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REVIEW

TESTS AND MODELS OF NOCICEPTION AND PAIN IN RODENTS

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Abstract—Nociception and pain is a large field of both neuroscience and medical research. Over time, various tests and models were developed in rodents to provide tools for fundamental and translational research on the topic. Tests using thermal, mechanical, and chemical stimuli, measures of hyperalgesia and allodynia, models of inflammatory or neuropathic pain, constitute a toolbox available to researchers. These tests and models allowed rapid progress on the anatomo-molecular basis of physiological and pathological pain, even though they have yet to translate into new analgesic drugs. More recently, a growing effort has been put forth trying to assess pain in rats or mice, rather than nociceptive reflexes, or at studying complex states affected by chronic pain. This aids to further improve the translational value of preclinical research in a field with balanced research efforts between fundamental research, preclinical work, and human studies. This review describes classical tests and models of nociception and pain in rodents. It also presents some recent and ongoing developments in nociceptive tests, recent trends for pain evaluation, and raises the question of the appropriateness between tests, models, and procedures.

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Key words: pain, nociception, test, model, rodents, analgesia.

Contents

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Abbreviation: CFA, complete Freund's adjuvant.

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NOCICEPTION AND PAIN

The distinction between nociception and pain is important to consider when using preclinical murine models (Table 1). According to the International Association for the Study of Pain (IASP), nociception is defined as "the neural processes of encoding and processing noxious stimuli," whereas pain is "an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage" (Loeser and Treede, 2008).

Nociception thus includes the mechanisms by which noxious stimuli are detected by the peripheral nervous system, encoded, transferred, and unconsciously treated by the nervous system. Detection is ensured by specific molecular transducers borne by nociceptive neurons whose cell bodies are grouped in the dorsal root or trigeminal ganglia. This afferent signal is then treated by complex networks within the dorsal horn of the spinal cord (Todd, 2010) or its equivalent in the brainstem. This treatment is under the influence of both sensory information and descending controls from the brain. Nociception also includes part of the information treatment by the brain as well as some reflex responses to protect the organism. In contrast, pain is a conscious experience that requires the cortical treatment and the aversive interpretation of the nociceptive information. It is a subjective and complex experience with a necessary affective component, accompanied with sensory-discriminative, autonomic, and cognitive components. Although nociception and pain appear closely linked, clinical evidence also proved that they can be dissociated one from the other. In patients, pain is assessed and quantified by verbal expression, which is not possible in rodents. Thus, what is commonly referred as "pain tests" in rodents are in fact nociceptive tests, and the preclinical measure of pain itself still remains a challenge to the field.

When pertinent, nociception and pain are critical for survival (Le Bars et al., 2001). They offer an alarm system that has the capacity to initiate an immediate adapted response, which can further evolve toward better adaptive responses through emotional associative learning. Despite this major physiological function, it is also critical to "silence" nociception or pain when they lose their pertinence as an alarm system, which is the case when the lesion or risk of lesion is already identified, when pain is anticipated, or when pain becomes chronic or dissociated from an actual lesion. This requires adequate treatments, whose

Table 1. Pain terminology (Loeser and Treede, 2008)

Term	Definition
Pain	An unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage
Nociception	The neural processes of encoding and processing noxious stimuli
Noxious stimulus	An actually or potentially tissue-damaging event
Nociceptive pain	Pain arising from activation of nociceptors
Neuropathic pain	Pain arising as a direct consequence of a lesion or disease affecting the somatosensory system
Allodynia	Pain in response to a non-nociceptive stimulus
Hyperalgesia	Increased pain sensitivity
Pain threshold	The minimal intensity of a stimulus that is perceived as painful

development may benefit from preclinical tests and models (Negus et al., 2006; Mogil et al., 2010a).

NOCICEPTIVE TESTS

For a long time, the basic science of pain and the preclinical research on pain treatments essentially relied on nociceptive tests that were done on naive animals. Although it brought major advances to the pain field, the benefit for developing new treatments was more limited. Thus, therapeutic preclinical research should associate pain models to nociceptive tests to be more relevant.

Nociceptive tests use electrical, thermal, mechanical, or chemical stimuli (Le Bars et al., 2001). Some of them rely on the latency of appearance of an avoidance behavior, usually a withdrawal reflex of the paw or the tail. In this case the stimulus may be considered as fixed. The concerned tests that use thermal stimulation include the tail flick test, the hot- or cold-plate tests, and the radiant heat paw-withdrawal test. Nociceptive tests can also rely on the stimulus threshold necessary to elicit an avoidance behavior. In this case, the stimulus is either variable, with increasing value, or the test may use successive incremental stimuli at a fixed value. These tests concern mechanical stimulation and include the von Frey filaments, the Randall–Selitto analgesimeter, and recent tests based on strain gauges held by forceps or fingers. The development of dynamic hot and/or cold plates also allowed the assessment of thermal thresholds in awake rodents. Electrical thresholds are also studied, particularly as a control for other behavioral experiments. Lastly, some nociceptive tests can rely on the observation and scoring of specific behaviors. This is the case for assessing cold allodynia with acetone or for tests using inflammatory or irritating chemical stimuli.

The results obtained in most nociceptive tests show a relatively low interindividual variability compared with what is observed in other fields of behavioral studies, such as mood disorder-related studies or operant behavior studies. As a consequence, experiments on nociceptive responses can often be conducted with fewer animals than what would be necessary for these other studies. Still, the measure of nociceptive response in rodents requires expertise in the behavioral field to avoid experimental pitfalls and potential artifacts. The choice of test is a critical step. Indeed, different nociceptive modalities are, at least partially, processed through different molecular transducers and fibers (Delmas, 2008; Scherrer et al., 2009). Moreover, genetic or pharmacological manipulation may dissociate these various modalities (Scherrer et al., 2009). In this review, the classical tests performed in awake rodents will be shortly presented (Table 2), and particular attention will be given to the recent development of new tests and to yet unanswered needs in the field.

Electrical thresholds

Electrical stimulation is an unnatural and non-specific stimulation, and electrical thresholds are rarely studied for themselves. They are more frequently evaluated as a control for other behavioral experiments in which electric shocks are involved (Simen et al., 2006). This is, for example, the case with learned helplessness, fear conditioning, active or passive avoidance. When phenotypes are observed with these procedures, it may be necessary to control whether these phenotypes could result from differ-

Table 2. Nociceptive tests

Test	Modality	Stimulus	Usual parameter	Species	Standard
Tail flick	Thermal, heat	Fixed T°	Withdrawal latency (s)	Rat, Mouse	+++
Hot plate	Thermal, heat	Fixed T° (48–55 °C)	Withdrawal/jump latency (s)	Rat, Mouse	+++
Plantar®	Thermal, heat	Fixed T°	Withdrawal latency (s)	Rat, Mouse	+++
Cold plate	Thermal, cold	Fixed T°	Scoring (nb)	Rat, Mouse	+/−
Acetone test	Thermal, cold	Drop application	Scoring (nb) or duration (s) of nociceptive behavior	Rat (Mouse)	+
Dynamic hot plate	Thermal, heat	T° ramp	Scoring and response threshold (T°)	Rat, Mouse	+
Dynamic cold plate	Thermal, cold	T° ramp	Scoring and response threshold (T°)	Rat, Mouse	+/−
von Frey	Mechanical	Multiple fixed pressure	Withdrawal threshold (g)	Rat, Mouse	+++
Randall–Selitto	Mechanical	Pressure ramp	Withdrawal or vocalization threshold (g)	Rat	+++
Strain gauges	Mechanical	Pressure ramp	Withdrawal threshold (g)	Rat	++
Formalin test	Chemical	Paw injection	Scoring (nb)	Rat, Mouse	+++

+++, classic, standardized tests; ++, recent test; +, tests with some technical difficulties; +/−, delicate procedures; s, seconds; nb, number; T°, temperature; g, grams.

ences in sensitivity to the shocks. An easy way to assess electrical thresholds is to deliver shocks at given time intervals, with a fixed increment (e.g. 0.05 mA) in intensity, until a behavioral response is observed (Evans, 1961). Observed responses include the presence of a flinch, a vocalization, or an escape response, either jumping or running. Their order of appearance depends on the species and the way the vocalization is detected, either by ultrasonography detector (Eschalier et al., 1988) or by simply assessing audible vocalization. With audible vocalization, the behavioral sequence with increasing shocks is flinch/vocalization/jump in rats and often flinch/run or jump/vocalization in mice. Ethically, this is typically a procedure that the scientist should test on himself or herself first; tickling sensation and reflex withdrawal of the hand should happen with thresholds within the range of the ones imposed to the rodents. Moreover, flinching in rodents is not a sufficient response to stop the test as it might reflect sensory thresholds rather than a nociceptive response. The complete behavioral sequence may thus be necessary to reveal significant differences (Barrot et al., 2002). As is the case for almost all nociceptive tests, testing must be stopped as soon as the expected response is observed, and a cut off (maximal intensity for which the test must be stopped even if no answer was observed) must also be defined before testing.

Nociceptive response to heat

Tests measuring the nociceptive response to heat can be experimentally used both in rats and in mice. The heat intensity of the commercially available tests can normally be controlled. It is usually set up to observe the nociceptive response between 5 and 10 s. Above these values, the risk increases for the presence of an animal movement unrelated to the nociceptive stimulus. Below, the differential power of the test may be strongly reduced. When preferentially studying analgesic response, one might choose to have low baseline values (higher heat intensity) to favor the detection of delayed response. Reciprocally, to assess the consequence of a pain model in these tests, one might prefer high baselines values (lower heat intensity) to favor the detection of faster responses. The stimulus may automatically stop when the animal responds, as it is the case with the tail flick or with the radiant heat paw-withdrawal test. For hot plate, the scientist must immediately retrieve the animal as soon as the response is observed. For all tests, a cut off time is always defined to avoid or limit the risk of burn. These tests often allow repeated measures, but they are sensitive to stress and stress-induced analgesia. As a consequence, the first measure(s) may give longer latency(ies) than subsequent ones (Le Bars et al., 2001), particularly in un or poorly manipulated rats or in non-habituated mice. Similar to any other behavioral study, the experiment should not be conducted within the first week of the rodent arrival to the animal facility. When done properly, these tests allow the experimenter to obtain stable values within the same day (for acute time course) or between days even in the long term.

The tail flick is one of the oldest nociceptive tests (D'Amour and Smith, 1941). The measured parameter is the latency, in seconds, for tail flick reflex following tail exposure to a heat stimulus. The stimulus may be applied by dipping the tail tip into a bath at a controlled temperature or by exposing the tail to a controlled infrared heat beam. In the latter case, the apparatus allows an automated detection of the tail flick and measure of its latency. The tail flick is a spinal reflex, but it is subject to supraspinal influences that can affect this reflex (Yaksh and Rudy, 1978; Millan, 2002). This test is highly sensitive to opiates (Le Bars et al., 2001). Because it has mostly been used to study the response to analgesic drugs, the heat intensity is usually set up for fast withdrawal latencies (around 2–4 s), but it should be adjusted when pain models are studied. Tail flick test is relatively easily done in rats with habituation to manipulation and may require more expertise in mice. A potential difficulty of the test is to maintain the animal in a correct posture without inducing unwanted stress. Another pitfall may be related to the role of the tail in the thermoregulation of rodents (Le Bars et al., 2001).

The hot-plate test (Woolfe and MacDonald, 1944; O'Callaghan and Holtzman, 1975) is another classic test in the field. The temperature is often set at 52 or 55 °C, more rarely at 48 °C. A 52 or 55 °C set up allows observing baseline latencies between 5 and 10 s for paw licking, depending on the material of the plate. The plate material may indeed influence heat conduction and explain small differences in latency values between the available brands of hot plates. These temperatures are 10–15 °C higher than the response threshold of heat nociceptors (Yeomans and Proudfit, 1996), which reflects the time required for skin temperature to increase until detection of the nociceptive stimulus, and the delay to elicit the withdrawal response. Higher temperatures are less relevant because of the risk of burn. Responses in the hot-plate test are supraspinal. The measured parameter is usually the latency for paw licking or the first observed response, which allows repeated measures. Some studies are specifically based on jump latency, particularly in mice. However, this parameter should be used with caution as it results in longer latencies, which may sometime raise ethical issues, and may also lead to a learning/anticipation process limiting the possibility to repeat measures on the same animal. Small differences in the plate temperature can result in important differences in response latency. Using reliable plates specifically designed for behavioral tests, with fast adjustment of temperature changes and with a 0.1 °C precision in the temperature control, is thus important. Although the test is easy to conduct, it is not automated. The timer is started and stopped by the experimenter.

In the late 80s, Hargreaves et al. (1988) described a test that differentiates the left and right hind paw responses to heat in freely moving rodents. This is the radiant heat paw-withdrawal test. In articles, it has also been referred to as the Hargreaves method or by a particular brand name, "Plantar®." In fact, various models are commercially available with the same principle: animals are placed in clear boxes on a glass surface, a controlled heat beam system

is present below the glass and is moved under a hind paw; and starting the stimulus starts the timer, whereas the paw withdrawal automatically stops the timer. This test takes longer to complete than the hot-plate test. Indeed, it requires a period of habituation to the box before each testing procedure. Moreover, each paw is tested independently, often with alternative measures of left and right hind paw withdrawal that are done repeatedly to average the results. However, with adequate set ups, a few animals can be tested in parallel, and this test has the advantage to allow differentiating the response of both hind paws. It is thus valuable when working with unilateral models of pain with injections in the paw or the knee or with manipulations of the sciatic nerve. It is also useful for tests requiring topical application of a substance, either proalgic or analgesic, the contralateral paw providing an internal control for the experiments.

The previous tests are now classics, but the measured parameters mainly remain responses to a stimulus known to be nociceptive, that is, hyperalgesia (Table 1). Despite its relevance to clinical conditions, thermal allodynia (pain in response to a non-nociceptive stimulus, Table 1), which would require measures of thresholds, is more difficult to assess in awake animals. To access this parameter, it is possible to use a modified hot-plate test in which the temperature is slowly increased from non-noxious to noxious levels (Hunskaar et al., 1985). Recently, such dynamic hot plates with a precise control over the ramp of temperature were made available by most test providers and demonstrated their usefulness for experimentally differentiating thermal allodynia from thermal hyperalgesia (Yalcin et al., 2009a, 2011a) (Fig. 1). When the temperature increment is slow (1 °C/min), the paw and plate temperatures are likely at a close temperature, and the temperature threshold for behavioral response is around 39–40 °C (Yalcin et al., 2009a), which is in agreement with thermonociceptor thresholds as measured by *in vivo* electrophysiology (Yeomans and Proudfit, 1996). Although of great interest, this dynamic test, however, has some limits. The relevant nociceptive behaviors are not easy to score and may vary between species or strains. It is thus necessary to identify the most relevant parameters (paw licking, jumps. . .) in naive animals before experiments. Moreover, depending on the speed of the increment, it may require a few minutes to more than 20 min to test one animal, whereas only a few seconds are necessary with a classical hot-plate test. The increment duration can also be a limit for some acute pharmacological manipulations. As a consequence, the dynamic hot plate may be more adequate when thermal allodynia appears scientifically relevant or is the primary topic of the study.

Nociceptive response to cold

Testing the nociceptive response to cold stimuli is more difficult than testing response to heat. To assess cold allodynia, a drop of acetone can be applied on the hind paws (Choi et al., 1994; Smith et al., 2004). Its evaporation produces a cold stimulus, which is usually not detected as nociceptive by naive animals but results in cold allodynia in neuropathic pain models. In rats, no or few response to acetone is present in naive animals. The procedure consists of five repeated applications, and the results are often expressed as a number or frequency of brisk foot withdrawal among the trials. In mice, responses to acetone are easily observed in naive animals, which limit number- or frequency-based procedures. The protocol then relies on the time spent reacting to the acetone, by licking or shaking the paws, measured over a minute.

Cold plates can also be used to assess nociceptive responses (Bennett and Xie, 1988; Choi et al., 1994). Because of strong behavioral variability, this test is rarely used compared with hot plates, and the scoring of nociceptive behaviors over a given period is often preferred to the latency to first response. As for heat stimuli, dynamic plates with a precise control over the temperature decrement may allow identification of the threshold temperature for escape behavior (Yalcin et al., 2009a), but the procedure can be challenging.

Nociceptive response to mechanical stimuli: the von Frey test

The von Frey test remains the only mechanical test that can be reliably used not only in rats but also in mice. It is derived from a clinical procedure to assess allodynia, particularly in patients with neuropathic pain. The von Frey filaments (or von Frey hairs) are plastic hairs, 5 cm long and of various diameters, fixed on applicators. Their end is not sharp but blunt. They are applied locally until they

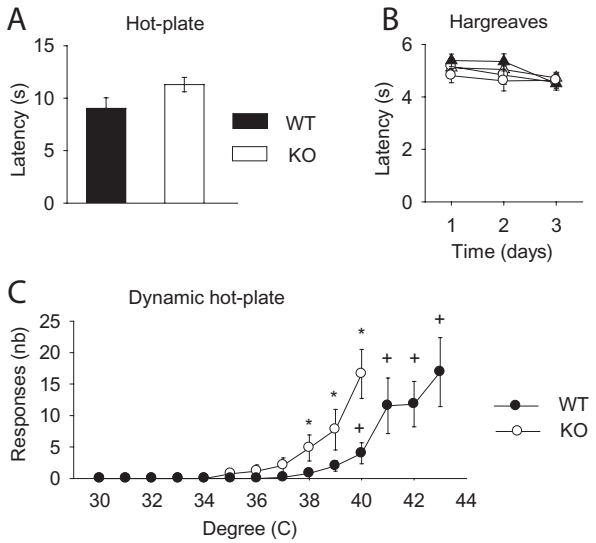

Fig. 1. Example of dissociation between heat allodynia and hyperalgesia. Mice deficient for the β_2-subunit-containing nicotinic acetylcholine receptors (KO) were compared with their wild-type littermates (WT) for nociceptive responses to heat. As previously reported (Marubio et al., 1999), KO mice displayed no thermal hyperalgesia as tested with the conventional hot-plate test at 55 °C (A) or with the Hargreaves test (B). However, thermal allodynia was evidenced in KO using the dynamic hot-plate test (C). (Yalcin et al., 2011a; this figure has been reproduced with permission of the International Association for the Study of Pain® (IASP®). The figure may not be reproduced for any other purpose without permission).

bend, at which point they exert a calibrated pressure. For the most common brand, the thinner filaments may go down to 0.008 g, and are far below detection threshold, whereas the larger ones can lead to a pressure up to 300 g. In rodents, they are, for the most part, used on the plantar surface while the animal is on a grid. The expected response is the paw withdrawal. Whether the threshold response to von Frey filaments in naive animals is a sensory or a nociceptive response remains debatable. Indeed, the flexor reflex can also be elicited by non-nociceptive stimulation. However, in models of pain, responses can be observed for filaments that never elicit paw withdrawal in controls, which may be considered as mechanical allodynia. Some groups use only one or few filaments, all with pressure value below response threshold in naive animals. In this case, a given number of trials are done with the selected filament, and the considered parameter is the number of positive responses. This simple procedure may be mildly sensitive, but is fast and respects the idea that von Frey filaments should not elicit a nociceptive response in the absence of allodynia. Other groups test a series of filaments to identify the lowest filament eliciting a response (Chaplan et al., 1994). Various standardized procedures may be used to identify this threshold, but each filament is always tested repeatedly. For mice, the withdrawal response is usually observed between a tenth of gram and a few grams, but may go down to tens of mg with some protocols and severe allodynia. For rats, the response is usually observed between grams and tens of grams. The von Frey test allows differentiating the response of both hind paws, and the threshold values are stable over time, allowing repeated measures.

Values from von Frey filament testing in rodents can depend upon the protocol and the type of filaments that are used. As a consequence, there is a strong variability in published values for mice. Mechanical thresholds for paw withdrawal in naive mice can range from 0.3 g up to 7–10 g (Leo et al., 2008; Osikowicz et al., 2008; Scherrer et al., 2009; Yalcin et al., 2009b). Although differences may be present between strains (Mogil et al., 2010b), variability also relates to protocols. Some groups observe baseline values around 0.5 g, some around 1–2 g, and others around 4–6 g, which is the case for our laboratory and others (Osikowicz et al., 2008; Yalcin et al., 2009b). When "Each probe [is] applied to the foot until it just bend" (Osikowicz et al., 2008), Swiss albino mice have a 5–6 g mechanical threshold, whereas neuropathic mice are around 1–1.5 g. With the same filaments and procedure, we obtained similar values in C57BL/6J mice (Choucair-Jaafar et al., 2009) and CD1 mice (unpublished data). However, even for a given strain such as CD1, threshold values either above 5 g or below 1 g were reported within the same article (Mogil et al., 2010b). The former value was obtained with a Dynamic Plantar Anesthesiometer (Ugo Basile, Collegeville, PA, USA), an automated version of von Frey test, while the latter value was obtained with classical von Frey filaments that ". . .were firmly applied to the plantar surface of the hind paw [. . .] until they bowed for 5 seconds." When the filament pressure is maintained

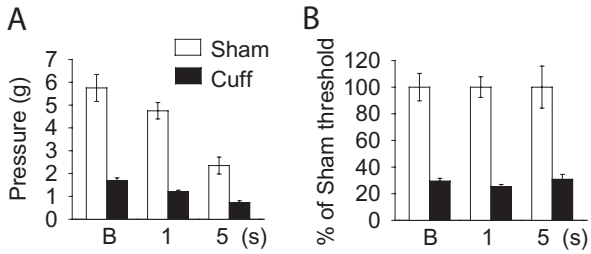

Fig. 2. Threshold response in the von Frey test for C57BL/6J mice. Mice underwent surgery to induce neuropathy by sciatic nerve cuffing (Cuff group) and were compared to their sham controls according to previously published procedures (Bohren et al., 2010). Two weeks postsurgery, the von Frey test was done on the same animals using various delays of filaments' application: until they just bowed ("B"), bowed for 1 s or bowed for 5 s. (A) Neuropathic mice displayed a mechanical allodynia, as revealed by the major difference between sham and cuff paw withdrawal thresholds. This mechanical threshold for paw withdrawal decreased both in sham and cuff mice with increased duration of filament bending. (B) When these data were expressed as percent change from sham controls, the relative allodynia remained similar for the three filament bending delays that were tested. $n=8$ per group (Mice were tested in our facilities by Salim Megat).

over a longer duration, then lower thresholds are observed. Testing control and neuropathic mice with a previously described procedure (Bohren et al., 2010), we evaluated paw withdrawal thresholds by applying filaments at various durations. We observed that longer application indeed led to lower values of paw withdrawal threshold (Fig. 2A). Interestingly, this shift concerned both control and neuropathic mice, and the relative allodynia remained similar whatever the protocol (Fig. 2B).

The various procedures are thus likely to be equally valid if they give stable and reproducible values, if they allow to readily measure allodynia in painful conditions, and if they allow to detect analgesic actions. The filament value per se may be of limited significance, even if it is controlled using precision scales, and raw values mostly depend on the procedure that is used. These raw values are, however, useful as they easily allow to compare experimental groups. The filament brand and the value of the different filaments that are used, the speed of filament application, the degree of bending, and the duration of the application are critical factors, but they are rarely given in the method section of articles. Adding this information would be of interest for the field and could favor more direct comparisons between published studies.

In the past years, automated von Frey apparatuses were made available by various providers, and their use gave relevant data (Leo et al., 2008; Mogil et al., 2010b). Compared with the logarithmic scale of classical von Frey filaments, the automated versions have the advantage of providing a continuous scale of stimulation. These apparatuses can be handheld or motorized. The former appears valid and useful for rat testing; however, they often rely on systems with a variability within the gram range and are thus of limited interest for mouse testing. The latter are more sensitive as the application does not rely on hand movement, but they can be slower and more delicate to

use than classical testing procedures. For intensive use of the test, and fast testing of large number of animals, the classical von Frey filaments probably remain the most appropriate approach to assess mechanical allodynia in mice. In rats, however, the handheld apparatuses may provide an experimental improvement.

Nociceptive response to mechanical stimuli: other tests

The other available tests to study nociceptive response to mechanical stimuli are used on rats, but are not appropriate for mice in their present form.

The Randall–Selitto test is a classical way to measure mechanical thresholds, mechanical allodynia or hyperalgesia, and analgesic activities in rats (Randall and Selitto, 1957; Kayser et al., 1990). In this test, the hind paw is placed between a fixed element, either a surface or a blunt point, and a mobile blunt point exerting a controlled pressure. This pressure is usually provided by a sliding counterweight system. The measured parameter is the threshold (in grams) for appearance of a given behavior, which may be a reflex withdrawal, struggling, or a vocalization, depending on the protocol. Done correctly, this test gives highly stable and reproducible values (Célèrier et al., 2001; Rivat et al., 2008), but requires a strong behavioral expertise from the experimenter and a large number of animals. Indeed, the rat is restrained in a vertical non-natural position to maintain its paw on the apparatus. This imposes overtraining of the animal and prehabituation to the posture and procedure to obtain reliable values.

To overcome this difficulty and to offer easier procedures, simpler apparatuses were designed relying on strain gauges (Hu, 2006). These gauges can be fixed on blunt forceps (Luis-Delgado et al., 2006); the animal is loosely restrained on the bench, the tips of the forceps are placed around the hind paw, and an incremental force is applied until the paw is withdrawn. Stress level and data variability are low, allowing to reduce the number of animals. Moreover the procedure is fast and does not require intensive habituation or pretraining. The drawback of the test is that the pressure increment is not automated but directly exerted by the experimenter who must be careful to use standardized procedures. The practical advantages, particularly for large experiments, greatly overcome this limitation. The force transducer can also be simply mounted on a unit fitted to the operator's thumb (Barton et al., 2007). Various providers are now offering apparatuses relying on strain gauges, and their use is rapidly developing in rats for which these apparatuses facilitate testing procedures.

Nociceptive response to chemical stimuli

Different irritating chemical agents can be used as nociceptive stimuli (Le Bars et al., 2001); Negus et al., 2006) to assess pain and preclinically evaluate analgesic drugs. They induce a tonic pain state that is evaluated by behavioral scoring. The formalin test (Dubuisson and Dennis, 1977; Tjølsen et al., 1992) is the most commonly used procedure. Formalin mainly acts through transient receptor potential ankyrin 1 (Macpherson et al., 2007; McNamara et al., 2007), a member of the transient receptor potential family of ion channels. It can be intradermally injected in the dorsal or plantar surface of either a forepaw or hind paw, resulting in paw withdrawal, licking, biting, or shaking, which are quantifiable. In rodents, the formalin injection produces a biphasic behavioral reaction, with an initial phase within the first minutes postinjection, followed by a quiescent period of around 10 min and a second phase of nociceptive behaviors lasting 20–40 min. The first phase is related to the direct stimulation of nociceptors and is sensitive to local anesthetics, whereas the second phase involves both inflammatory mechanisms and central sensitization within the dorsal horn (Tjølsen et al., 1992). This second phase responds to various drugs with established clinical analgesic action, such as opiates (Dubuisson and Dennis, 1977), steroid or non-steroidal antiinflammatory drug analgesics (Hunskaar and Hole, 1987), N-methyl-D-aspartate antagonists (Coderre and Melzack, 1992), or gabapentin (Singh et al., 1996). In the writhing test, the irritating agents are administered intraperitoneally, inducing a stereotyped behavior characterized by abdominal contractions, which are quantified (Le Bars et al., 2001).

PAIN MODELS

The various nociceptive tests offer useful and often easy-to-use tools for basic science. However, this toolbox, based on acute nociception assays, is not sufficient to perform translational research on pain and its treatments (Negus et al., 2006). There is the necessity for methods to induce more clinically relevant pain states, for example by using models of sustained or chronic pain as may be observed clinically.

Models of inflammatory pain

Following tissue damage, as in autoimmune diseases or with exposure to irritating agents, the immune system releases inflammatory mediators that activate and sensitize the nociceptive system (Marchand et al., 2005). Most models of inflammatory pain rely on the administration of substances that induce an immune response or the administration of inflammatory mediators themselves (Negus et al., 2006). The formalin test can thus be considered as a short-term inflammatory pain model. However, studies on inflammatory pain more often use compounds with strong antigenic potential, either complete Freund's adjuvant (CFA) or carrageenans. The former is a suspension of heat-killed *Mycobacterium butyricum* or *Mycobacterium tuberculosum*, whereas the latter are sulfated polysaccharides extracted from seaweed. Paw injection of these compounds induces both thermal and mechanical allodynia and hyperalgesia for at least several hours.

Knee or ankle joint injections of kaolin/carrageenan mix, carrageenan, zymozan, or CFA are used as painful inflammatory monoarthritis models (Neugebauer et al., 2007). The time course of local inflammation and the alteration of the synovia, cartilage, and bone differ between these models of arthritis and are well described (Neuge-

bauer et al., 2007). The CFA model is reliable in rats, but more difficult in mice for which either repeated injections at high concentration or the use of sensitive strains might be required (Gauldie et al., 2004; Neugebauer et al., 2007).

More chronic autoinflammatory or autoimmune painful conditions can also be modeled in rodents. These animal models of polyarthritis may raise ethical issue related to their duration (weeks to months) and to their pain-related consequences. However, they are important for preclinical translational research on rheumatoid arthritis and its treatments. Detailing these models is beyond the scope of the present review, but they are described elsewhere in the literature (Bendele, 2001; Holmdahl et al., 2001; Neugebauer et al., 2007; Bevaart et al., 2010; Billiau and Matthys, 2011; Bolon et al., 2011).

Models of neuropathic pain

Neuropathic pain arises as a direct consequence of a lesion or disease affecting the somatosensory system (Loeser and Treede, 2008; Jensen et al., 2011). It is usually a chronic condition, which affects the quality of life of patients. In most cases, neuropathic pain is consecutive to a peripheral nerve injury, either a nerve section or compression, or is a consequence of diabetes (Attal et al., 2008). However, it can also result from infectious diseases, exposure to neurotoxic compounds, or be of central origin. Neuropathic pain symptoms include abnormal sensations, spontaneous pain, which may be continuous or paroxysmal, or provoked pain-like allodynia or hyperalgesia. Patients may also experience sensory deficits such as local hypoesthesia or anesthesia. Clinically, neuropathic pain remains challenging to treat. The recommended first-line treatments consist either of anticonvulsant drugs, such as gabapentin or carbamazepine, or antidepressant drugs, such as tricyclic antidepressants or more recent serotonin and noradrenaline reuptake inhibitors (Moulin et al., 2007; Saarto and Wiffen, 2007; Attal et al., 2010; Barrot et al., 2010).

Numerous models of neuropathic pain have been developed in rodents. They are based on most of the known etiologies in humans, aiming to reproduce peripheral nerve injuries, central injuries, trigeminal neuralgia, diabetic neuropathies, chemo-induced neuropathies, postherpetic neuralgia, and so forth. Recent reviews of the literature detail these models (Sorkin and Yaksh, 2009; Colleoni and Sacerdote, 2010; Jaggi et al., 2011), thus only some of them, related to sciatic nerve manipulation, will be rapidly presented here.

Most murine models of peripheral nerve injury target the sciatic nerve and rely on either compression or section. This nerve is relatively easy to access; the nociceptive tests can be ideally done on the hind paws, and in some cases, a unilateral injury allows using the contralateral paw as control. The model for which most data are available is the chronic constriction injury (CCI), consisting of three or four loose ligatures around the main branch of the sciatic nerve (Bennett and Xie, 1988), but various other models of chronic nerve compression are frequently used. These consist of the implantation of a polyethylene cuff around the main branch of the sciatic nerve (Mosconi and Kruger, 1996; Benbouzid et al., 2008a), tight ligation of the sciatic nerve (partial sciatic nerve ligation or PSL; Seltzer et al., 1990), tight ligation of L5 and L6 spinal nerves (spinal nerve ligation or SNL; Kim and Chung, 1992), or common peroneal nerve ligation (Vadakkan et al., 2005). Before the development of these models, researchers mainly used a complete sciatic nerve transaction, or axotomy, to induce neuropathic pain in rodents. Although this approach was beneficial to the field and brought advances on the neurobiology of neuropathic pain, it often resulted in autotomy behavior in rodents, that is, a self mutilation of the denervated limb. Originally considered as an index of neuropathic pain, this autotomy behavior is now ethically questionable, and more refined models implying sections were designed. In these spared nerve injury (SNI) models, two of the three terminal branches of the sciatic nerve are tightly ligated followed by a distal axotomy, the third branch being left intact (Decosterd and Woolf, 2000; Shields et al., 2003). All neuropathic pain models targeting the sciatic nerve result in lasting mechanical allodynia, and some of these models also induce notable changes in sensitivity or response to thermal stimuli.

RECENT TRENDS FOR PAIN EVALUATION IN RODENTS

Despite criticism on the translational value of rodent models of pain and on tests used by basic researchers (Langley et al., 2008; Craig, 2009), progress brought by the preclinical pain field and its relevance should be acknowledged. The interactions are rather strong between clinicians and basic researchers, and research effort appears balanced between human studies, preclinical work, and fundamental research. As a consequence, the knowledge on the anatomo-molecular basis of physiological and pathological pain progressed quickly in the past decades, even though it did not fully translate into new analgesic drugs. In this context, research continues to improve nociceptive tests and models of pain previously evoked. In parallel, a growing preclinical research effort is being put forward trying to assess pain, rather than nociceptive reflexes, in rodents. It is, however, a challenge as pain is a subjective experience. Some of the approaches that were developed are using tools initially developed in other fields of behavioral neuroscience.

Giving rodents the choice

One experimental strategy to assess pain is to give the animal the choice between environments that are or not associated with painful experiences. This can be done by adapting place conditioning tests or active avoidance tests to pain paradigms. Conditioned place aversion to noxious stimuli in naive animals, for example, with formalin (Johansen et al., 2001), or conditioned place preference to analgesics in models of pain (Sufka, 1994; King et al., 2009), are examples of such strategy. These approaches brought major information to the field, by giving preclinical causal evidence for the role of the anterior cingulate cortex in the

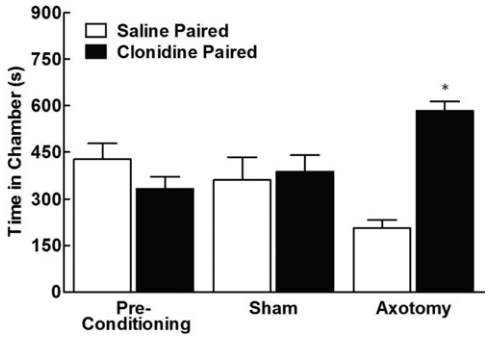

Fig. 3. Evidence for spontaneous pain in a model of peripheral neuropathy. Rats with sciatic nerve axotomy or sham surgery underwent a place conditioning procedure with spinal clonidine administration. Rats with axotomy, but not sham animals, developed place preference to the analgesic action of spinal clonidine, revealing the presence of a tonic-aversive state in neuropathic rats (Qu et al., 2011; this figure has been reproduced with permission of the International Association for the Study of Pain® (IASP®). The figure may not be reproduced for any other purpose without permission).

aversive component of pain (Johansen et al., 2001) or by demonstrating the presence of a spontaneous tonic aversive state in animal models of neuropathic pain (King et al., 2009; Qu et al., 2011) (Fig. 3). To test the aversive component of pain without going through a heavy conditioning procedure, avoidance tests can also be used. In this case, animals with a pain condition are exposed to a mechanical stimulation (LaBuda and Fuchs, 2000), a thermal gradient or a two-temperature choice test (Moqrich et al., 2005) with the possibility to avoid or limit their exposure to the evoked pain. These various tests are crucial to dissect the anatomo-molecular basis of the aversive component of pain.

Facial coding scales

Emotions are often associated with specific facial expression signatures, and the facial expression of pain can be a parameter of interest. Its quantification by facial coding scales has been useful to assess pain in non-verbal human population (Williams, 2002), and recent evidences suggest that facial expression of pain could also be used in rodents (Langford et al., 2010; Sotocinal et al., 2011). Assessing internal emotional states of rodents by analyzing their facial expression is difficult. Nevertheless, it has for example been previously used for taste liking/disliking assessment (Berridge and Kringelbach, 2008). In the case of pain expression, the “grimace scale” that was defined for mice (Langford et al., 2010) and rats (Sotocinal et al., 2011) involves scoring the orbital tightening (Fig. 4) as well as changes in the position of nose, cheeks, ears, and whiskers. With the exception of nose and cheeks, the facial expressions are similar between both rodent species (Sotocinal et al., 2011). This new approach to pain evaluation in rodents may be facilitated by partial automation. These scales have the advantage of giving access to spontaneous pain when most tests rely on evoked pain, but the approach may be limited to acute or short-term pain. Indeed, as can also be observed in chronic pain patients, this facial signature of pain is not necessarily present in chronic models (Langford et al., 2010).

Other approaches

Vocalizations can also be a way to express emotions. Concerning rodents, audible and ultrasonic vocalizations occur in various behavioral situations, but their usefulness for pain-related research is still debated (Calvino et al., 1996; Jourdan et al., 2002; Han et al., 2005; Wallace et al., 2005; Kurejova et al., 2010). Rodents can emit ultrasonic vocalizations in the 20–28 kHz range with acute pain exposure, 22–25 kHz vocalizations being known to be associated with aversive stressful situations (Covington and Miczek, 2003). However, this measure does not appear to allow evaluation of spontaneous pain in a chronic pain context (Jourdan et al., 2002; Wallace et al., 2005). A recent study suggested that vocalizations at higher frequencies may be an indicator of chronic pain in mice (Kurejova et al., 2010), but these findings are still to be confirmed by other research groups.

The impact of chronic pain on autonomic controls, sleep regulation, reward and mood-related phenotypes, cognitive functions, social interactions, and so forth, is subject to growing scientific interest. These works are important to understand the complex consequences of chronic pain, but they won’t necessarily offer markers that are always present. Indeed, they often require very specific conditions for a given phenotype to be seen, and variability because of interindividual differences, species, strain, and model may also be expected, which is somewhat similar to the complex consequences of pain in patients. For exam-

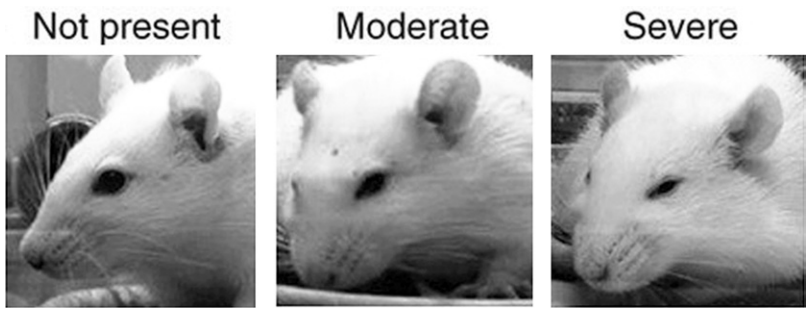

Fig. 4. Facial expression of pain in rats. Example of orbital tightening scoring as part of the rat grimace scale (Adapted from: Sotocinal et al., 2011, with permission).

ple, anxiety- and depression-related phenotypes in a neuropathic pain model are not always observed (Kontinen et al. 1999; Hasnie et al., 2007; Suzuki et al., 2007; Benbouzid et al., 2008a; Gonçalves et al., 2008; Matsuzawa-Yanagida et al., 2008; Urban et al., 2011; Yalcin et al., 2011b), and it appears that these mood-related phenotypes display delayed (Suzuki et al., 2007; Benbouzid et al., 2008a; Yalcin et al., 2011b) and time-dependent development (Yalcin et al., 2011b) with chronic neuropathy. Studying these complex states may also bring advances to the definition and relevance of chronic pain models. Indeed, the duration parameter and the nociceptive phenotype are presently the only parameters that are considered. It remains an open question whether a preclinical model should also contain a parameter reflecting quality of life to be considered as "chronic pain."

BEYOND TESTS AND MODELS PROCEDURES ARE CRITICAL

Using the finest tests and models available, increasing research efforts to refine them as well as looking for new parameters, developing new automated tests to limit experimenter bias, or increase experimental throughput, is important, but it may be of limited impact with inappropriate procedures. Indeed, the relevance of preclinical research on rodents relies on the appropriateness between the chosen models, tests, and procedures, that is, on relevant protocols.

In this context, the choice of the test should depend on the model that is used and on the scientific question that is addressed. As previously evoked, shock sensitivity may be more appropriate than the hot plate as control for shock-using experiments. For neuropathic pain models, and based on clinical information, cold may be more relevant than heat as thermal stimulus, and mechanical allodynia is a parameter of choice but is still imperfect. Indeed, present procedures in rodents evaluate static allodynia (pressure stimulation) while patients mainly complain about dynamic allodynia (brush stimulation), which can imply different mechanisms. Dynamic allodynia can be assessed in rats using paint brushes (Thibault et al., 2008), but this procedure remains rarely used.

Drug doses in pharmacological studies are critical. A major gap in the range of doses is often present between rodents and humans. It is partially justified by differences in drug metabolism or by differences in affinities, but it strongly limits the relevance of the study when doses are out of selectivity range or when they induce overall behavioral perturbations that interfere with the nociceptive measure. Deliberately increasing doses until some response is observed is not always appropriate. The treatment duration is also a main parameter. Studying acute drug effects is adequate when considering transitory pain or primary analgesia, that is, analgesia resulting from the direct action of the drug on its molecular target, whether receptor or channel. However, such approach might be challenged in a chronic pain context. When facing a condition lasting weeks to months, acute analgesia may be of limited interest, unless its action is maintained over time and after chronic treatment. Moreover, in such a chronic pain condition, one might expect some drugs to act indirectly, through downstream plasticity mechanisms, which could require days of treatment before any benefit can be seen. This would be in agreement with therapeutic delays in clinical practice. In this case, drug screening through acute administration and testing may not be the best choice, even if it is of lower cost and higher throughput than chronic treatments. The idea that a drug displaying analgesic action after chronic administration should also have some action at first injection may also be challenged (Benbouzid et al., 2008b). The idea that a drug can display both acute and delayed analgesic actions and exert both through the same mechanism should be questioned.

Temporal aspects of protocols are important to consider, particularly when working on chronic pain models. Beside treatment duration, testing time is another aspect to look at. Neuropathic pain is an evolving condition, and different mechanisms underlie its early and late stages. Early treatment procedures may then be of interest for preventive aspects, whereas late treatments may model curative clinical management of neuropathic pain. Unfortunately, the transition between both aspects remains unclear: does it happens in rodents after a week, 2 weeks, or later? Does it require the presence of a non-nociceptive phenotype reflecting an alteration of the animal's "quality of life?" Ethical and economical arguments favor fast, short-term experiments, which may sometimes be detrimental to preclinical relevance.

CONCLUSION

Nociception and pain is an important field of both neuroscience and medical research. Over time, various tests and models were developed in rodents to provide tools for fundamental and translational research on the topic. Tests using thermal, mechanical and chemical stimuli, as well as measures of hyperalgesia and allodynia, models of inflammatory or neuropathic pain, are part of the toolbox available to researchers. These tests and models allowed rapid progress on fundamental aspects of physiological and pathological pain, but effort is still needed to reach translational value for patient treatment. In this respect, the relevance of protocols, that is, the adequate association of models, tests, and procedures, is a critical point. Temporal aspects, related either to the treatment or to the model durations, are important, and the question of markers of the transition from tonic pain to a pathological "chronic pain" state remains challenging. The search for pain-related parameters, rather than simple nociceptive reflexes, is another challenge for the field. In parallel, the study of pain consequences on other complex states, including parameters that may reflect impairments in the "quality of life," is growing. Thus, it appears that the field does not simply rely on the existing toolbox, but is in constant progress to improve the quality of research and access new parameters.

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