesic, animals will stay longer in the environmental location associated with drug administration.

8.2. Mechanical Allodynia Assessment Methods

8.2.1. Von Frey Test

Von Frey filaments have a long history of use. Chaplan et al. [53] standardized and optimized the Up-Down data reading method in 1994, which is currently regarded as the gold standard for evaluating static mechanical allodynia. This method uses a series of standardized filaments with different diameters, applied to the paw pads of animals (mainly rats or mice), with the force range of the filaments usually being 0.008-300g. By sequentially applying Von Frey filaments of different grams, the minimum stimulus intensity that causes the animal's paw withdrawal response can be measured, and this value is the mechanical withdrawal threshold (MWT).

The classic Von Frey test adopts the "up-down method", where the gram weight of the next filament used is determined according to the animal's response to the previous stimulus, thereby determining the threshold. During the test, the filament is vertically applied to the surface of the paw pad, bent and held for 2-3 seconds, with an interval of 10 seconds for the next stimulus. Positive responses include clear behaviors such as paw withdrawal, paw licking, or paw shaking. Usually, 3-5 rounds of tests are required, and the average threshold is calculated.

In recent years, the electronic Von Frey system has made the test more automated and accurate. It replaces the original nylon filaments with electronic force sensors and can record the force magnitude and response threshold in real time, avoiding human errors. The advantage of the electronic system is that it can provide continuous force readings and capture changes in the minimum force, making it very suitable for detecting subtle changes in mechanical sensitivity.

8.2.2. Brush Test

The brush test is a method for detecting dynamic mechanical allodynia, i.e., pain induced by gentle tactile stimulation (such as pain caused by clothing touching the skin), which is regarded as a behavioral manifestation of central sensitization [54, 55]. Under normal circumstances, the light tactile signals transmitted by A β fibers may be amplified due to neuronal sensitization and incorrectly interpreted as pain sensations.

Commonly used detection tools are soft brushes or soft art brushes of a certain standard size for stimulation. The brush (or art brush) is brush past the skin surface at the same speed and force, and pain-related actions such as paw withdrawal, paw licking, or paw shaking are observed and recorded. At present, most studies on the mechanism of neuropathic pain combine the Von Frey method and the brush method to evaluate mechanical allodynia and hyperalgesia.

8.3. Thermal Hyperalgesia Assessment Methods

8.3.1. Thermal Radiation Method – Hargreaves Method

This method evaluates the thermal pain sensitivity of unrestrained animals and is a classic method first proposed by Hargreaves et al. [56] in 1988. The principle of this detection method is to focus thermal radiation on the animal's plantar surface and record the latency of its paw withdrawal response, i.e., paw withdrawal latency (PWL). The test instrument consists of a glass floor, a movable thermal radiation source, and an automatic timing device. The radiation intensity is usually set to 50-100 mW/cm², the focal diameter is about 5 mm, and the cutoff time is generally set to 20-30 s.

During detection, the animal is placed in a transparent plexiglass cage, and thermal radiation is concentrated on the plantar surface of the animal's hind paw on the glass floor. The time from the start of irradiation to the occurrence of the paw withdrawal response is recorded. The same test point is repeated more than 5 times (at least 5 minutes apart each time) to eliminate the effects of sensitization and habituation. Finally, the average value of the 5 test results is taken as the PWL. Its advantage is that it is non-contact, basically eliminating skin tactile interference.

8.3.2. Hot Plate Test

The hot plate test is also a classic method to evaluate thermal pain sensitivity, first described by Macdonald et al. [57] in 1946. After Eddy and Leimbach [58] improved the hot plate method in 1953, indicators such as paw licking/jumping were determined as the basis for judging animal pain responses. The animal is placed on a metal plate with a temperature of 52-55 °C, and the time of paw licking or jumping is recorded. A cutoff time (such as 60 s) is usually set to avoid tissue damage.

8.4. Cold Hyperalgesia Assessment Methods

8.4.1. Cold Plate Test

Allchorne et al. [59] proposed the cold plate method in 2005, which can accurately quantify the paw withdrawal response of freely moving rats to cold stimulation, providing a standardized quantitative method for detecting cold allodynia and cold hyperalgesia in animals.

By placing the animal on a temperature-controlled cold metal plate (usually 0–4 °C), its behavioral responses related to cold stimulation are observed. The main evaluation indicators include PWL (time from placement to the first paw lifting), number of paw lifts, and total duration of paw lifting. The test time is usually limited to a maximum of 120 seconds to prevent low-temperature damage.

8.4.2. Acetone Evaporation Method

Choi et al. [60] reported the use of the acetone evaporation method to evaluate the cold sensitivity of animals in 1994. That is, model animals have obvious paw lifting and paw licking behavioral responses to acetone application on the plantar surface, proving that the acetone test can be used as one of the common standards for judging cold hyperalgesia. First, a small amount of acetone (about 50–100 µl) is spotted on the animal's paw pad. The evaporation of acetone takes away heat, causing cold stimulation to the skin. The number of paw lifting and paw licking responses and the duration of paw lifting of mice or rats within different time periods after the experiment are observed and counted.

8.5. Precautions for Pain Behavioral Assessment

Selecting an appropriate detection method needs to consider various factors such as research objectives, pain models, detection dimensions, and equipment conditions. It is recommended to apply multiple detection methods in combination to comprehensively reflect various phenotypes of pain. To obtain reliable and reproducible pain behavioral data, the following aspects should be noted:

8.5.1. Standardized Operation

Experimental operators need to be trained to ensure standardized operation procedures (especially when using subjective scoring methods). In addition, the detection process also needs to be standardized: before each test, animals should be fully adapted to the test environment and familiar with the experimenter in advance; tests should be conducted at a fixed time every day to exclude the impact of diurnal fluctuations; the ambient temperature and humidity should be constant, etc.

8.5.2. Blinding Design

The experimental grouping design must be blinded to the actual detector, that is, the detector does not know the grouping of experimental animals in each group (for example: control group vs model group, solvent group vs administration group), especially for detection methods that require subjective judgment, to avoid unconscious bias.

8.5.3. Positive Control

It is recommended to set a positive control when adopting a certain detection method for the first time or applying a known detection method in a new pain model to verify the sensitivity of the detection method or the credibility of the model.

Abbreviations

CPDs	Chronic Pain Diseases
ICD-11	The 11th Revision of the International Classification of Diseases
CCI	Chronic Constriction Injury
SNI	Spared Nerve Injury
SNL	Spinal Nerve Ligation
CCI-ION	Chronic Constriction Injury to Infraorbital Nerve
PHN	Postherpetic Neuralgia
VZV	Varicella-Zoster Virus
HSV-1	Herpes Simplex Virus Type 1
RTX	Resiniferatoxin
DPN	Diabetic Peripheral Neuropathy
STZ	Streptozotocin
PSCIP	Post-Spinal Cord Injury Pain
CPSP	Central Post-Stroke Pain
MCAO	Middle Cerebral Artery Occlusion
CFA	Complete Freund's Adjuvant
AWR	Abdominal Withdrawal Reflex
VMR	Visceromotor Response
LDH	Lumbar Disc Herniation
CCD	Chronic Compression of Dorsal Root Ganglia

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Conflict of Interest

The authors declare no conflicts of interest.

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