Video Article

Measuring Pain Behavior in Animals

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Abstract

Pain is a multidimensional and subjective experience that can be extremely difficult to measure, since no pain marker exists. Measuring pain quality and quantity in experimental preclinical models can be even more difficult since verbal ratings cannot be obtained. Current methods rely on either electrophysiological measurements of increased activity in the neural pain pathways or behavioral testing of conscious animals, both in response to painful stimulation. Both types of methods are unspecific and at best represent an indication of the actual level of perceived pain.

An infinite number of different behavioral methods have been described. In general, these methods either consist of thermal (cold spray, ice bath, ice probe, heat plate, infrared light beam, diode light) or mechanical stimulation (brush, pin prick) followed by an observer's evaluation of the response, but the individual variations in the protocols may vary extensively, or may not even be described, thus rendering replication and comparison difficult or impossible.

As inspiration, we present a video demonstration of our protocol for quantitative sensory assay (QSA) comprising a cold test (acetone drop), a dynamic mechanical test (brush), and a static mechanical test (von Frey filaments), covering the most common clinical pain qualities of central spinal cord injury pain. The von Frey test is widely used to test for pain-like behavior, but the two other tests are also frequently used. In the video we illustrate different sites for stimulus application (trunk/thorax and hind-paws) and, furthermore, present different responses to stimulation (spinal or spinal-brainstem-spinal reflex). A model of spinal cord injury, the spinal cord contusion, is used as an example. We hope the visualization of a protocol will promote better understanding of the experimental procedure, thus allowing standardization, minimizing of common biases, and enabling generalization of experimental results.

Video Link

The video component of this article can be found at http://www.jove.com/video/2372/

Protocol

The following protocol demonstrates how we performed the test in our laboratory. We hope it will assist new scientists in experimental pain research, and form the basis for a discussion with more experienced scientists. All parameters can of course be adapted to fit other needs and facilities.

Preparation

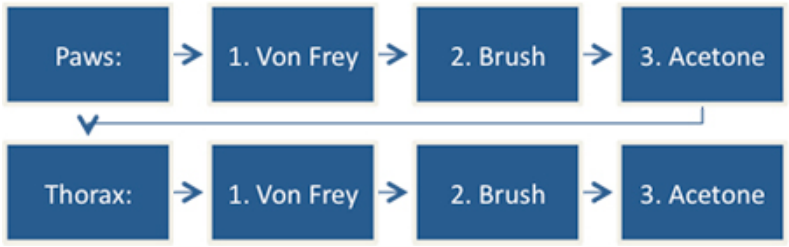
Animals: Animals are housed according to current Danish legislation and the guidelines of the Danish Animal Inspectorate (DAI), and all experiments are performed according to the DAI and the international guidelines of the IASP [6]. Rats (female Sprague-Dawley) are housed in pairs with a 12-h light/dark cycle (lights on at 7:00 AM).

Test facility: An isolated room with low levels of noise and evenly distributed light.

The test box: An elevated test box with a grid floor allowing the paws to be stimulated.

Test conditions: Behavioral testing is performed during the inactive period between 9:00 AM and 6:00 PM. Animals should be habituated to the test (box and procedure) before study start. The test can be repeated daily without habituation bias. The animals are tested in randomized sequence on each test day. Each animal is habituated to ambient temperature, noise and odour in the test box and the test facility for at least 30 min. prior to test start. Four rats are tested concurrently, though without visual contact to each other [3]. Animals must be awake, non-grooming, and in upright position (weight-bearing) when stimulation is applied [1]. Investigators must be trained in the procedure.

Test procedure



The test sequence can be randomized, but it is recommended that the most aversive stimulus is applied as the last one, in each location, to avoid wind-up like phenomenon, hypersensibilisation, or after-sensations. Furthermore, all repetitive stimulation of an area should be separated by ample time.

Von Frey (static mechanical stimulation): Performed according to up-down method described by Chaplan *et al*.[2] using von Frey monofilaments. Filaments are applied perpendicular to the skin surface until bending. Our sequence of filaments is: 0.69, 1.20, 2.04, 3.63, 5.50, 8.51, 15.14 g for paw stimulation and 0.07, 0.17, 0.69, 1.20, 2.04, 3.63, 5.50 g for thorax stimulation. The sequence is chosen based on the sensitivity of the model determined in a pilot study. The filament that elicits the first response in a test animal should be the second or third applied stimulation in the series. Filaments for paw stimulation should preferably not exceed 28.84 g since this based on our experience will only lift the normal paw (in a 200-400g rat) before bending. All test animals should be of similar size to minimize size bias.

Brush test (dynamic mechanical stimulation): 5 single applications with a soft brush.

Acetone (cold stimulation): Application of 10 µL of acetone from 0.1 ml syringe.

Response definition: A definition of a positive response must always be clearly defined and included in the method section. The shown responses are divided into spinal and spinal-brainstem-spinal reflexes.

Spinal reflex	Spinal-brainstem-spinal reflex
•Immediate brisk withdrawal in response to stimuli	•Licking •Guarding (prolonged lifting of the paw) •Struggling •Vocalization •Jumping •Flinching •Biting •Orientation towards stimuli
•This response can be applied to monitor the reflex circuitry and is thus mediated through primary sensory afferents, the dorsal horn of the spinal cord and primary motory efferents. It is not necessarily correlated to pain perception. This type of behaviour only applies to paw stimulation.	•These responses are preserved after spinal transaction and are thus considered to involve processing at least at the level of the least brainstem[5]

Other frequently observed behavior: Transient scratching of the stimulated skin area, attacking of the filament or brush, avoidance (moving repeatedly away from the approaching stimulation), escaping attempts immediately after stimulation, persistent grooming frequently initiated in the stimulated area and propagating to normal grooming pattern, freezing (lack of response to stimulation or manipulation) can also be observed. Furthermore, attention and vigilance to stimulation are frequently observed and also seen in normal alert control and sham animals.

Preparation of test box: After each trial the test boxes are thoroughly cleaned to prevent bias based on olfactory cues.

Data registration: All results of the QSA are entered into an Excel spreadsheet, which is appended to the article. Completion of an extended animal sheet [4] for each investigation is recommended.

Data analysis: A von Frey threshold (VFT) for each animal/stimulation site is calculated, e.g., according to [2]. Responses to brush and cold stimulation are evaluated separately as either positive or negative according to the chosen response definition, and the percentage of responding animals is calculated. Results are compared to a proper reference, e.g., the same site on the same animal before treatment or a sham group, depending on study design and experimental model.

Discussion

The above-described protocol can be applied to most strains of rats and most disease models. The range of von Frey filaments should however, be modified as described to each experiment. The method is easy to set up, requires little equipment, and can produce relatively reproducible results (low inter investigator and animal variability). Many factors may influence the obtained results, primarily variations in stress and anxiety levels of the animals and the subjective evaluation of responses made by the investigator. Thus, an appropriate testing facility, strict adherence to a standardized study protocol, and a limited number of investigators who are properly trained are essential in minimizing confounding factors.

The described QSA is a surrogate measurement of hypersensitivity and cannot be extrapolated directly to the complex experience of pain. The method is a useful research tool, but caution should always be taken in the conclusion made based on the results.

Disclosures

No conflicts of interest declared.

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