REVIEW ARTICLE

Methods for Evaluating Sensory, Affective and Cognitive Disorders in Neuropathic Rodents

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Abstract: The animal models of neuropathic pain that faithfully reproduce the symptoms that occur in humans are a fundamental tool for understanding the mechanisms underlying the disease, identifying new targets, and developing effective drugs. So far, the studies aimed at describing the animal models of neuropathic pain have been focused mainly on the sensory symptoms associated with the disease consisting of mechanical allodynia and hyperalgesia, cold allodynia and hyperalgesia, and heat hyperalgesia. However, affective and cognitive comorbidities occur in patients suffering from neuropathic pain, arising in a closely associated and dependent manner on the sensory symptoms. The same occurs in animal models of neuropathic pain in which anxiety- and depressive-like behaviors and cognitive disorders are observable at different time points from the induction of neuropathy. Today there are several tests available that exploit different paradigms in rodents for measuring sensorial, affective, and cognitive behavior. This review will describe those mainly used in the scientific community. The tests mainly used are based on the motor activity of the animals tested, so it is fundamental that it remains unaffected in the model used for inducing neuropathic pain. We hope that this review will be useful to the scientific community to direct the choice towards the best, most suitable, and simplest tests for the study of the sensory, affective, and cognitive symptoms associated with neuropathic pain.

1. INTRODUCTION

Neuropathic pain is a chronic pain condition caused by a lesion or disease affecting the somatosensory nervous system. Injuries to this system alter the signal transmission to the spinal cord and the brain and its following processing leading to abnormal sensorial symptoms such as burning and electrical-like pain, allodynia (pain resulting from innocuous stimuli), hyperalgesia (increased pain sensation resulting from noxious stimuli), dysesthesia, tingling, and numbness. These symptoms become chronic and are resistant to traditional analgesic medications. Causes and forms of neuropathic pain include post-herpetic or trigeminal neuralgia, diabetes mellitus, HIV infection, leprosy, amputation, painful radiculopathy, peripheral nerve injury due to compression or trauma, and stroke. Neuropathic pain occurs in 1.5–6.9% of the general population, more frequently in women (8%) than in men (5.7%) and in patients over 50 years (8.9%) than under this age (5.6%) [1]. It affects mainly the lower back, lower limbs, neck, and upper limbs [2]. The most frequent causes of neuropathic pain are the radiculopathies of the back and neck [3]. Understanding the plastic changes affecting the nervous system and the pathophysiological mechanisms underlying the initiation, development, and maintenance of neuropathic pain is fundamental for the identification of new targets on which addressing effective drugs. The discovery of new therapies goes hand in hand with the need for animal models of neuropathic pain to transfer the knowledge acquired to clinical practice [4, 5]. Moreover, sensory anomalies are only part of the symptoms of the disease, which extend to the alteration of affective and cognitive behavior. Neuropathic pain is often associated with affective disorders such as depression, anhedonia, impaired family and social interactions, anxiety, and cognitive disorders [6-8]. The prevalence of anxiodepressive disorders in patients suffering from neuropathic pain reaches 30% [9-11]. This is since chronic pain causes anatomical and functional alterations in the main neural circuitries that control pain, affectivity, and cognition in both, humans and rodents. The prefrontal cortex, including anterior cingulate, prelimbic, and infralimbic cortices, through its reciprocal functional connections with mesolimbic dopaminergic system, hippocampus, and amygdala, represents the neural substrate regulating both, sensorial or affective/cognitive components of pain [12]. Apart from the neural circuitries, there is

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also an overlap of neurotransmitters and mechanisms underlying pain, affective behavior, and cognition justifying why sensory alterations are associated with anxiety- and depressive-like behavior and cognitive disorders [13, 14]. Maladaptive alterations in neurotransmitter release, neuron activity, immune cell infiltration, glia-neuron communication, and gut microbiota appear to be closely associated with sensory, affectivity, and cognitive disorders in the spared nerve injury model of neuropathic pain [15]. Considering a large number of high-quality literature in the field, the current review, based on structured research of electronic databases (medline, google scholar, embase, *etc.*), will briefly summarize and outline the main conclusions of the key articles dealing with the most used models and tests for evaluating the sensorial, emotional and cognitive symptoms associated with neuropathic pain.

1.1. Animal Models of Neuropathic Pain

The most used animal models of neuropathic pain are those that are based on the injury to a peripheral nerve. Often the injury is produced by the compression of the nerve as in the chronic constriction injury (CCI), spinal nerve ligation (SNL), partial sciatic nerve ligation (PSNL), and polyethylene cuff models. The CCI model consists of applying four loose ligatures around the sciatic nerve [16]. A variant of this model, based on the application of a single loose ligature around the sciatic nerve, produces the same sensorial, affective, and cognitive disorders. Furthermore, compared to the CCI model with four ligatures, it induces fewer motor impairments and does not cause autotomy [17]. The spinal nerve ligation (SNL) model is produced by the tight ligation of the L5 and L6 spinal nerves [18]. A variation of the latter model is the tight ligation of the spinal nerve L5 only [19]. The partial sciatic nerve ligation (PSNL) is also based on a tight ligature applied on one third to half of the sciatic nerve [20]. Another way for producing nerve compression is the implantation of a polyethylene cuff around the main branch of the sciatic nerve [21]. The spared nerve injury (SNI) is among the most used models of neuropathic pain. It is based on the axotomy of two branches of the sciatic nerve (common peroneal and tibial), leaving the sural component intact [22]. A variant of the SNI model is the tibial nerve transection (TNT), which consists of the axotomy of the tibial branch only, leaving the sural and common peroneal components intact [23]. The spinal cord injury (SCI) model represents an animal model of neuropathic pain in which the injury affects the central nervous system and not a peripheral nerve. The injury is produced by a weight falling on the spinal cord, which has been previously exposed [24] or by inducing occlusion of small vessels feeding the spinal cord and thrombosis [25]. Neuropathic pain models may also reproduce trigeminal neuralgia such as the infraorbital nerve constriction (CION), which exploits the same technique of the CCI on the infra-orbital nerve [26], and trigeminal inflammatory compression (ICT) in which a suture is applied on the same nerve [27]. Alternatively, neuropathic pain can be induced by the administration of neurotoxic anti-tumoral agents, such as vinca alkaloids, platinum compounds, and taxols [28-30] or the antiretroviral stavudine [31]. Another way to induce neuropathic pain is based on reproducing diseases that are associated with its development in humans, such as diabetes, which is induced by streptozotocin [32], human immunodeficiency virus-1 (HIV-1), which is induced by virus coat protein gp120 [33] or postherpetic neuralgia induced by the infection with varicella-zoster virus [34, 35].

1.2. Measurements of Pain Responses

Sensory symptoms associated with neuropathic pain have been widely described in both, humans and rodents. Clinically, pain is evaluated verbally, which is not feasible in rodents. Therefore, the choice of the test to evaluate the pain threshold assumes critical importance in preclinical studies. Moreover, most of the nociceptive tests commonly used to measure pain in rodents are evoked by stimuli (mechanical, thermal, chemical), while in humans, spontaneous pain is the predominant symptom. Novel tests such as the Grimace scale, burrowing assay, gait analysis, weight-bearing, and automated behavioral analysis have been recently introduced for measuring spontaneous pain in rodents [14, 36, 37].

1.3. Measurements of Pain Evoked by Stimuli

Tests for measuring pain evoked by the stimuli quantify the latency or force evoking a nocifensive reaction in rodents. Among these, manual and electronic von Frey, Randall-Selitto, tail-flick, and Hargreaves' tests are those widely used. In most cases, the stimulus is applied on the hind paw, so in neuropathic pain models that are based on the unilateral injury, the contralateral hind paw to the insult provides an internal control for the experiment. Alternatively, the stimulus can be applied to the tail.

The Von Frey test represents the reference test for measuring mechanical allodynia. The animals are positioned in cages with a mesh floor, which is penetrated by a metal filament applied for 2–5 s on the plantar surface of the hind paw with a predetermined and constant force (0.2-13.7 mN for mice and 5.9–98 mN for rats). The nocifensive response consists of the rapid withdrawal, shaking, or licking of the stimulated paw. Alternatively, other body surfaces such as the abdomen or dorsal surface of the hind paw can be stimulated using the same filaments. Recently, an automated version of the Von Frey has been introduced: the plantar dynamic aesthesiometer. It uses a single filament applied with increasing force to evoke the withdrawal of the paw; this force value is automatically recorded. The advantage of this automated version of von Frey is that the same filament can always be used, thus greatly saving the time of the test. However, the discrimination between proper from false responses due to touch or normal walking or hind paw movements remains critical.

The Randall-Selitto, which is also called the paw pressure test, consists of applying a mechanical stimulus with increasing force on the plantar or dorsal surface of the forepaw, hind paw or tail up to the withdrawal or vocalization evoked by the pressure [38]. In this way, the test allows for

measuring mechanical hyperalgesia. The main disadvantage of the test is the restraint, particularly critical in mice, in which the test does not apply to the paw [39-41] and is preferred the application of the mechanical stimulus on the tail [42]. The previous handling of the rodent is necessary to avoid stress-induced false responses.

The hot plate test [43] consists of placing a mouse or rat on a metal surface kept at a constant temperature (between 50 and 55 °C). It measures thermal sensitivity. A limit of the test is that the nocifensive response is highly variable, consisting of the withdrawal, licking, stamping, shaking of the paws, freezing, or jumping [44]. The dynamic version of the hot plate exploits a heat flow, starting with a non-harmful temperature (<42), which increases at a constant speed. The nocifensive reaction, based on the same responses of the static version, stops the heating through a sensor and records the time of latency and the temperature evoking the response [45, 46]. Manual removal of the rodent from the heated surface is a critical point of the test since the slightest delay, in addition to distorting the outcome, could induce tissue damage [47]. Furthermore, these tests have the limit of the tendency to learn (especially in rats) that may shorten the latency [48, 49].

The limitations of these tests have been overcome by the Hargreaves' test, which consists of the focused application of radiant infrared heat on the plantar surface of the hind paw and the automatic or manual quantification of the latency of the nocifensive response recorded as thermal withdrawal latency [50]. The test apparatus consists of plexiglass transparent enclosures in which rodents can stay unrestraint, anti-refraction glass floor, and a mobile infrared heat source unit below the latter. The method allows measurement on both ipsilateral and contralateral hind paws and does not require restraint. The habituation allows minimizing the exploratory walking, a factor which is particularly critical in the mouse, which requires a longer habituation time [51-53].

The tail-flick is a test based on the application of a heat stimulus, radiant heat or hot water, on the tail of mice or rats and the recording of the time, which evokes the abrupt twitch of the tail from the thermal stimulus (tail flick) [54]. Although the test is relatively quick and easy to perform, it requires the restraint of both, rats and mice. The tail-flick response is a spinal reflex rather than an integrated response to pain involving the supraspinal centers [55]. However, the slope of heating and temperature are believed to be crucial elements that can involve, at least in part, the supraspinal areas [56]. The test has often been associated with single-unit extracellular recordings of the rostral ventromedial medulla (RVM) to characterize a population of pain-responding neurons: the ON and OFF cells [57]. Simultaneous measurement of the spontaneous and/or induced activity of ON and OFF neurons and behavioral responses to tail-flick allow identification of the analgesic (or pain-facilitating) potential of pharmacological compounds or treatments [58, 59].

The thermal probe test is a novel method which permits to evaluate the thermal threshold using the same apparatus for the von Frey or dynamic plantar aesthesiometer. The wire mesh floor is penetrated by a slightly rounded, 2 mm large mobile thermal probe. It is applied to the plantar surface of the hind paw and heats on contact. The mobile probe starts heating from 37°C until the cut-off (60°C), the withdrawal response automatically stops the heating and the temperature at which this occurs is automatically recorded. The thermal thresholds of both, ipsilateral and contralateral hind paws can be quantified in unilateral models of neuropathic pain. The opportunity to measure the mechanical and thermal thresholds in the same apparatus permits apart from avoiding the restraint to save the time necessary for habituation [60].

The acetone evaporation test quantifies cold allodynia. It consists of buffering or spraying a drop of acetone on the plantar surface of the hind paw and recording the nocifensive reaction evoked by the cooling due to the evaporation [61-65]. The control is easily made by applying a drop of water at 30 °C [61, 62]. The difficulty of the test consists of the fact that acetone has a low surface tension, which makes the formation of uniform drops through pipette or syringe difficult [66, 67]. The quantification of nociceptive responses is based on the number and duration of the nocifensive responses or the use of a score based on the intensity of the nocifensive behavior (no response = 0, rapid withdrawal or twitch = 1; repeated flicking of the paw = 2; repeated flicking of the hind paw and licking of the paw = 3). The use of video recording in a slow-motion setting makes the score quantification easier [64, 68].

The cold plate test permits to measure cold-induced allodynia and hyperalgesia, quantifying the behavioral responses to innocuous and noxious cold, respectively [69]. The animal is placed on a cooling plate and the latency of the nocifensive response is recorded. The nocifensive reaction consists of the shaking or licking of the fore or hind paws or jumping. This test is particularly suitable for unilateral pain models because it allows the guarding of the injured paw while in bilateral pain models, the nocifensive reactions are identified more critically.

The cold plantar assay also measures cold allodynia and hyperalgesia. It consists of applying a cold stimulus to the hind paw using a cut off syringe filled with dry or wet ice through the glass serving as floor. The time of latency to the withdrawal of the hind paw is recorded. The rodent needs to be placed in an enclosure and restraint is avoided. The limit of the test is that the paw to be tested needs to remain in contact with the glass floor for achieving efficient temperature transfer so that any guarding or altered weight distribution of the hind paw may lead to false measurements [70, 71].

Ultrasound vocalizations (22-kHz) measured by condenser microphones associated with software sensitive to high frequencies are also used as an indicator of pain in rodents [72]. However, the quantification of ultrasound vocalization emissions of rodents subjected to pain has yielded mixed results [73-77]. In particular, Wallace and colleagues did not find any correlation between ultrasound vocalizations and withdrawal responses evoked by thermal and mechanical stimulations in rats rendered neuropathic by the partial sciatic

nerve ligation. Indeed, it seems that the measurement of ultrasonic vocalizations gives convincing results only when these are evoked by acute nociceptive stimulations [74].

1.4. Measurements of Spontaneous Pain

Animal tests for the measurement of pain responses evoked by stimuli are difficult to translate to the neuropathic pain condition in humans in which spontaneous pain is the most disabling symptom. Novel methods for measuring spontaneous pain have been now introduced, such as burrowing assays, weight-bearing, Grimace scale, gait analysis, and automated behavioral analysis [78].

The burrowing test is based on the paradigm that the burrowing represents a spontaneous and self-motivated behavior in rodents, so any modification of normal behavior can represent a state of "malaise" and can be quantified as a measure of spontaneous pain or anxiety. The test apparatus consists of a transparent tube closed at one end and raised at the other filled with material suitable for building a burrow such as pellets, sand, or marbles. The quantity of material displaced is weighed and measured. A reduction in the amount of material pushed out from the burrow is an index of malaise, such as that generated by spontaneous pain [79, 80].

In the weight-bearing, also called incapacitance test, the rodent is placed on an inclined holder with the hind paws resting on two separate pressure sensors able to measure the weight distribution of the body on the hind paws. Unequal weight distribution between the ipsilateral and the contralateral paw is related to the degree of spontaneous pain [81-84]. The test is suitable only for unilateral hind paw pain models. The advanced dynamic weight-bearing apparatus represents an evolution of the weight-bearing permitting to measure the weight ratio and the weight-bearing for each paw in unrestrained animals [85].

In the Grimace scale, facial expressions of rats or mice are scored for quantifying spontaneous pain intensity. Orbital tightening, nose and cheek bulge, ear and whisker position are the facial expressions that are scored varying from normal (0), moderate (1), and severe (2) [86, 87]. The limit of the Grimace scale is that it can only be used in pain models that evoke intense and short-lived pain while neuropathic pain models, including CCI and SNI, do not determine changes in facial expression of rodents.

Gait analysis measures gait changes in rodents. Rodents are left free to walk and any change played out to guard the painful limb is recorded and analyzed [88]. Initially, this test was performed by coloring the animal's paws with ink and letting it walk freely on a sheet of paper, then scanned for analysis [89]. Currently, several automated gait analysis devices have been introduced for measuring gait alterations. These devices exploit reflected light illuminating paw prints of the animal freely walking on an elevated glass floor and video recordings associated with software [90-92]. The parameters used to analyze gait changes are the paw pressure intensity, print area, stance phase duration (time spent on paw), and swing phase duration (time spent off paw) of the ipsilateral hind paw. The reduction of paw pressure, print area, and the increase in the swing phase duration correspond to guarding behavior and reducing weight-bearing, which are positive indexes of spontaneous pain [91, 92]. Apart from gait analysis, other behaviors may be indicative of spontaneous pain such as motor activity (still, walking, trotting, running), velocity, distance traveled, posture, eating/drinking, grooming, and foraging.

The automated behavioral analysis, which uses automated video analysis, vibration sensors, photobeams, and combinations thereof, has been developed and can be performed in rodents' home cages or dedicated apparatus. The behavioral spectrometer consists of an enclosed box endowed with a ceiling-mounted fish-eye lens, accelerometer, and a row of wall-mounted photo beams for the real-time recording of different behaviors (ambulation, grooming, rearing, distance traveled and average velocity) [93]. The HomeCageScan exploits an automated video analysis for quantifying predefined behaviors such as sleeping, walking, sniffing, rearing, stretching, foraging, jumping, digging, drinking, eating, hanging, grooming in the rodent home cage [94, 95]. While these tests have the caveats of being no specific, they have the advantages of the unrestraint, unconsciousness, and lack of any stress applied to the tested animals.

1.5. Measurements of Anxiety-like Behavior

There are several tests to measure anxiety-like behavior in rodents, among these, the open field is one of the oldest tests and consists of a lighted square arena which permits to quantify the number of entries and the time spent in the central part. The test is based on the paradigm of the conflict generated by the innate exploratory activity and the fear of the illuminated and unprotected environment in rodents. The anxiety-like behavior corresponds to the preference of exploring the part of the arena close to the walls, whereas the time spent in the center area represents a negative index of anxiety-like behavior. This test is based on the rodent's motor activity, so that it is often used to measure motor activity. It does not permit to discriminate between general locomotor activity and novel environment exploration, and it is often considered for providing an initial screen for anxiety-related behavior in rodents [96].

The light-dark box is another test exploiting the same conflict paradigm between two preferred and non-preferred environments: two communicating chambers, one is brightly lit and open and the other is covered and dark. The rodent's preference is for the darker and covered area, however, the innate tendency to explore a new environment pushes it to enter the non-preferred environment as well. The low propensity to explore the open and illuminated chamber is an index of anxiety-like behavior [97].

Elevated plus-maze is certainly one of the widely used tests for measuring anxiety-like behavior in rodents. The apparatus consists of a cross formed by two open arms and two closed ones separated by a central platform, set at 40 to 80 cm above the floor [98]. This creates a conflict between the

rodent's innate predisposition to explore the spaces available and the fear generated by the open and raised arms. The entrances and time spent in open arms are measured for a period ranging from 5 to 15 minutes and represent a negative index of anxiety. The validity of the test to measure anxiety is out of the question, even if it is based on the locomotor activity of the animal tested, the motor deficit that can arise from the nerve injury could thus compromise the outcome. Another critical issue is the repetition of the test, which could lead to a reduction in the time spent in the open arms [99].

The elevated zero maze is a variant of the elevated plus-maze in which the closed and open arms are sequentially placed in an annular way to avoid any ambiguity in the interpretation of time spent in the central square and interruption of exploration. Rodents tend to stay in the central part of the elevated plus-maze for 20-30% of the duration of the test. Alterations in the time spent in the central area have often been attributed to changes in anxiety-like behavior, but the interpretation remains ambiguous [100].

The test of social interaction is widely used to evaluate anxiety and is based on allowing the rodent freely exploring an unfamiliar congener in its home cage or another neutral environment and measuring the time spent sniffing, following, allogrooming, biting, mounting, or wrestling the congener [101]. This test can be performed in rodents of the same sex or in male-female pairs. Furthermore, both in male-female and female-female social interaction tests, the emission of concomitant ultrasound vocalizations, ranging from 40 to 80 kHz, represents a further measurable event considered as an index of interest and social motivation [102-104].

Marble burying is a test used to measure either anxiety-like [105] or compulsive-like behavior [106]. It is performed in cages of the same dimension of the home cage containing 3-5 cm of sawdust and 12-15 marbles of 1 cm diameter each. Rodents are left undisturbed for 15-30 min after which the number of marbles buried, duration of burying, and the number of burying events are scored, representing an index of anxiety- or compulsive-like behavior.

The hole board test was developed to overcome the limits of the open field test, which, being simply a free area, does not allow discrimination between locomotion and exploratory behavior. The hole board apparatus consists of an arena in which floor 9 to 16 holes are distributed. The latest generation devices are equipped with infrared sensors and software for measuring nose pokes into the holes, rearing, and locomotion. These behaviors are monitored for periods ranging from 5 to 15 min and the more they occur, the less anxious the animal is and, reciprocally, if these behaviors are reduced, then the animal is more anxious [107].

1.6. Measurements of Depression-like Behavior

The forced swim or Porsolt's test is certainly among the most common tests for measuring depression-like behavior in rodents [108, 109]. The test is slightly differently carried out in the rat and mouse. The rat is placed in a cylindrical tank filled with water at 23-25 °C for 15 min and the day after, it is re-exposed to the same apparatus for measuring the immobility time in a 5-min session [100]. In the mouse, the pre-exposure to the test apparatus is not necessary and it is directly monitored for the immobility time in a 6-min trial [110]. Mice require a longer observation time since in the first two minutes of the test no immobility is observed, the latter can only be measured in the remaining last 4 minutes of the session. Immobility, considered as the rodent's floating with the minimum movements necessary to keep the head out of the water, corresponds to a state of despair caused by the inevitability of escaping from an aversive condition. The duration of immobility is, therefore, a positive index of depression-like behavior and, reciprocally, swimming and climbing are indicators of antidepressant activity. Interestingly, an increase in the duration of immobility in forced swimming was observed only 8 weeks after the induction of neuropathic pain, while anxiety-like behavior was evident already after 6 weeks [111].

The tail suspension is also used for measuring depression-like behavior. It is based on the same paradigm of despair. The mouse is hanging from the tail with adhesive tape on a bar at 50 cm from the floor and the duration of immobility time is measured in a 6-minute session. Reciprocally, escape oriented behaviors can be quantified. The tail suspension is not suitable for rats or heavy mice (obesity models) since supporting the weight of heavier animals by the tail can be potentially painful. Another critical issue of the test that can compromise the result is represented by the tail-climbing behavior [112].

In sucrose preference and novelty-suppressed feeding tests, the paradigms used to measure depression-like behavior are different.

The sucrose preference test measures anhedonia [113, 114], a reduced propensity to experience pleasure associated with depression in humans and rodents. Rodents are trained for the preference of sucrose for including in the experiments, only those with a clear propensity to consume the sweet solution. In the test, lasting from 15 min to 48 hours, the rodents are left free to drink water or the solution containing sucrose from 1% to 20%. The decrease in the ratio between the intake of the sweet solution compared to the total solution drunk is an index of anhedonia associated with depressive behavior. Saccharin can also be used to avoid excessive caloric intake that could lead to errors. The test requires habituation during which the bottles are interchanged to avoid side preference and, sometimes, water deprivation from 2 to 24 hours. Water, sucrose, and total fluid intakes are measured by weighing the bottles containing the respective drinking solutions before and after the test. Noteworthy is the fact that in neuropathic mice, anhedonia develops in parallel with allodynia and hyperalgesia, is dependent on tumor necrosis factor, and is associated with hippocampal neuroplasticity [115].

The novelty suppressed feeding test measures the latency to eat of rodents left fasted for 24 hours and subsequently exposed to a single food pellet [116]. The latency to feed is the result of the conflict between the need to eat and the fear

of an un-preferred environment. The latency to feed is a measure of depression- (and anxiety-) like behavior [116].

The splash test exploits the paradigm of the innate grooming behavior in rodents, a deficiency of which is an indication of depression-like behavior. A 10% sucrose solution is sprayed on the rodent's back. Cleaning the body fur by licking or scratching it, strokes along the snout, cleaning the face with semicircular movements of the paws above the top of the head, and behind the ears are considered as grooming and recorded in sessions of 5 min duration [111, 117].

The nesting test exploits the innate behavior of rodents to reproduce and protect themselves from the cold. The reduction in nesting behavior can, therefore, be considered as an index of malaise. Mice are housed, individually in cages for habituation of 30 minutes, after which compressed cotton sheets are weighed, cut into 6 pieces, and placed on the lid of the metal cage. The session lasts 120 min and at the end of the test, the material left on the cage lid is measured and scored: no nest (0) poor nest (1) and complete nest (2). A poor nest score is indicative of depression-like behavior [118].

1.7. Measurements of Cognitive Performance

Pain affects cognition in humans and rodents since it can divert attention, memory, and decision-making. Neuropathic pain has a negative impact on cognition in rodents, however in preclinical studies, unlike clinical studies, caution is required when interpreting behavioral test outcomes to measure cognitive performance. These tests are mainly based on space learning, memory, or attention and depend strictly on locomotor activity, exploration, and appetite that are compromised in the models of neuropathic pain [119]. The devices most used to test memory and learning in rodents are the mazes. Mazes exploit the innate learning and remembering of the location associated with safety, food, or any other reward in rodents [120]. The simplest procedure to study spatial working memory, the capability to retain spatial information for a short time, is the scoring of the spontaneous alternation in devices either in "T" or "Y" shapes. The alternation is the result of the innate propensity of rodents to explore the less recently visited arm, implicating that the rodent remembers the last arm visited. Positive reinforcements such as food or sweetened water may be used to reward alternation.

The radial maze measures spatial learning and memory and consists of eight equidistantly spaced arms radiating from a central platform. The rodent is gently placed on the center of the maze and a food reward at the ends of each arm, not visible from the center of the maze, serves as motivation to visit all the arms. The test allows us to measure two types of memory: the reference memory when mice visit the arms of the maze that contains the food (reward) and the working memory when mice enter an arm that has not been visited before [121].

The Morris water maze is one of the most used tests for assessing spatial learning and memory in rodents. In this paradigm, rodents are placed in a cylindrical pool with an invisible platform submerged under the surface of the water. The innate aversion of rodents to water gives the motivation to perform the test. The test consists of firstly training rodents to locate the hidden platform in the target quadrant, defined by ideally dividing the surface of the water into 4 quadrants of equal size. Rodents learn to locate the platform by accidentally touching it during swimming in daily trials (typically 4) of a predetermined duration (cut-off). After that, it is possible to verify the mnestic capacity of the rodent (short and long term memory) by removing the platform and measuring the behavior in the swimming pool, the elapsed time, the number of crossings and the distance traveled in the target quadrant [122]. Compared to rats, mice have greater difficulty in finding the platform due to species differences: rats build burrows near water and normally swim, whereas mice tend to avoid water as much as possible [123].

Sustained attention over several seconds may be measured throughout operant tasks, such as pressing a lever or nose poke into a window. The 5-choice serial reaction time task, depending on its configuration, is used to measure attention, memory, and impulsivity [124]. Rodents are trained to exploring 5 apertures, one of which lights up when food is gained. The duration of the light signal is reduced overtime, requiring increased attention [125]. The nose poke carried out before the light stimulus indicates an impulsive response and it determines the end of the trial. The test is typically used in rats, although it can also be set up for mice [126].

The novel object recognition is one of the most common tests used to evaluate recognition memory in rodents and it exploits the spontaneous tendency of rodents to explore novel objects. The test is carried out in an open field arena and consists of habituation, training, and testing trials. During the habituation period, rodents are freely allowed to explore the arena without any object. During the training period, which is carried out 24 hours after habituation, rodents are freely allowed to explore 2 identical objects. Finally, in the test trial, one of the identical objects is replaced with a new one and the time spent to explore the novel object is scored [127, 128]. Overall, the amount of studies investigating the cognitive deficits associated with neuropathic pain is less than those that have focused on affective disorders. These fewer studies targeting memory deficits could reflect the complexity, high number and diversity of paradigms available for measuring cognition.

CONCLUSION

The main concern in the study of the sensory, affective, and cognitive symptoms associated with neuropathic pain in rodents is, first of all, the choice of the neuropathic pain model, which must be as faithful as possible to the same condition reported in humans. There are several models available today, mainly induced by the injury to a peripheral nerve, nerve roots or spinal cord. Alternatively, neuropathic pain can be caused by anti-cancer chemotherapic drugs,

virus infections, or metabolic diseases such as diabetes. The choice of model should take into account the one associated with minimal motor impairment because most behavioral tests are based on motor activity. In rodents, moreover, the study of sensory disturbances is mainly assessed through the responses to pain evoked by stimuli (thermal, chemical, and mechanical), while in humans, the most debilitating sensory disturbance is spontaneous pain. In humans, spontaneous pain is self-referred and obviously, this is not doable in rodents, however, in the last years, new methods for measuring spontaneous pain have been developed. The close correlation between chronic pain in general and neuropathic pain in particular and affective/cognitive disorders is due to maladaptive plasticity observed in brain areas involved in the control of pain, emotions, and cognition that occur in both, humans and rodents. The *anthropomorphization* of rodent behavior and reciprocally the translation of rodent behavior on humans are the main complexities of studying depressive- and anxiety-like behavior in laboratory rodents. There are several tests to measure anxiety and depressive-like behavior, that exploit the innate behavior of the animal and introduce an "anxio/depressio-genic" element, such as the height, lighting, novelty, water, hunger, thirst or simple monitor the normal rodent behavior such as nesting, grooming, borrowing and burying. The studies on cognitive deficits associated with neuropathic pain are scarce compared to those on emotional deficits and this is probably due to the wider number, and variability of tests available to study cognitive performance. Indeed, cognition includes different components, such as attention, learning, and different types of memory. The most used tests are the mazes and the novel object recognition. This review has treated very briefly the tests available today for the study of sensory, affective and cognitive disorders associated with neuropathic pain because rather than describing the various existing models, it was intended to suggest that the complete description of neuropathic pain symptoms should include those affective/cognitive beyond the purely sensory ones.

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