Chao Ma
Yuguang Huang *Editors*

Translational Research in Pain and Itch

Second Edition

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Editors
Chao Ma
Department of Anatomy, Histology and Embryology,
Institute of Basic Medical Sciences,
Neuroscience Center, School of Basic Medicine, Peking Union Medical College,
Chinese Academy of Medical Sciences
Beijing, China

Yuguang Huang
Department of Anesthesiology,
Peiking Union Medical College Hospital,
Peiking Union Medical College Chinese Academy of Medical Sciences
Beijing, China

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Preface

The past decade has witnessed exciting advances in the basic research of pain and itch—both are our most basic yet still mysterious sensations. Clinically, the treatment of prolonged and intractable pain (chronic pain) and itch (pruritus) cost billions of dollars every year worldwide while the results are often unsatisfying or accompanied with serious side effects. Despite the slow progress in drug discovery, scientists all over the world have recently acquired more insights into the mechanisms underlying pain and itch in both physiological and pathological conditions. The gaps between basic research and clinical application are eagerly waiting to be filled by translational research. This book provides a comprehensive review of recent advances in the translational research on pain and itch. The contributing authors are world-renowned scientists and have made important discoveries in the relevant field of research. Their findings not only shed light on the mechanisms but also pave the way for developing novel strategies for the effective and safe treatment of chronic pain and pruritus. Hopefully not long from now, medical practitioners can be more confident and patients can be more optimistic when facing these annoying (and often terrible) conditions. We sincerely appreciate all the contributing authors, our editorial team from the Joint Laboratory of Anesthesiology and Pain in Peking Union Medical College, and the Springer publisher. This book would not be possible without their time and effort.

Beijing, China

Chao Ma
Yuguang Huang

Contents

Chapter 1
Assessment of Itch and Pain in Animal Models and Human Subjects

Tangmi Yuan, Juan Li, Le Shen, Wanying Zhang, Tao Wang, Yinyan Xu, Jie Zhu, Yuguang Huang, and Chao Ma

Abstract For the past century, scientists have developed a variety of methods to evaluate itch and pain in both animal models and human subjects to throw light on some of the most important pathways mediating these unpleasant sensations. Discoveries in the mechanisms underlying itch and pain in both physiological and pathological conditions relied greatly upon these studies and may eventually lead to the discovery of new therapeutics. However, it was a much more complicated job to access itch and pain in animal models than in human subjects due to the subjective nature of these sensations. The results could be contradictory or even misleading when applying different methodologies in animal models, especially under pathological conditions with a mixed sensation of itch and pain. This chapter introduces and evaluates some of the classical and newly designed methodologies to access the sensation of itch and pain in animal models as well as human subjects.

Keywords Itch · Pain · Animal model · Human subject

T. Yuan · L. Shen
Department of Anesthesiology, Peking Union Medical College Hospital, Beijing, China

J. Li
Department of Anesthesiology, Peking Union Medical College Hospital, Beijing, China

Department of Anatomy, Histology and Embryology, Institute of Basic Medical Sciences, Neuroscience Center, School of Basic Medicine, Peking Union Medical College, Chinese Academy of Medical Sciences, Beijing, China

W. Zhang · T. Wang · Y. Xu · J. Zhu · C. Ma (✉)
Department of Anatomy, Histology and Embryology, Institute of Basic Medical Sciences, Neuroscience Center, School of Basic Medicine, Peking Union Medical College, Chinese Academy of Medical Sciences, Beijing, China

Y. Huang (✉)
Department of Anesthesiology, Peking Union Medical College Hospital, Peking Union Medical College, Chinese Academy of Medical Sciences, Beijing, China

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1.1 Introduction

Itch and pain are both unpleasant sensations that may indicate actual or potential tissue damage. Despite the ability to clearly discriminate between itch and pain in human subjects, it has never been an easy job to access such information in animal models. Itch, often defined as a "desire to scratch," is actually a multifaceted sensation. Although the general discourse mainly deals with histaminergic and nonhistaminergic itch (Davidson and Giesler 2010; Johanek et al. 2007), more sub-classifications could be beneficial. Pain faces a similar situation. In 1979, the International Association for the Study of Pain (IASP) defined pain as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage" (Iasp 1979). This definition also clearly indicates that pain is a multidimensional experience. This chapter provides an overview of the methods used to assess experimentally induced itch and pain and analytically outlines the recently introduced animal models and human study protocols for itch and pain that have been reported in the research literature (Andersen et al. 2015).

1.2 Assessment of Itch in Animal Models and Human Subjects

Itch, also known as pruritus, is an unpleasant sensation that may prompt the sufferer to scratch the affected area that is aimed at alleviating or eliminating the effects of the stimulus and the on-going irritation or discomfort (Frese et al. 2011; Patel and Yosipovitch 2010; Shim and Oh 2008).

1.2.1 Assessment of Itch in Animal Models

1.2.1.1 Assessment of Itch in the Nape of Mice

An intradermal injection of histamine and capsaicin each elicited hind limb scratching behavior when injected into the nape of the neck of the mouse indicated that there may be only one type of behavior toward an injection into the nape of the neck (Shimada and LaMotte 2008).

1.2.1.2 Assessment of Itch in the Cheek of Mice

In 2008, LaMotte's study modified Kuraishi model (Kuraishi et al. 1995) by administering intradermal injection of histamine and capsaicin, known to evoke predominantly itch and pain, respectively, in humans; each elicited hind limb scratching behavior when injected into the nape of the neck of the mouse. When the same

chemicals were injected into the cheek of the mouse, there were two site-directed behaviors: histamine elicited scratching with the hind limb, capsaicin evoked wiping with the forelimb, no crossover any more as happened in the nape intradermal injection (Shimada and Lamotte 2008).

Other pruritic chemicals, such as chloroquine, and cowhage spicules evoked both scratching and rubbing of the face, indicating a mixture of itch and nociceptive sensations after the application of these stimuli (Akiyama et al. 2010; Kim et al. 2011). Thus, the "cheek model" allows the animal to report differential responses to the application of a stimulus similar to the multiple choices available to humans. This could be advantageous in evaluating whether candidate therapeutic drugs applied to mice will be selective for blocking itch or pain in humans.

The cheek model might also be useful in determining whether an agonist selective for a specific isoform of a receptor elicits one type of site-directed behavior rather than a mixture of behaviors that might be evoked by a less selective chemical that activates multiple isoforms. For example, scratching the site of a histamine injection (Dunford et al. 2007) or an allergic contact dermatitis (Rossbach et al. 2009) on the rostral back of the mouse was reduced but not eliminated by either an H1 or an H4 antagonist. If the experiment were repeated on the cheek, it might be possible to determine whether the reduction produced by each antagonist was more related to pain, to itch, or to both.

1.2.1.3 Assessment of Itch in the Legs of Mice

In 2011 LaMotte's study, when different doses of histamine or capsaicin were injected into the calf of the mouse, there were two site-directed behaviors: capsaicin produced mainly licking, whereas histamine elicited more of a mixture of responses with more biting than licking for most animals (Lamotte et al. 2011), in which biting was characterized by contact of the incisors with the skin in a fairly high-frequency and low-excursion motion of the head. In contrast, licking was characterized by repeated protrusions of the tongue toward the skin over a longer excursion and lower frequency that could be readily distinguished from biting.

1.2.1.4 Assessment of Itch in the Eyes of Mice

Compared to other models described above, the eye model is relatively new and less used for itching research. However, it shows great potential for its obvious advantage: easy for experimenter to establish and measure. In fact, the eye model is mainly used in allergic conjunctivitis studies. The allergic conjunctivitis eye model was first established in guinea pig and then developed in mice (Laidlaw et al. 2002). Acute or chronic allergic conjunctivitis is induced by the instillation of histamine and other contact sensitizers (Nakano et al. 2009). Like many other models, scratching behavior is still the indication of an itch sensation in the eye model. It has been reported that ICR mice show the most marked scratching

behavior in response to histamine; therefore, ICR mice are considered the most suitable strain for studying mediators and/or mechanisms for itching (Inagaki et al. 2001). A bout of eye scratching was defined as when a mouse stretched its hind paw on the treated side toward its eye, leaned its head toward the paw, rapidly scratched its eye several times for approximately 1 s, and then lowered its hind paw (Andoh et al. 2012).

The allergic conjunctivitis model might also be used to find candidate therapeutic drugs because it shows different symptoms by inhibiting specific receptors by selective antagonists. For example, histamine H1 receptor antagonists inhibited not only eye scratching behavior but also allergic-like symptoms such as edema and hyperemia, while the histamine H4 receptor antagonist inhibited only scratching behavior induced by histamine (Nakano et al. 2009).

1.2.1.5 Assessment of Itch in the Rats

Similar methods have been applied to assess itch sensing in rats, although with fewer studies up to date (Table 1.1). In the cheek model, rats present the same behavioral responses to pruritogens and algogens as mice, that is, hind limb scratching and fore limb wiping, respectively (Klein et al. 2011). However, rats have a different pruritogen and algogen pattern compared with mice (Akiyama and Carstens 2013). For example, intradermal injection of histamine evoked pain-related behavior instead of itch sensing in rats. When injected intradermally in the rostral back, 5-HT and formalin stimulated hind limb scratching. In addition, cowhage spicules failed to elicit significant itch or pain behavior when injected into the cheek of rats (Klein et al. 2011). Other investigators (Minami and Kamei 2004) attempted to evaluate itch behavior in a rat model in the eye, by applying eye drops containing histamine locally and creating a conjunctivitis model. However, "forelimb

Table 1.1 Assessment methods and chemicals applied to evaluate itch in rats

Assessment methods	Application site	Behavioral response	Sense implication	Chemicals
Intradermal injection	Cheek	Hind limb scratching	Itch	5-HT, formalin, chloroquine, SLIGRL-NH2, capsaicin
		Fore limb wiping	Pain	Histamine, SLIGRL-NH2[a], capsaicin[a], AITC
	Rostral back	Hind limb scratching	Itch	5-HT, formalin
Cowhage spicules insertion	Cheek	Not significant	–	–
Eye drops dripping	Eye	Fore limb movements	Itch	Histamine

[a]SLIGRL-NH2 and capsaicin cause both itch- and pain-related behaviors in rats, therefore occurring in both boxes

movements directed to the ocular surface" were regarded as an implication of itch, which seems to contradict the cheek model and requires further evidence.

1.2.2 Assessment of Itch in Human Subjects

In humans, a pruritic stimulus elicits two types of response: one related to the sensations such as a verbal report ("I have a weak itch") and the other, a reaction to the sensation, such as a feeling of discomfort and behavior directed toward the stimulus site to reduce or eliminate the sensation and the source of irritation (e.g., scratching) (Lamotte et al. 2011).

1.2.2.1 Assessment of Itch Intensity and Quality

With the exception of mechanically and electrically evoked itch, most human surrogate models produce itch lasting 5–15 min with a peak intensity rating elicited between 1 and 3 min after induction. In the case of clinically, as well as experimentally, induced itch, the sensation frequently presents with one or more associated sensations, such as pricking or burning (Papoiu et al. 2011; Sikand et al. 2009). The most common approach is to instruct the participating subject to separately rate the sensory qualities of itch, pricking, and burning on a generalized labeled magnitude scale (gLMS), a visual analogue scale (VAS), or a numerical rating scale (NRS), frequently (every 10–30 s) upon itch induction. This allows for a temporal overview of the itch and other sensory qualities and reporting of itch latency, peak, area under the curve, etc. (Andersen et al. 2015).

1.2.2.2 Defining Histamine-Dependent Itch

Histamine is by far the most studied pruritogen, having been widely used as the prototypical experimental proxy of itch, and to induce itch, histamine can be applied epicutaneously in combination with iontophoresis, by epidermal penetration with a lancet or functionally inert cowhage spicules coated with histamine or as an intradermal injection (Hagermark 1973; Papoiu et al. 2011; Shim and Oh 2008). All routes of administration are shown to produce a moderate to strong sensation of spontaneous itch, with slight differences in the reported presence of nociceptive sensations, alloknesis, and hyperknesis (Sikand et al. 2011; Simone et al. 1991).

Histamine-dependent itch has some disadvantages in particular, when injecting histamine the induced response ratio between nociception and itch appears to shift away from itch toward a more nociceptive sensation characterized by burning and pricking (Sikand et al. 2011). Lastly, the use of histamine is accompanied by a significant wheal and flare reaction regardless of the route of administration (Bickford 1938; Bromm et al. 1995; Schmelz et al. 2000; Sikand et al. 2011).

1.2.2.3 Defining Histamine-Independent Itch

Unlike histamine-dependent itch, histamine-independent itch is thought to rely mainly on a subpopulation of mechano-heat-sensitive/polymodal c-fibers (CMH) incapable of producing the extensive flare that is characteristic of histamine-induced itch (Johanek et al. 2007; Simone et al. 1991).

In the nonhistaminergic pathways, the key second messenger role is played by transient receptor potential cation channel, subfamily A, member 1 (TRPA1), a downstream target of proteinase-associated receptor 2 (PAR) and Mas-related G-protein-coupled receptor (Mrgpr) member G signaling (Terada et al. 2013; Wilson et al. 2011, 2013).

For practical purposes, in the experimental setting, a distinction between histamine-dependent and histamine-independent itch can be determined by showing that preadministration of topical antihistamine, such as doxepin, reduces the itch intensity (Johanek et al. 2007; Sikand et al. 2011). Since the terms "histamine- independent" and "nonhistaminergic" are essentially negative definitions, it is necessary to recapitulate on histamine as an itch inducer. Although the focus of this review is histamine-independent itch modalities, histamine-induced itch deserves mention.

1.2.2.4 Human Surrogate Models of Itch

Electrically Evoked Itch

A few studies have explored the opportunity of using transcutaneous electrical stimulation to induce itch, with varying success (Ikoma et al. 2005; Tuckett 1982; van Laarhoven et al. 2010). Ikoma et al. (2005) explored numerous electrical stimuli paradigms designed to produce itch and found that a 2 ms, 50 Hz, 0.05 mA stimulation with a 0.1 × 7 mm electrode induced a highly selective sensation of moderate itch rated ≈3 on a NRS (VAS 0–10), while increasing the current intensity to 0.12 mA produced the most intense itch sensation, 4.5 (VAS 0–10). At this higher intensity level, itch occurred alongside a modest level of pain at 2.2 (VAS 0–10).

Mechanically Evoked Itch

Apart from the above-mentioned electrical approach, itch can also be induced nonchemically with the use of mechanical stimulation. In a recent study, microvibration of the facial vellus hairs in a stimulus paradigm of 0–1 mm probe amplitude, at 1–50 Hz for 90 s, resulted in a mean peak itch intensity at 5 (VAS 0–10). The chin was by far the most sensitive location, while the cheek and the forehead were considerably less responsive (both ~ 2.5, VAS 0–10), and stimulation on the forearm did not produce any itch. The mechanically evoked itch was unresponsive to

antihistamine and did not entail flare or nociceptive sensations at any stimuli intensity, making the itch model unique (Andersen et al. 2015).

Proteinase-Activated Receptor 2/4 (PAR)-Mediated Itch

Cowhage spicules. The spicules found on the pod of the leguminous plant cowhage (Mucuna pruriens) and, more importantly, the sensory effects that these induce when inserted into the epidermis were described by Broadbent, who wrongfully concluded their itch inducing properties to be a consequence of an unknown substance causing histamine release (Broadbent 1953). A few years later, Shelley and Arthur isolated mucunain, identified it as a proteinase, suggested it to be the principal itch-inducing compound in cowhage, and reported that the itch sensation it induced was "very unlike that of histamine" (Reddy and Lerner 2010; Shelley and Arthur 1955). Cowhage spicules are 1–3 mm in length, with a diameter of 1–3 μm at their tip. Inserted into the epidermis the spicules evoke a moderate-to-intense sensation of itch and, to a lesser extent, sensations of burning and stinging pain (Johanek et al. 2007; Sikand et al. 2009).

Other proteinases. The use of various proteinases, such as papain and tryptase, has been attempted to mimic nonhistaminergic itch (Reddy and Lerner 2010). The results are relatively sparse and variable.

Mas-Related G-Protein-Coupled Receptor-Mediated Itch

Mrgprs are a family of approximately 50 receptors, of which several are exclusively expressed on small diameter neurons of dorsal root ganglia. In humans these include MrgprX1, a receptor for chloroquine and bovine adrenal medulla 8–22 peptide (BAM8-22), and MrgprD, which is restricted to axons innervating the epidermis and is responsive to the itch-inducing amino acid, that is, β-alanine (Dong et al. 2001; Lembo et al. 2002; Zylka et al. 2005). Itch is induced by algogens: serotonin, bradykinin, and substance P.

1.3 Assessment of Pain in Animal Models and Human Subjects

For patients with pain, based on their verbal report, diagrammatical representation of cutaneous spread, completion of pain questionnaires such as the McGill Pain Questionnaire, and pain scales such as the visual analogue scale and neuropathic pain scale provide health specialists with information about the intensity, duration, and location of the pain. While we cannot ask an animal directly about the ongoing nature of its pain experience, many of the behaviors have been reported in different animal models of temporary, persistent, inflammatory, and neuropathic pain (Xie 2011).

1.3.1 Assessment of Pain in Animal Models

This part highlights several types of nociceptive stimuli (thermal, mechanical, or chemical), which have been used in different pain models such as acute pain, chronic pain, inflammatory, and visceral pain (Xie 2011).

1.3.1.1 Tests Based on Thermal Stimuli

The Tail-Flick Test

There are two variants of the tail-flick test. One consists of applying radiant heat to a small surface of the tail. The other involves immersing the tail in water at a predetermined temperature. This test has proved particularly sensitive for studying the analgesic properties of pharmacological substances. It can also be used to evaluate basal thermal pain sensitivity or to study putative genetic differences among animals without drug ("naïve") (Carstens and Wilson 1993; D'Amore et al. 1992; Hardy et al. 1940).

The Paw Withdrawal Test Using Radiant Heat

Radiant heat was applied to a paw that had already been inflamed by a subcutaneous injection of carrageenan. Basically, the animal moves freely on a glass surface. A focused infrared source is moved under the animal when the animal is not moving, and a button press applies the heat to the plantar surfaces of the foot. When the animal feels the pain and moves the paw, a photosensor stops the clock and shows the latency from heat onset to paw withdrawal. In each test session, each animal is tested in three to four sequential trials at approximately 5-min intervals to avoid sensitization of the response (Hargreaves et al. 1988; Randall and Selitto 1957).

The Hot Plate Test

This test consists of introducing a rat or mouse into an open-ended cylindrical space with a floor consisting of a metallic plate that is heated by a thermode or boiling liquid up to 65 °C. Animals are brought to the testing room and allowed to acclimatize for 10 min before the test begins. Pain reflexes in response to a thermal stimulus are measured using a hot plate analgesia meter (Ankier 1974).

Tests Using Cold Stimuli

Cold is very rarely used to test acute pain. On the other hand, it is more common to test cold allodynia in animal models of neuropathies. The techniques are directly inspired by those that use heat by contact: immersion of the tail or a limb (Attal

et al. 1990), or placing the animal on a cold surface (Bennett and Xie 1988), a cold plate cooled by cold water circulating under it. The temperature (−5 °C to 25 °C) of the cold plate, which is equipped with a Plexiglas box to contain test animals, is set and allowed to stabilize for 5 min (ambient temperature of testing room 21 ± 1 °C). The animal is then placed onto the cold plate, and the time taken for the first brisk lift or stamp of the ipsilateral hind paw to occur is recorded.

1.3.1.2 Tests Based on Mechanical Stimuli

Randall and Selitto Test

The preferred sites for applying nociceptive mechanical stimuli are the hind paw and the tail. A common way to assess acute mechanical sensitivity is using withdrawal threshold to paw/tail pressure using the Randall–Selitto test (Randall and Selitto 1957). The analgesy meter for the rat paw allows for the application of a steadily increasing pressure to the dorsal surface of the rat's hind paw, tail, or muscle via a blunt point (dome-shaped plastic tip) mounted on the top of a system of cogwheels with a cursor that can be displaced along the length of a graduated beam. These devices permit the application of increasing measurable pressures and the interruption of the test when the threshold is reached. The measured parameter is the threshold (weight in grams) for the appearance of a given behavior. The intensity of pressure causing an escape reaction is defined as the withdrawal threshold. The threshold (in g) for either paw/tail withdrawal or vocalization is recorded.

Pricking Pain Test

Another approach to testing mechanical sensitivity is to use a pinprick, applying painful pressure to the plantar surface of the hind paw. This is similar to the pricking pain test performed during the neurological exam in patients and represents an alternative to the "Randall and Selitto" test. In practice, the animal is gently restrained and maintained in a natural position. The force is applied between the two tips of a rodent pincher and is independent of the movements of the limb. The rodent pincher displays the force, at which the animal reacts, and reports the mechanical nociception threshold. The behavior can be measured by the duration of paw lifting following the pinprick application or recorded as a frequency of withdrawal (% of response to the pinprick in ten trials) (Xie 2011).

Von Frey Test

Finally, mechanical hypersensitivity can also be tested with von Frey monofilaments. The von Frey filament test, developed more than 100 years ago, is still widely used today for the assessment of tactile allodynia. Von Frey monofilaments are short calibrated filaments (nylon filaments are mainly used today) inserted into a holder

that allows the investigator to exert a defined pressure on a punctiform area of the rodent paw. The animal is repeatedly stimulated with increasingly stronger filaments to determine the threshold where a nocifensive paw withdrawal response is reliably elicited. In this paradigm, testing is initiated with 2.0 g hair, in the middle of the series. Stimuli are always presented in a consecutive fashion, either ascending or descending. In the absence of a paw withdrawal response to the initially selected hair, a stronger stimulus is presented; in the event of paw withdrawal, the next weaker stimulus is chosen. According to Dixon, optimal threshold calculation by this method requires six responses in the immediate vicinity of the 50% threshold (Chaplan et al. 1994; Dixon 1980).

Electronic Von Frey Hair

Based on the von Frey test, electronic von Frey hair (Electronic VFH) was first developed by Jensen (Jensen et al. 1986) and later adapted to Rodents research by Cunha and colleagues (Cunha et al. 2004). Electronic VFH is also called an electronic pressure meter and has three components: a von Frey filament, a hand-held force transducer, and a display. The animal is stimulated with the von Frey filament similar to the classical von Frey test, and the pressure is processed by the force transducer and displayed simultaneously on the screen. The maximum applied pressure, which is the withdrawal threshold, is automatically recorded on a paw withdrawal response in one single test. This method requires three to four repeated tests to get optimal threshold calculation. Animals displaying paw withdrawal thresholds more than 2 standard deviation (SD) below the mean threshold of the un-operated are considered neuropathic (Chaplan et al. 1994). The electronic VFH has several advantages over the classical von Frey test. First, there is no need to change filaments, so stimulation areas have an equal size (the area varies with the diameter of the von Frey filaments). Second, the withdrawal thresholds are automatically recorded in every single test and have a higher level of resolution because pressure can be recorded continuously rather than in increments in the form of weights of manual filaments. Third, there is a reduction of the number of attempts required, so animals spend less time confined in the testing box and are therefore less stressed during an experiment (Cunha et al. 2004; Martinov et al. 2013). Recently, an automated von Frey equipment has been developed using a mechanically advancing probe as the stimulator can record time to withdrawal along with withdrawal thresholds (Bradman et al. 2015). This automated von Frey equipment inherited almost all the advantages from electronic VFH except the limitation by the position or placement of the hind paw (Nirogi et al. 2012).

Q-tip Test

The terms Q-tip, cotton wisp, or cotton swab test are often used interchangeably. It is a common approach to assess allodynia, especially tactile allodynia, in both animal models and human beings. A wisp of cotton pulled up but still attached to a

cotton swab was lightly stroked on the plantar surface of the rodent's paw through the floor of a wire mesh cage (Song et al. 1999). The duration of each stroke is at least 1 s, and the inter-stroking interval is 10–15 s. A single, quick withdrawal response is considered to indicate the presence of tactile allodynia. At least three measurements are taken at each time point. The threshold is expressed as the percentage of withdrawals of the total strokes (Zhang et al. 2000).

1.3.1.3 Tests Based on Spontaneous Pain-Related Behavior

Spontaneous Foot Lifting, Biting, and Licking to Estimate the Spontaneous Pain of Rats

One of the most common measures of spontaneous pain behavior in models of neuropathic pain is the quantification of foot lifting, biting, and licking (Choi et al. 1994). Each rat is placed on a brass plate kept at a neutral temperature (30 ± 1 °C) and covered by a transparent plastic dome (8 × 8 × 18 cm) without apparent external stimulus. After 5 min adaptation, use a camera to capture the behavior of the rat for the next 5 min and quantify the cumulative duration of time that the rat lifts, bites, and licks its paw.

Behavior of foot lifting, biting, and licking is interpreted as a kind of guarding action of the injured paw. Foot lifting is the behavior to increase weight on the intact hind limb and decrease the weight of injured hind paw, which indicates spontaneous pain in the injured hind paw.

However, paresthesia and dysesthesia (tingling and numbness), which are common sensory complaints of peripheral neuropathic patients, can also induce pain-like behavior as described above (Mogil 1999). Therefore, the observation of pain-like behavior may not only be implied as spontaneous pain but may also include paresthesia and dysesthesia.

Formalin Test

The formalin (37% solution of formaldehyde) test was first conducted in rats to study the analgesic effects of morphine and meperidine (Dubuisson and Dennis 1977) and was later modified for use in mice (Hunskaar et al. 1985). Depending on the specific goal of the experiment, formalin can be injected into different body regions such as forepaw or hind paw, either subcutaneously or intramuscularly. A number of other chemicals have also been used to induce pain, such as hypertonic saline, ethylene diamine tetra-acetic acid, Freund's adjuvant, capsaicin, and bee venom (Xie 2011). Different experiments can adopt different doses, depending on the object of the experiment. Usually, the average dose is 10–20 μl for mice and 50 μl or 80–150 μl, occasionally 250 or 400 μl, for rats. Most commonly, the rats receive subcutaneous injection of 5% to the plantar surface of the hind paw (Watson et al. 1997). Animals should be allowed to accommodate in the observation

chamber 15 min before and recorded up to 60 min after injection. The first 10 min and the 20–40 min after injection are for early-phase responses and late-phase responses, respectively, with a quiescent period of 5–10 min in between.

A four-level pain rating scale can be used to evaluate formalin-evoked painful behaviors. The rating criteria are the following: 0, both paws are placed on the floor with even distribution of weight; 1, the injected paw rests lightly on the ground with little or no weight placed on; 2, the injected paw is obviously elevated; and 3, the injected paw is licked, bitten, or shaken, while the uninjured paw remained stable (Dubuisson and Dennis 1977). Additionally, the number of licks or twitches of the paw per unit of time or the cumulative time spent biting/licking the paw, or even a measure of the overall agitation of the animal obtained by a strain gauge coupled to the cage, can be used as a criterion to evaluate formalin-evoked pain.

A small necrotic area will produce after formalin injection, which requires 7–10 days to recover, and an analgesic drug should be injected after the test.

1.3.1.4 Tests Based on Limb Function

Weight-Bearing Analysis Using Incapacitance Tester or CatWalk Setup

Normal rats and mice distribute weight on the paws equally. However, when one limb is injured, the weight distribution between injured and noninjured paw changed. Thus, by measuring the weight distribution, we can easily estimate the level of discomfort caused by pain. Incapacitance tester is an ideal instrument for automatically measuring the weight distribution on the two hind paws of small animals, especially in the osteoarthritis models, neuropathy, peripheral nerve injury models, cartilage degeneration, and inflammation models. By detecting the force exerted by each limb, it indicates the tendency for animal shifting its weight from one side to the other, hence facilitating a quantitative measurement of incapacitance.

During the static weight-bearing test, the animal is placed into a holder with its hind paws resting on two separate sensor plates. If one of the limbs or paws is injured, it will adjust its weight distribution on both hind paws according to the level of pain.

Moreover, with the application of an automated quantitative gait analysis system, CatWalk, it is possible to quantify several gait parameters, including the duration of each phase of the step cycle and pressure applied during locomotion (Gabriel et al. 2007). Because the parameters in the CatWalk method show great correlation with those determined by von Frey filament, the CatWalk method serves as an additional tool in the investigation of mechanical allodynia. In CatWalk, the animal traverses a walkway with a glass floor located in a darkened room. Light from a fluorescent bulb enters the distal end of the glass floor. It strikes the surface and entirely internally reflects. When the animal's paw touches the glass, light exits the floor and scatters at the paw. Images are reflected by a mirror and recorded by a CCD video camera. The intensity of the signal is relevant to the depth of paw floor contact and pressure applied (Vrinten and Hamers 2003).

Posture and Gait Analysis with Stainless Steel Cylinder

It is a computer-assisted device for analyzing the abnormal posture of the hind paw and gait, which is used for rating pain-related spontaneous behavior especially in knee joint arthritis models (Tonussi and Ferreira 1992).

The animal is placed on a stainless steel cylinder of 30 cm in diameter, rotating at 4 rpm. Then the animal is forced to walk in the stainless steel cylinder. When the electrode on the animal's paw contacts the floor, the circuit is closed. The period during which the circuit is closed is recorded. Gait disturbance is detected by paw elevation time, which is defined as the period during which animal's hind paw fails to touch the surface for 1 min. Pain score is calculated by comparing static (standing) and dynamic (walking) behaviors, including complete touch of foot pad, partial touch, or one foot stand (standing) and slight limping, severe limping, or one foot gait (walking).

Its quantitation is independent of the observer and is sensitive to all kinds of analgesics.

Assessment of Spontaneous Mobility with Biotelemetry System or Activity Boxes

In animals with knee joint arthritis, loss of spontaneous mobility is detected. Biotelemetry system is a biological technology evaluating the spontaneous activity and body temperatures in rodents. It comprises a transmitter in the peritoneal cavity of the rodent and a receiver beneath the cage. The transmitter sends signals including locomotion activity and temperatures to the intermediated processor. Then the receiver detects the signal and interprets it in the computer system (Gegout-Pottie et al. 1999). Moreover, activity box is another way for detecting spontaneous mobility. It is divided into several zones by photobeams consisting of infrared light emitting diodes (LEDs) and phototransistors. When the animal has spontaneous mobility, the pattern of photobeam will be disrupted, which will be recorded on the computer.

1.3.1.5 Tests Based on Pain Emotion and Memory

Conditioned Place Paradigm

Conditioned place paradigm (CPP) has been regarded as the most classic model for assessing the motivational effects of drug rewards and addictions. In the recent years, it is increasingly used to study the affective components of pain, the mechanism of spontaneous pain, and the selection of analgesic drugs. It has several advantages over the traditional animal models. First, since the traditional animal model is based on evoked pain, it cannot reflect the drugs' effects on the persistent spontaneous pain. So it is no wonder that many drugs selected by traditional animal model are finally proved to be useless on releasing chronic pain. Second, the reflex

behavior measured by traditional methods only indicates the sensory discriminative component of pain, but not any negative affective components.

The main principle for CPP is to regard specific locus or environmental signals as conditioned stimulus (CS) and reward/punitive stimulus as unconditioned stimulus (UCS). CS pairs up with UCS to form conditioned reflex, which promotes the approaching to or avoidance of similar situation. CPP is an ideal tool for studying pain emotional component and spontaneous pain, which plays a significant role in uncovering the mechanism of pain and evaluating new analgesic drugs. It consists of conditioned place aversion (CPA) and conditioned place preference (CPP).

Conditioned Place Aversion (CPA, Fear Based)

In conditioned place aversion (CPA), two distinct neural compartments are paired with distinct unconditioned stimulus, such as drug vs. saline. Animals have the same opportunities to enter each compartment. The time they spent in each compartment is used as the index of reinforcing value of each UCS. Animals tend to spend less time in compartments with aversive reinforcing stimulus compared with those with neutral stimulus. As a result, the previous compartment cues become the secondary negative reinforcers (Swerdlow 2000).

Johansen et al. are the first to apply CPA to study the negative affective component of pain. On preconditioning day, each rat was allowed to move freely between each compartment. The time they spent in each compartment was recorded as the "baseline" preference. On conditioning day, distinct treatment is paired with conditioning compartment. Rats received an injection of aversive reinforcing stimulus (hind-paw injection of formalin) in one compartment (A) or control treatment (no drug) in another compartment (B). They are allowed to enter each conditioning compartment freely. The result shows the rats tend to spend less time in compartment A, which is paired with aversive reinforcing stimulus. It indicates the successful establishment of formalin-induced conditioned place aversion (F-CPA), which provides great opportunities to study the negative affective components of pain (Johansen et al. 2001).

Conditioned Place Preference, CPP (Award Based)

Since the CPA can reflect animal's negative affective components of pain and avoidance motivation, it can be used as an indicator of spontaneous pain. Conversely, if the spontaneous pain can be controlled, the avoidance motivation to the previous environment can be reversed. Because the relief of pain is rewarding, it sheds light on the idea of conditioned place preference (CPP).

Chronic neuropathic pain model was established in rats with spinal nerve ligation (SNL). Different drugs are given in different compartments to see whether it can reverse SNL-evoked tactile allodynia or not. In one compartment (A), the rats with SNL are given analgesic agents such as clonidine or conotoxin, while in the other compartment (B), no drugs are given. As a result, the rats developed preference to compartment A, which indicates the establishment of CPP (King et al. 2009).

We used CPP to concomitantly demonstrate the presence of automatic spontaneous pain and evaluate the efficacy of analgesic drugs.

1.3.2 Assessment of Pain in Human Subjects

Experimental human pain models have improved our understanding of the physiology and pathophysiology of clinical nociception, inflammation, and analgesia (Bingel and Tracey 2008; Handwerker and Kobal 1993). They represent sophisticated tools to assess the efficacy of analgesic drugs in humans. They also have the potential to limit the costs of analgesic drug development by predicting clinical success with fewer resources than are needed for large clinical trials.

1.3.2.1 Requirements for Human Subjects for the Measurement of Pain

In human experimental pain models, subjects can be selected for age, sex, body measures, ethnicity, genetic and epigenetic background, health, or disease. The assay by which pain is assessed involves the pain stimulus, which can be electrical, thermal, mechanical, and chemical. This can be applied to different body parts to evoke superficial, muscle, or visceral pain.

Common criteria apply to the use of the stimuli (Beecher 1957). These include administration to body parts exhibiting minimal individual variation in terms of neuronal histological characteristics, ability to provoke minimal or no tissue damage, correlation between stimulus strength and perceived pain, and differential discrimination between strong stimuli with high resolution. In addition, the responses to stimuli should be largely time-invariant to allow for repeated measurements. The stimuli should evoke responses that can be measured by a variety of readouts.

The measure of pain involves surrogate markers, as pain cannot be measured directly, being a subjective phenomenon defined as “unpleasant sensory and emotional experience associated with actual or potential tissue damage.” The measures by which pain is quantitatively determined (Barrett 2015) range from psychophysical responses, obtained by questionnaires during most experimental pain studies or by measuring the length of visual rating scales or the number of items describing pain (Melzack 1975), to cortical evoked potentials (Chapman 1986), magnetoencephalographic, positron emission, and functional magnetic resonance tomographic assessments of the brain representation of pain (Price 2000).

1.3.2.2 Assessment of Pain in Human Subjects Using Capsaicin

In humans, intradermal injections of capsaicin always evoked only pain, typically described as burning or stinging. The localized pain began immediately upon injection, peaked within a minute later, then gradually declined. The duration of sensations produced by the highest doses was 10–15 min (Shimada and Lamotte 2008).

In the capsaicin study, the subjects were not asked to judge the intensity of any itch they may have felt. It is well known that some chemical stimuli applied to the skin can evoke nociceptive sensations such as pricking, stinging, or burning that are not rated as painful, that is, does not hurt. However, capsaicin can produce significant itch if applied topically by soaked filter paper or by capsaicin soaked, inactivated cowhage spicules. Thus, the quality of a chemically evoked sensation may depend in part on how the chemical is delivered to the skin (Andersen et al. 2015).

1.4 Relationship Between Animal Models and Human Subjects

1.4.1 Similarities Between Animal Models and Human Subjects

The basic question at hand is whether these "site-directed behaviors" differ in relation to whether the chemical evokes predominantly itch or nociceptive sensations in humans (Lamotte et al. 2011).

Although differences probably do exist in comparison with humans, notably with respect to certain cerebral structures, generally, the most reliable signs of pain are physical ones (Xie 2011). Thus, if the models are well designed and conditions are well controlled, results of the animal models can have significant accordance with the human subjects. For example, when histamine or capsaicin was injected into the cheek, mice behaved in an appropriate manner that was consistent with the respective sensations reported by humans. The mice wiped their cheeks to a substance that produces pain in humans and scratched to a chemical that evokes itch. The present finding that mice emit different behaviors in response to capsaicin and histamine applied to the cheek is in agreement with human observations that the former is nociceptive and the latter pruritic (Shimada and Lamotte 2008).

1.4.2 Differences Between Animal Models and Human Subjects

We must always bear these factors in mind because they can influence the pharmacokinetics and pharmacodynamics of administered substances just as much as the physiological mechanisms that underlie the recorded responses.

Variability can also relate to the anatomy of the nervous system: noradrenergic neurons from the locus coeruleus project toward the dorsal or ventral horn, depending on whether Sprague–Dawley rats belong to the Harlan or the Sasco stock (Cizza and Sternberg 1994). At a pharmacological level, the effects of morphine are also genetically determined, at least in the mouse. There is another problem that itch and

pain cannot be monitored directly in animals but can only be estimated by examining their responses to nociceptive stimuli; however, sometimes such responses do not necessarily mean that there is a concomitant sensation (Iasp 1979).

Interspecies variability is undoubtedly even greater. Like in the hot plate test, the behavior is relatively stereotyped in the mouse but is more complex in the rat, including sniffing, licking its forepaws or hind paws, straightening up, stamping its feet, and starting and stopping washing itself. Because so many of these behaviors exist, observation of them is difficult. All these factors make this test a very delicate one to use (Bardo and Hughes 1979; Van Ree and Leys 1985). Another example is that NK1 receptors in humans are identical to those in the guinea pig but different from those in the rat and mouse (Watling et al. 1994).

Recent advances in neuroimaging technology have reinforced the concept that the recognition of pain in humans is a multifaceted process that involves the parallel integration of sensory, emotional, and noxious perceptual information by multiple brain structures. The absence of verbal communication in animals is undoubtedly an obstacle to the evaluation of pain (Rainville 2002). Humans can be tested on psychophysical measurements, while animals cannot. This makes human itch and pain models more diverse and complicated than animal models.

1.5 Limitations of Animal Models and Human Subjects

1.5.1 Limitations of Animal Models

Animal models, no matter how carefully designed and assessed, will never be able to 100% accurately simulate human conditions. Unlike human subjects, animals cannot speak a language to accurately describe the sensations of itch and pain and the related qualities (burning, pricking etc.). Therefore, one may never understand the actual feelings of an experimental animal. In addition, animals are genetically different from humans in terms of itch- or pain-related receptors, cellular pathways, and anatomical structures. These limitations may partially underlie the difficulty of translational medical research and drug development in pain and itch. However, under proper control and training, the margin of error could be minimized for the assessment of itch or pain when applying the above testing methods in certain animal models.

Mice and rats are easily disturbed and have to adapt to the experimental condition, especially in the assessment of itch-related behaviors. Animals should be handled, restrained, and placed in containers several times on different days before the experiments began. It is worth noting that training the animal for at least 3 consecutive days prior to the operation will help to obtain a more stable response and increase the sensitivity of the assay (Xie 2011). Efforts should be made to reduce distractions to a minimum. For example, to achieve a relatively accurate assessment for the itch- or pain-related behaviors, experiments should be conducted inside a sound proof room (Shimada and Lamotte 2008). Pseudo-white noise was delivered

from a radio tuned in between stations to mask extraneous laboratory noises. When monitoring behavior in a closed test chamber, vacuum lines could be used to allow ambient air through the containers at a rate of about 300–500 ml/min so that mouse odors did not circulate between containers. Animals should be tested individually or effectively isolated so that they could not see each other during an experiment. A small amount of bedding was placed in each container to absorb any urine voided by the animals. Ambient temperature was maintained between 23 °C and 27 °C (Shimada and Lamotte 2008).

1.5.2 Limitations of Human Subjects

Experimental human pain models, like all models, provide a limited reflection of reality (Fioravanti et al. 2008). This reality is clinical pain, which is the most frequent reason for visits to a doctor and chronically affects one-fifth of adults in Europe, North America, and Australia (http://www.iasp-pain.org). Why, then, should analgesic efficacy be studied with models and not directly? In contrast to spontaneous clinical pain, experimental pain is controllable with regard to its spatial (localization), temporal (duration), quantitative (intensity), and qualitative (e.g., "pricking" or "pressing") properties.

Major confounders, such as analgesic therapy, can be avoided and placebo- controlled cross-over designs can be applied to healthy subjects. Withholding analgesic therapy would be unethical in pain patients. However, models capture not all attributes of the original pain but only those considered as relevant, and these obviously vary in their ability to reflect clinical pain. This is the background to the present comparative analysis that made use of a further characteristic of models, which can itself be subject to modeling (Trentin et al. 2006), namely, the agreement between analgesic efficacy under experimental and clinical conditions.

1.6 Conclusion

Experimental methodologies for the measurement of itch and pain have been widely used in animal models and human subjects under controlled conditions. Efforts to improve the reliability and feasibility of these approaches have been encouraging and largely facilitated our understanding of the underlying mechanisms for itch and pain. Further development of methodologies to assess itch and pain in both animal models and human subjects will be required to overcome the gaps between the bench and the bedside.

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