EXPERIMENTAL MODELS AND TESTS FOR NOCICEPTIVE AND NEUROPATHIC PAIN EVALUATION

Vesela Kokova
Department of Pharmacology, Toxicology and Pharmacotherapy, Faculty of Pharmacy, Medical University-Plovdiv, Bulgaria, vesela.kokova@mu-plovdiv.bg
Elisaveta Apostolova
Department of Pharmacology, Toxicology and Pharmacotherapy, Faculty of Pharmacy, Medical University-Plovdiv, Bulgaria, elisaveta.apostolova@mu-plovdiv.bg

Abstract: Pain, both acute and chronic, is an extremely prevalent symptom and remains a significant health problem with a great impact on function and quality of life. There are many experimental tests and models for pain evaluation, which are used during the preclinical characterization of drugs. Rodents are widely used to study the pathophysiology of pain as studies in humans are difficult to perform and ethically limited. The use of animal models can reveal the mechanisms, side-effects, and new effective therapeutic possibilities for patients suffering from acute and chronic pain syndromes, which could alter our clinical outlook. The wide range of animal models allows exploration of pain mediators and mechanisms, different pain etiologies and manifestations, and could reveal the mode of action of analgesics. In this review, the most commonly used animal models and experimental tests used in the study of nociceptive and neuropathic pain are discussed. For nociceptive pain examination, stimulus-evoked methods and temperature preference tests are used. The tests with a mechanical stimulus include the Paw pressure test, Pin-prick test, Incapacitance Test, and Von Frey Test. *Tests with a chemical stimulus, such as the* Formalin test *and Abdominal* writhing *test are* described. *The tests with a heat stimulus include* the Hot Plate Test, Plantar Test, and Tail-Flick Test. Acetone Spray Test *like* a test with a cold stimulus is also *summarized*. To study neuropathic pain, the ideal methods should induce solely sensory neurons dysfunction, such as allodynia and hyperalgesia for short periods. Models of diabetic polyneuropathy, chemotherapy-induced painful peripheral neuropathy (CIPN), chronic constriction injury of *n. ischiadicus*, and spinal nerve ligation in rats are described. CIPN is most often induced by vinca alkaloids (vinblastine, vincristine), platinum derivatives (cisplatin, oxaliplatin, carboplatin), and taxanes (docetaxel, paclitaxel). Depending on the chemotherapeutic agent used, CIPN can be pure sensory neuropathy or mixed sensorimotor neuropathy with or without autonomic nervous system dysfunction. The occurrence and severity of the neurotoxicity depend on the type of drug used and are dose-dependent. The rate of CIPN correlates to the cumulative dose delivered and dose per treatment cycle. For neuropathic pain examination, tests with a mechanical stimulus (Von Frey Hair Test), a heat stimulus (Heath Plantar Test), and a cold stimulus (Cold plate test) are used. The methodology of each animal model is specific and results can be greatly influenced even by slight changes related to the study design. Different experimental models for pain evaluation were developed over the years and the search for new methods will continue in the future.
Keywords: neuropathic pain, nociceptive pain, animal models, experimental tests, rodents

1. INTRODUCTION

Pain, both acute and chronic, is an extremely prevalent symptom and remains a significant health problem with a great impact on function and quality of life. There are many experimental tests and models for pain evaluation, which are used during the preclinical characterization of drugs. Rodents are widely used to study the pathophysiology of pain as studies in humans are difficult to perform and ethically limited. However, pain in animals cannot be measured directly, and therefore pain-suggesting behavior is reported [32]. This is usually a limb withdrawal reaction when a nociceptive stimulus is applied. The animal models of pain can reveal the mechanisms, side-effects, and new effective therapeutic possibilities for patients suffering from acute and chronic pain syndromes, which could alter our clinical outlook. The wide range of animal models allows exploration of pain mediators and mechanisms, different pain etiologies and manifestations, and could reveal the mode of action of analgesics [16].

2. EXPERIMENTAL TESTS USED FOR NOCICEPTIVE PAIN EXAMINATION

2.1. Tests with a mechanical stimulus

Paw pressure test (Randall–Selitto test)

The test is usually performed on the hind paw or tail and uses an apparatus, known as Analgesimeter. The method was initially described by Randall and Selitto [27]. It measures the force of pressure at which the pain threshold is

reached and the animal withdraws the limb or tail. The test consists of applying gradually increasing mechanical pressure to the rat's hind paw. The level of pressure (in relative units PPT on the linear scale of the apparatus) at which the motor reaction occurs is related to the severity of the pain [36].

Pricking pain test

This test uses a device known as a "Rodent pincher" and is based on applying pressure by pinching a rodent's hind paw. The force applied at the moment when the animal tries to pull or lift the limb correlates to the pain threshold. The nociceptive threshold is defined as the time from the starting point of pressure application to the moment of reaction [36].

Incapacitance Test

The animal is positioned in a chamber, a part of an apparatus. The apparatus measures the distribution of the body weight of the animal on the hind limbs. Both of its hind paws are placed on different plates, which detect the pressure that the animal applies on every limb. Usually, one of the limbs is injured (a model of inflammation or nerve injury). The distribution of the body weight correlates with the level of pain in the injured paw [40].

Von Frey Hair Test (FHT)

Fibers with different rigidity (Von Frey hairs) or electronic von Frey devices are used. They apply gradually increasing pressure in the middle of the lower surface of the animal's hind paw. The animal withdraws its paw when the pain threshold is reached [12]. The apparatus measures the force of the pressure that causes the motor reaction, and the latency time for withdrawal of the paw.

2.2. Tests with a chemical stimulus

Formalin test

Two hundred microliters 0,2% formalin solution is injected subcutaneously in the lower surface of the hind paw of the animal. The animal is placed for observation in a transparent cage. The formalin injection causes specific behavior, namely trembling, licking, and/or biting of the injected paw. The licking/biting time of the affected paw during the first 10 minutes after the injection (1st phase) and during the period 20th-30th minutes after the injection (2nd phase) is recorded. The first phase is a result of direct chemical stimulation of the nociceptive receptors. The second phase is a result of peripheral inflammation and subsequent excitation of nociceptive spinal neurons [17,20].

Abdominal writhing test

Pain is induced by intraperitoneal injection of irritants and 3% acetic acid is the most commonly used. Acetic acid induces chemical irritation and an inflammatory response in the abdominal cavity, with subsequent activation of nociceptors. The animals react with stretching of the spine which is called writhing. The number of writhings during the first 20 minutes after the injection is determined [39].

2.3. Tests with a heat stimulus

Plantar Test (HPT)

The method consists of the application of an infrared ray on the lower surface of the animal's hind paw. When the pain threshold is reached, the animal withdraws its paw. The latency of the withdrawal is measured and used as a criterion for nociception [40].

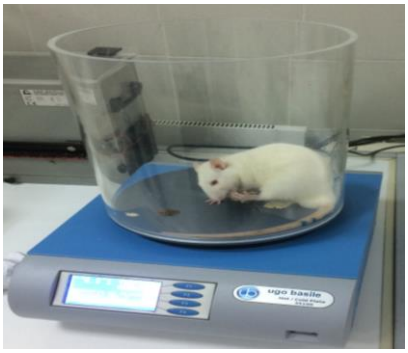
Tail-Flick Test (TFT)

The test is similar to the Plantar test (see above), however, the thermal stimulus is applied on the rat's tail. When the pain threshold is reached, the rat withdraws the tail with a swift movement (tail flick), which terminates the thermal stimulation [12]. The time from the application of the stimulus to the withdrawal of the tail is measured, three consecutive tests are performed and the average value is determined. The time of exposure to the heat stimulus should not exceed 10-20 seconds due to the risk of skin burns.

Hot Plate Test (HP)

The animal is placed in a transparent container. The floor represents a hot plate, which temperature could be adjusted (Fig.1). The pain threshold is determined via the animal behavior. The plate temperature is kept constant at 55°C (±5°C) and the time for vocalization, escape attempt, or licking of the hind paw is evaluated. Three more tests are performed – on the 60th, 120th, and 180th minute. The time of exposure to the heat stimulus should not exceed 30 seconds to avoid thermal injury of the paws [1,38].

Figure 1. Hot/Cold Plate (Ugo Basile, Italy) Test.



2.4. Tests with a cold stimulus

Acetone Spray Test

This test is used mainly for the evaluation of cold-induced allodynia and consists of dripping acetone on a rat's paw. The animal is placed in a cage with a mesh floor. After a period of adaptation, the skin of the lower surface of one of the paws is sprayed with 50μL of acetone. Evaporation of acetone cools the skin to a temperature of 15-21°C. The time of rapid withdrawal, shaking, licking, or biting of the limb is measured for a period of 30 seconds to 5 minutes after each application of acetone. Uninjured rats do not respond (or respond very poorly) to the sensation of cooling that acetone causes by evaporation, while animals with neuronal damage respond with an enhanced response [38].

2.5. Temperature Preference Test

In this test, the animal can move freely within a cage, which floor is divided into two areas and each of them has different temperature. The animals are placed on a floor with a normal temperature but can reach the area with a specific temperature (between 5°C and 55°C). The time spent on the two areas was determined and compared to that of the control animals.

In another variant of the test, the floor temperature of the cell changes with a constant gradient linearly or circularly. Placed in the cage, the animal initially explores the new environment, but then stays for a long time in a certain part of the cage, whose temperature it prefers [38].

3. ANIMAL MODELS TO INVESTIGATE NEUROPATHIC PAIN

The ideal model should induce solely sensory neurons dysfunction, such as allodynia and hyperalgesia for short periods. The most frequently used animal models are summarized below.

3.1. Diabetic neuropathy

A single injection of 75 mg/kg streptozocin intraperitoneally in rats induces diabetic polyneuropathy that initially is characterized by changes in tactile sensitivity (allodynia) followed by decreased thermal nociceptive threshold [40].

3.2. Chemotherapy-induced painful peripheral neuropathy

Chemotherapy-induced peripheral neuropathy (CIPN) is most often induced by vinca alkaloids (vinblastine, vincristine), platinum derivatives (cisplatin, oxaliplatin, carboplatin), taxanes (docetaxel, paclitaxel), thalidomide, bortezomib, and ixabepilone [10,26]. The toxic attack is directed against the peripheral nerves and the targets of therapy-induced toxicity are the neuronal soma, the axonal microtubules, the axonal transport system, the myelin sheath, and glial cells [31]. Abnormalities in mitochondrial structure and function in peripheral sensory fibers that are associated with neuropathic pain induced by chemotherapeutic agents have been reported. [8,37]. Oxaliplatin has been shown to affect voltage-gated sodium channels in sensory neurons which leads to sensory hyperexcitability [10, 23, 24].

Depending on the chemotherapeutic agent used, CIPN can be isolated sensory neuropathy (cisplatin, oxaliplatin, carboplatin) or mixed sensory and motor neuropathy with or without autonomic nervous system dysfunction (vincristine, taxanes). The occurrence and severity of the neurotoxicity depend on the type of drug used and are dose-dependent. The rate of CIPN correlates to the cumulative dose delivered and the dose per treatment cycle. A wide variety of factors, including treatment schedule, duration of therapy, concomitant medications, and co-morbidities, affect the incidence of CIPN [21]. Neuropathic pain is an important side effect manifested in many patients and it may occur early in therapy or can be delayed in time. CIPN can develop even after a single drug application (with oxaliplatin) [2]. Moreover, its psychological aspects in malignant diseases should not be underestimated [14]. Many patients develop anxiety, depression, and suicidal thoughts that affect the perception of

pain. It is also reported that the recovery from neuropathic symptoms is often incomplete and a long period of regeneration is required to restore function.
Table 1 presents the most commonly used experimental models causing CIPN in male rats. The table includes the injection scheme of an anticancer agent (taxane, vinca alkaloid, or platinum derivative).

Table 1. Experimental models of CIPN in male rats.

Anticancer agent causing neuropathy	Experimental design	Time	Application	Cumulative dose (mg/kg b.w./animal)	Reference
Vincristine	0,1 mg/kg/day, 5 days/week	12 days	i.v.	1	[35]
Vincristine	0,075 mg/kg/day	10 days	i.v.	0,75	[3]
Vincristine	0,1 mg/kg/day	14 days	i.v.	1,4	[22]
Vincristine	0,15 mg/kg/2 days	10 days	i.v.	0,75	[5]
Vincristine	0,1 mg/kg/day, 5 days/week	12 days	i.p.	1	[34]
Vincristine	0,05 mg/kg/day	10 days	i.p.	0,5	[30]
Paclitaxel	16 mg/kg/week	5 weeks	i.p.	80	[7]
Paclitaxel	32 mg/kg X 1	1 day	i.p.	32	[7]
Paclitaxel	2 mg/kg/2 days	7 days	i.p.	8	[25]
Paclitaxel	1 mg/kg/day, 5 days/week	2 weeks	i.p.	10	[13]
Docetaxel	10 mg/kg, 1/week	4 weeks	i.v.	40	[28]
Cisplatin	2 or 1 mg/kg/3 days	4 weeks	i.p.	15	[4]
Cisplatin	3 mg/kg, 1/week	5 weeks	i.p.	15	[6]
Cisplatin	2 mg/kg, 1/week	5 weeks	i.p.	10	[33]
Cisplatin	0,5 mg/kg/day	3 days	i.p.	1,5	[9]
Cisplatin	2 mg/kg X 1	5 days	i.v.	2	[15]
Oxaliplatin	2 mg/kg, 2/week	4 weeks	i.v.	16	[19]
Oxaliplatin	6 mg/kg X 1	30 hours	i.p.	6	[18]
Oxaliplatin	2 mg/kg X 1	5 days	i.v.	2	[15]

3.3. Chronic constriction injury (CCI) of n. ischiadicus

Different models of CCI have been described. One of the modifications of CCI is reported by Seltzer et al., 1990 [29]. In this model of partial nerve ligation, nerve injury is created by tying loosely double ligature (the dorsal one-third to one-half) of the rat's sciatic nerve. Sciatic nerve ligation results in allodynia 12 to 15 days after injury.

3.4. Spinal nerve ligation (SNL)

Many different variations of the SNL model have been reported. The original model was described by Chung et al. and it was developed by tightly ligating one (L5) or two (L5 and L6) segmental spinal nerves in the rat. The operation results in long-lasting pain and behavioral signs of mechanical allodynia, cold allodynia, and heat hyperalgesia [11].

4. EXPERIMENTAL TESTS USED IN THE STUDY OF NEUROPATHIC PAIN

1. Test with a mechanical stimulus

Von Frey Hair Test (see above)

2. Test with a heat stimulus

Heath Plantar Test (see above)

3. Test with a cold stimulus

Cold plate test

The experimental setup is similar to that of the hot plate test, but the set plate temperature is in the range of −5 to +25°C. The maximum time spent by the animal on the plate is 150 seconds. No more than one test per day is performed to avoid the analgesic effect of low temperatures [38].

4. CONCLUSION

The study of pain and analgesia is an important area of pharmacological research that has led to several significant breakthroughs in the treatment of acute and chronic pain. Different experimental models of pain evaluation were developed over the years and the search for new methods will continue in the future.

REFERENCES

Апостолова, Е. (2020). Експериментални модели на зависимост и болка при животни. ИПК „Екобелан“-Асеновград, ISBN 978-619-7561-17-3.

Костадинов, И. (2013). Кломипрамин и флуоксетин – аналгетично и противовъзпалително действие в експеримент. Автореферат на дисертация за присъждане на образователна и научна степен „Доктор“. Пловдив, Медицински Университет, Медицински факултет.

Краевски, П. (2011). Модулация от нестероидни и стероидни адюванти на ноцицепцията и ефекта на някой аналгетици. Невропатични и възпалителни експериментални модели и клинични наблюдения. Автореферат на дисертация за присъждане на образователна и научна степен „Доктор“. София, Българска Академия на Науките, Институт по невробиология.

Abboud, C., Duveau, A., Bouali-Benazzouz, R., Massé, K., Mattar, J., Brochoire, L., Fossat, P., Boué-Grabot, E., Hleihel, W., & Landry, M. (2021). Animal models of pain: Diversity and benefits. *Journal of neuroscience methods*, *348*, 108997.

Authier, N., Balayssac, D., Marchand, F., Ling, B., Zangarelli, A., Descoeur, J., Coudore, F., Bourinet, E., & Eschalier, A. (2009). Animal models of chemotherapy-evoked painful peripheral neuropathies. *Neurotherapeutics : the journal of the American Society for Experimental NeuroTherapeutics*, *6*(4), 620–629.

Authier, N., Coudore, F., Eschalier, A., & Fialip, J. (1999). Pain related behaviour during vincristine-induced neuropathy in rats. *Neuroreport*, *10*(5), 965–968.

Authier, N., Fialip, J., Eschalier, A., & Coudoré, F. (2000a). Assessment of allodynia and hyperalgesia after cisplatin administration to rats. *Neuroscience letters*, *291*(2), 73–76.

Authier, N., Gillet, J. P., Fialip, J., Eschalier, A., & Coudore, F. (2003). A new animal model of vincristine-induced nociceptive peripheral neuropathy. *Neurotoxicology*, *24*(6), 797–805.

Authier, N., Gillet, J. P., Fialip, J., Eschalier, A., & Coudore, F. (2003b). An animal model of nociceptive peripheral neuropathy following repeated cisplatin injections. *Experimental neurology*, *182*(1), 12–20.

Authier, N., Gillet, J. P., Fialip, J., Eschalier, A., & Coudore, F. (2000). Description of a short-term Taxol-induced nociceptive neuropathy in rats. *Brain research*, *887*(2), 239–249.

Bennett, G. J. (2010). Pathophysiology and animal models of cancer-related painful peripheral neuropathy. *The oncologist*, *15 Suppl 2*, 9–12.

Cata, J. P., Weng, H. R., & Dougherty, P. M. (2008). Behavioral and electrophysiological studies in rats with cisplatin-induced chemoneuropathy. *Brain research*, *1230*, 91–98.

Cavaletti, G., & Marmiroli, P. (2010). Chemotherapy-induced peripheral neurotoxicity. *Nature reviews. Neurology*, *6*(12), 657–666.

Chung, J. M., Kim, H. K., & Chung, K. (2004). Segmental spinal nerve ligation model of neuropathic pain. *Methods in molecular medicine*, *99*, 35–45.

Deuis, J. R., Dvorakova, L. S., & Vetter, I. (2017). Methods Used to Evaluate Pain Behaviors in Rodents. *Frontiers in molecular neuroscience*, *10*, 284.

Dina, O. A., Chen, X., Reichling, D., & Levine, J. D. (2001). Role of protein kinase Cepsilon and protein kinase A in a model of paclitaxel-induced painful peripheral neuropathy in the rat. *Neuroscience*, *108*(3), 507–515.

Hough, S.W., & Kanner, R.M. (2004). Cancer pain syndromes. *Principles and practice of pain medicine*, 456-464.

Joseph, E. K., Chen, X., Bogen, O., & Levine, J. D. (2008). Oxaliplatin acts on IB4-positive nociceptors to induce an oxidative stress-dependent acute painful peripheral neuropathy. *The journal of pain*, *9*(5), 463–472.

Kaliyaperumal, S., Wilson, K., Aeffner, F., & Dean, C., Jr. (2020). Animal Models of Peripheral Pain: Biology Review and Application for Drug Discovery. *Toxicologic pathology*, *48*(1), 202–219.

Kostadinov, I., Delev, D., Kostadinova, I., & Georgieva-Kotetarova, M. (2014). Involvement of 5-HT2 and 5-HT3 receptors in the analgesic effect of clomipramine according to the formalin pain test in rats. *Medicine*, *4*(1).

Ling, B., Coudoré-Civiale, M. A., Balayssac, D., Eschalier, A., Coudoré, F., & Authier, N. (2007a). Behavioral and immunohistological assessment of painful neuropathy induced by a single oxaliplatin injection in the rat. *Toxicology*, *234*(3), 176–184.

Ling, B., Authier, N., Balayssac, D., Eschalier, A., & Coudore, F. (2007). Behavioral and pharmacological description of oxaliplatin-induced painful neuropathy in rat. *Pain*, *128*(3), 225–234.

Meunier, C. J., Burton, J., Cumps, J., & Verbeeck, R. K. (1998). Evaluation of the formalin test to assess the analgesic activity of diflunisal in the rat. *European journal of pharmaceutical sciences: official journal of the European Federation for Pharmaceutical Sciences*, *6*(4), 311–316.

Murtagh, F.E., & Higginson, I.J. (2007). Cancer neuropathic pain. *Neuropathic pain*, 67-75.

Nozaki-Taguchi, N., Chaplan, S. R., Higuera, E. S., Ajakwe, R. C., & Yaksh, T. L. (2001). Vincristine-induced allodynia in the rat. *Pain*, *93*(1), 69–76.

Pachman, D. R., Barton, D. L., Watson, J. C., & Loprinzi, C. L. (2011). Chemotherapy-induced peripheral neuropathy: prevention and treatment. *Clinical pharmacology and therapeutics*, *90*(3), 377–387.

Park, S. B., Lin, C. S., Krishnan, A. V., Goldstein, D., Friedlander, M. L., & Kiernan, M. C. (2009). Oxaliplatin-induced neurotoxicity: changes in axonal excitability precede development of neuropathy. *Brain: a journal of neurology*, *132*(Pt 10), 2712–2723.

Polomano, R. C., Mannes, A. J., Clark, U. S., & Bennett, G. J. (2001). A painful peripheral neuropathy in the rat produced by the chemotherapeutic drug, paclitaxel. *Pain*, *94*(3), 293–304.

Quasthoff, S., & Hartung, H. P. (2002). Chemotherapy-induced peripheral neuropathy. *Journal of neurology*, *249*(1), 9–17.

Randall, L. O., & Selitto, J. J. (1957). A method for measurement of analgesic activity on inflamed tissue. *Archives internationales de pharmacodynamie et de therapie*, *111*(4), 409–419.

Roglio, I., Bianchi, R., Camozzi, F., Carozzi, V., Cervellini, I., Crippa, D., Lauria, G., Cavaletti, G., & Melcangi, R. C. (2009). Docetaxel-induced peripheral neuropathy: protective effects of dihydroprogesterone and progesterone in an experimental model. *Journal of the peripheral nervous system: JPNS*, *14*(1), 36–44.

Seltzer, Z., Dubner, R., & Shir, Y. (1990). A novel behavioral model of neuropathic pain disorders produced in rats by partial sciatic nerve injury. *Pain*, *43*(2), 205–218.

Siau, C., Xiao, W., & Bennett, G. J. (2006). Paclitaxel- and vincristine-evoked painful peripheral neuropathies: loss of epidermal innervation and activation of Langerhans cells. *Experimental neurology*, *201*(2), 507–514.

Stillman, M., & Cata, J.P. (2006). Management of chemotherapy-induced peripheral neuropathy. *Curr Pain Headache Rep.*, *10*, 279 –287.

Turner, P. V., Pang, D. S., & Lofgren, J. L. (2019). A Review of Pain Assessment Methods in Laboratory Rodents. *Comparative medicine*, *69*(6), 451–467.

Vera, G., Chiarlone, A., Cabezos, P. A., Pascual, D., Martín, M. I., & Abalo, R. (2007). WIN 55,212-2 prevents mechanical allodynia but not alterations in feeding behaviour induced by chronic cisplatin in the rat. *Life sciences*, *81*(6), 468–479.

Weng, H. R., Cordella, J. V., & Dougherty, P. M. (2003). Changes in sensory processing in the spinal dorsal horn accompany vincristine-induced hyperalgesia and allodynia. *Pain*, *103*(1-2), 131–138.

Windebank, A. J., & Grisold, W. (2008). Chemotherapy-induced neuropathy. *Journal of the peripheral nervous system: JPNS*, *13*(1), 27–46.

Xie, W. (2011). Assessment of pain in animals. In animal models of pain. *Humana press*, ISSN 0893-2336.

Zheng, H., Xiao, W. H., & Bennett, G. J. (2011). Functional deficits in peripheral nerve mitochondria in rats with paclitaxel- and oxaliplatin-evoked painful peripheral neuropathy. *Experimental neurology*, *232*(2), 154–161.