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PAIN ASSESSMENT IN ANIMAL MODELS OF OSTEOARTHRITIS

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Abstract

Assessment of pain in animal models of osteoarthritis is integral to interpretation of a model's utility in representing the clinical condition, and enabling accurate translational medicine. Here we describe behavioral pain assessments available for small and large experimental osteoarthritic pain animal models.

Keywords

rodents; dogs; horses; rabbits; guinea pigs

Introduction

Animal models of osteoarthritis (OA) include those that develop spontaneously or are surgically or nonsurgically (chemically) induced, all of which can provide insights into the molecular, pathological, or biochemical progression of changes in the joint during OA. Chronic pain is a hallmark of OA, and its evaluation in any animal model is integral to assessing the relevance and utility of that model in translation research. OA pain is typically localized and related to movement or weight-bearing of the affected joints. In animal models, these joints are typically the knee and/or hip joint.

Pain is difficult to evaluate objectively because of the inherent variability in the individual's interpretation of the sensory input. This variability represents the emotional and cognitive

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Conflict of Interest

The authors declare that there was no actual or potential conflict of interest that could inappropriately influence or be perceived to influence this work.

components of pain perception. In addition, little correlation exists between the objective measures of OA (e.g., radiologic or pathologic changes) and the degree of chronic pain experienced by the individual. Many assessments of pain in OA animal models are behaviorally based, and may require that the animals be acclimatized to any apparatuses before testing. Measures for assessing pain in animals can be direct or indirect. Indirect measures include static or dynamic weight-bearing, foot posture, gait analysis, spontaneous movement, and mechanical/thermal/cold sensitivity. Direct measures include hind limb withdrawal test, knee compression force, struggle threshold angle of knee extension, knee tissue edema, vocalizations after stimulation of the affected knee, and brain imaging.

Pain assessment methods for osteoarthritis models in small animals

Small animals (primarily mice and rats but also rabbits and guinea pigs) are used extensively in OA research, and a large repository of historical data, especially in rats and mice, exists to which research data can be compared. Their small size and typically lower cost for purchase and maintenance compared with large animals make them attractive animals in which to model OA. Some small animals, such as mice, can be genetically altered to enable the study of specific modulators in the development of OA, and while others, such as the Duncan Hartley guinea pig, can spontaneously develop OA. Methods used to assess pain in rodents include those that are mechanically, anatomically, or chemically based.

The Rotarod Test

This is a performance test that utilizes a rotating rod onto which a rodent is placed. The rotating rod creates forced motor activity so as to evaluate functional parameters, such as balance and coordination, by measuring riding time (seconds) or endurance. Each animal is trained for 5 minutes at a constant speed of 4 rpm on the rotarod (Rotamex, Columbus Instruments). The first trials start at least 1 hour after training. Every day, each animal receives a number of trials, separated by 30 minutes, at speeds accelerating from 4 to 40 rpm (with a 4 rpm increase every 30 seconds). Each animal is tested for a number of consecutive days (typically 3). Each trial is finished when the animal falls off the rotarod. The latency to falling off the rotarod is recorded and used in subsequent analyses (Figure 1).

Computerized Incapacitance Meter System

The Dynamic Weight Bearing (DWB) system is a computerized incapacitance meter (similar to gait analysis) in which behavioral observations are made on the animal during free ambulation on four paws, which is consistent with normal gait in quadrupeds. Computerization obviates observer bias. This test evaluates weight redistribution associated with pain. Redistribution of body weight to a portion of the paw surface is quantified as OA-induced impairments in paw positioning. During data capture, raw data for each paw is synchronized with images from a video camera, and the averaged values are encrypted and recorded on a computer that has a sampling rate of 10 Hz. This provides for accurate and non-biased pain assessment. The weight distribution of the animal, per limb, can be shown for each time period, with the mean and the variation coefficient (Figure 2).

Gait Analysis – CatWalk® (Noldus)

The Catwalk method is an automated quantitative gait analysis that allows the objective and rapid quantification of individual paw parameters as well as parameters related to inter-limb coordination (1). Briefly, light from a fluorescent tube is sent through a glass plate. Light rays are completely reflected internally. As soon as anything, e.g. a rat's paw, is in contact with the glass surface, light is reflected downwards. It results in a sharp image of a bright paw print. Locomotion can be considered as a daily life activity, i.e. it is a voluntarily initiated movement, repeated several times a day. Daily use of the painful limb can lead to

habituation, which further results in a subsequent attenuation of the behavioral gait adaptation. Analysis of all gait parameters is performed through the use of an appropriate software program.

Assessment of Spontaneous Behavior

Spontaneous exploratory activity is measured as a method to assess ongoing pain (2, 3). Animals are tested in clean, clear vivarium plastic cages (42×25×20 cm) enclosed in a cage rack. Photobeam Activity System (San Diego Instruments, San Diego, CA). Adjacent beams are 5 cm apart and beam interruptions are recorded automatically. One set of photo-beams is set at foot level to measure ambulation (movement from one beam to another), and an upper set of photo-beams is set 11 cm (rats) or 5 cm (mice) above ground to measure rearing (beam breaks in the vertical direction). Activity is monitored in a low lit room for a 60-minute period (Figure 3).

The Laboratory Animal Behavior Observation Registration and Analysis System (LABORAS) is an automated system that recognizes several normal and special behaviors of rats and mice by analyses of the forces that are induced by the movement of the animal. Pattern recognition software then determines and quantifies behaviors that may have been changed by OA-induced pain, including hind limb licking, scratching, wet dog shakes, head shakes, head twitches, purposeless chewing, grooming, locomotion, climbing, immobility, and feeding. Position tracking information is also monitored and quantified, including position (X, Y) and position distribution, speed, and traveled distance. Changed behavioral parameters in an experimental OA model can also be interpreted to be knee OA-induced pain response (4, 5) (Figure 4).

Mechanical sensitivity - von Frey test

Mechanical allodynia is measured by use of a series of calibrated von Frey filaments (Stoelting, Bioseb), which range in bending force from 0.008 to 300 grams (typical force range used in rat testing is 0.4–15 grams). The animal to be tested is placed on a wire-mesh floor with an inverted plastic shoebox-type rodent cage placed over it. In order to access the plantar surface of the animal's foot, the rodent cage and wire mesh flooring can be suspended on a stainless steel surgical instrument tray with the tray removed. The von Frey filaments are applied starting in ascending order at right angles to the midplantar surface of the hind paw through the mesh floor. Each filament is applied to the foot until it bends. Once the filament bends, continued advancement produces more bending but not more force. The time interval between consecutive filament administrations is at least 5 seconds. The threshold force corresponding to 50% withdrawal is calculated, using the up-down iterative method, as described previously (6, 7). An electronic von Frey unit – a dynamic plantar aesthesiometer (Bioseb) is available that allows measurement of the sensitivity threshold in one test with high repetitiveness. It consists of a moveable touch-simulator unit, a framed metal mesh, a 2-compartment animal enclosure, and a microprocessor controlled electronic unit. The animal moves freely within the enclosure positioned on the metal mesh. Once the animal has acclimatized to the apparatus and ceased exploratory behavior, the operator places the touch simulator below the animal's paw. The unit then automatically raises the filament at a preset force until a signal is received that the animal has either moved its paw or the greatest preset force has been met. Latency to paw withdrawal and force exerted are recorded. Observer bias and operator error are minimized as are the possible effects of room temperature and humidity, which are possible when using the von Frey filaments (Figure 5).

Thermal Sensitivity - Hargreaves Test

The pain threshold to high temperature is tested using the plantar test (Hargreaves Apparatus, Type 7370; Ugo Basile). Animals are placed into the Hargreaves apparatus, an

individual plastic cage that has a glass floor, 5 minutes before the experiment. A noxious thermal stimulus is focused through the glass onto the plantar surface of a hind paw until the animal lifts its paw away from the heat source. The paw withdrawal latency is automatically displayed to the nearest 0.1 second. A cutoff latency of 20 seconds is used to avoid tissue damage. The latency of nociceptive reaction is measured in seconds under baseline conditions and weekly after OA induction. A simpler version of the Hargreaves test involves the use of the hot plate test, wherein an animal is placed on the plate, within a surrounding enclosure. The latency to onset of brisk hind paw lifting and/or flicking/licking of the hind paw is assessed at different temperatures. A cold- or hot-plate analgesimeter (Bioseb) can also be applied to test temperature-induced pain sensitivity resulting from exposure to heat or cold. Heat-induced pain sensation can be measured by placing the animal into a plastic cylinder that is affixed to a metal plate, which can be heated to 65° C. The operator starts a timer, which begins altering the metal plate's temperature. Once the animal lifts its paw in response to discomfort, the operator stops the timer and the number of seconds that it took for the animal to react is displayed. Cold sensitivity is measured using a ceramic plate on a bed of ice, which is cooled in a -20°C freezer. During the test, the plate is allowed to warm to 0°C as measured by two independent temperature probes. The onset of brisk hind paw lifts and/or flicking/licking of the hind paw is assessed (Figure 6).

Intraplantar Injection of Noxious Substance

For the chemically-induced pain test, an intraplantar injection of a noxious substance [6 µl of capsaicin (1 mg in 10 ml saline/7% Tween-80) or formalin (2% formalin in saline)] is used. The time the animal spends licking and flinching is measured for 15 minutes (after the capsaicin injection) or for 1 hour (after formalin injection).

Hind Limb Withdrawal Test

Also called the paw withdrawal test or the calibrated forceps test, this test can be performed manually or, in rats, by use of an electronic unit (Rodents Pincher, Bioseb). The electronic unit allows the induction of a quantifiable mechanical stimulation on a linear scale by use of calibrated forceps. The animal is gently restrained in a natural position, and the forceps applied to the hind paw. A preset force (between 0 and 900 grams) is applied between the two tips of the forceps that is independent of the movements of the limb. The force at which the animal reacts is recorded and data are stored in the unit.

Vocalizations Evoked by Extension of the Knee and Knee Pressure Test

Knee extension test is performed for both legs by starting with knee in resting position (slightly flexed). While holding the animal's thigh, its knee is extended. The number of vocalizations that occur during 5 extensions is recorded (8) (Figure 7). Vocalizations can also be measured by knee pressure. A knee pincher is placed on the ipsilateral knee joint and careful pressure applied until the animal vocalizes, which is indicative of an uncomfortable feeling created by the knee joint pressure. The pressure measurement at which vocalization occurred is recorded and compared with that for the contralateral knee (Figure 8).

Pain assessment methods for osteoarthritis models in larger animals

Large animals that have been used for modeling OA include dogs, sheep, goats, and horses (9). These species are used experimentally to study OA in the knee induced by surgical techniques. In addition, dogs and horses can develop OA spontaneously and at a greater incidence than in rodents. Phylogenetically closer to humans than rodents, dogs represent the primary large animal used in modeling OA, and results in this species may be more accurate in predicting clinical relevancy (10).

Methods used to assess OA pain in dogs include direct observation by experts of the degree of lameness, gait analysis using a force plate apparatus, and subjective rating scales. Subjective rating scales include the Glasgow University Veterinary School questionnaire (GUVQuest) (<http://theses.gla.ac.uk/3193/1/2005WisemanOrrPhd.pdf>) and the canine brief pain inventory (CBPI) (11). Both are extensive questionnaire-based assessments, more typically used during clinical evaluation and completed by the animal's owners. GUVQuest consists of 109 descriptors rated on a scale of 1 to 7 and assesses pain through its impact on health-related quality of life. CBPI is based on the human-equivalent brief pain inventory (BPI) often used in human clinical trials to assess pain. No such subjective scoring systems exist for other large animal species.

Force plate (kinetic) gait analysis systems are available for use in large animals, such as the Animal Walkway® System, the equine Hoof® System (both from Tekscan, Boston, MA) or a biomechanical force platform (Advanced Medical Technology, Inc, Watertown, MA). Such systems provide for automated calculations of gait parameters. Animals are placed on the device that utilizes scanning electronics, sensors and software to collect data on gait, including discrepancies between pressure profiles of each paw or foot and ground reaction forces, such as peak vertical force. Studies have shown that such measures may be appropriate for evaluating the development of OA in dogs (12, 13). However, no consensus exists on the best method for pain evaluation in canine OA or in large animals in general. It has been reported that changes in force plate analysis are probably the result of both structural deficits (joint laxity) and pain sensation, making force plate analysis not the most sensitive sole measure of pain in dogs (10).

Conclusions

Rodent models of pain have historically played a dominant role in the study of pain mechanisms (14, 15) for many good reasons, including low cost, easy to handle, simplified ethical concerns (compared with larger animals), and the scientific value for new findings. For these reasons, rodent models contributed to a large historical important database in the field of drug discovery and development as pre-clinical studies, and there is no doubt that rodent animal models will continue to be the workhorses driving basic research as well as drug discovery. Unfortunately, in the use of small animal models, there are concerns whether the biological and pharmacological results in small animal models can be applied to clinical pain conditions in humans (16, 17), and underlies the necessity of biological validation steps using higher order pre-clinical species for better prediction and relevance for clinical pain. The use of appropriate larger animal may increase the probability of the successful development of new analgesic therapeutics offering enhanced efficacy and reduced adverse effects.

There are numerous small or large animal models invented to understand the mechanism of chronic OA pain and to develop drugs for OA-associated pain relief. Unfortunately, just as no animal model perfectly mimics the complex processes involved in the initiation and progression of OA as well as OA-associated symptom in humans, so too no single method of assessment is perfect.

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Abbreviation List

BPI	basic pain inventory
CBPI	canine basic pain inventory
DWB	dynamic weight bearing
GUVQuest	Glasgow University Veterinary School questionnaire
LABORAS	Lab Animal Behavior Observation Registration and Analysis System
OA	osteoarthritis

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Highlights

- We review methods for assessing pain in animal models of osteoarthritis.
- An individual's interpretation of pain sensory input impedes objective evaluation.
- Emotional and cognitive components of pain perception affect assessment.

Figure 1.
The Rotarod Test

Figure 2.
Computerized Incapacitance Meter System

Figure 3. Spontaneous photobeam activity system for rearing (vertical photobeam crossings) and ambulatory movement (horizontal photo-beam crossings).

Figure 4. Spontaneous behavior pattern recognition system by LABORAS. Vibration made by animals is measured by ultrasensitive sensors located in two corners of triangular platform

Figure 5.
Mechanical allodynia (von Frey filament testing)

Figure 6.
Thermal Hyperalgesia

Figure 7.
Knee extension

Figure 8.
Knee pressure