Rodent analgesiometry: The hot plate, tail flick and Von Frey hairs

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Rodent Analgesiometry: The Hot Plate, Tail Flick and Von Frey Hairs

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Purpose. The hot plate, tail flick and use of Von Frey hairs all test an animal's ability to sense and react to a mildly painful stimulus. The hot plate and tail flick tests each measure an animal's conscious removal of a body part from a heat source. These two tests provide models of acute thermal pain; this is analogous to the removal of one's hand from an open flame such as a candle. Although the tail flick and hot plate tests measure response to acute thermal pain, the neurologic mechanisms assessed by each test are different. The tail flick test measures a reflexive, spinally mediated response to noxious stimulation. In contrast, the hot plate test measures a more complex behavior that requires neurological processing within the brain and that results in lifting and then licking of a paw in response to acute heat. These two tests are commonly used as models of pain sensitivity, or nociception, and they provide a means to assess and compare analgesic properties of compounds. These tests have also been used to demonstrate differences in analgesic sensitivity between strains of mice (1, 2). Von Frey hairs are small calibrated filaments of various gauge and stiffness that provide the ability to measure the withdrawal threshold, or touch sensitivity, to mechanical stimulation of an animal's skin. All three of these tests have been proposed as components of a battery of tests for the behavioral phenotyping of genetically modified rodents (3, 4).

Methods. (i) Hot plate. The hot plate test can be performed manually on individual animals or by use of automated systems, some of which can test up to 16 mice simultaneously. The manual hot plate test is performed by placing an animal on a heated surface that is maintained between 52-55°C (Fig. 1). To prevent the animal from moving off the platform, a clear plastic cylinder is placed around the animal. After a brief period, typically several seconds, an animal will lift and lick a paw as the heat of the surface becomes uncomfortable. The animal is then immediately removed from the apparatus. The dependent variable in this test is the time, or latency, to lick the paw and it is manually recorded by use of a stopwatch. Some mice may jump or vocalize and these responses may also be used in place of paw licking. A cut-off time, generally 30 sec, must be established to minimize risk of an animal sustaining tissue injury from prolonged exposure to the heated surface. The automated version of this test measures the latency for an animal to jump, which is automatically recorded by the apparatus (2, 5). Administration of an analgesic, such as morphine, will result in an increased latency for an animal to respond to placement on the heated surface.

When an investigator initially begins using the hot plate test, and when first assessing a new strain of animal, care must be taken to identify the appropriate amount of stimulus (i.e., temperature of surface) that will produce the desired response (i.e., paw lick or jumping). One should also assess the effects of a reference analgesic such as morphine to determine that the planned hot plate temperature will result in the desired sensitivity (2).

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Figure 1. The hot plate test. Note that one animal is licking its paw, a behavior that signifies completion of the test. Photograph courtesy of the Stoelting Company, Wood Dale, Ill.

(ii) Tail flick. The tail flick test is performed by placing a rodent on a platform that includes a heat-generating beam of light (Fig. 2). The animal is gently restrained by being wrapped with a towel or placed in a Plexiglas tube, and then positioned so that its tail is exposed to the narrow beam of light. In a brief period of time, the heat produced by the beam of light becomes uncomfortable and the animal will reflexively move its tail away from the heat source. The time, or latency, between exposure to the heat source and flicking of the tail is recorded as the dependent variable. The latency can be measured manually with a stopwatch, or by a timer within an automated tail flick device. These timers usually work through the use of a photocell. When the animal's tail is in the test position, the photocell is blocked. When the animal moves its tail, the photocell is activated and turns off the timer and energy source.

Prior to starting the test, the intensity of the beam is adjusted to produce a latency of approximately 3-6 sec. This calibration series of tests is generally performed three times for each animal, at 3- to 5-min intervals, and the average of the three times recorded as the baseline value. The light beam should be delivered about 15 mm from the tip of the tail for mice, and approximately 50 mm from the tip of the tail for rats. Administration of an analgesic such as morphine will increase the latency, or time the animal's tail remains in the light beam. To minimize the chance of tissue trauma from heat exposure, a cut-off time such as 10 sec is established, at which time the animal is removed from the test (2).

(iii) Von Frey hairs. Von Frey hairs are nylon monofilament or metal hairs of varying diameter and stiffness that exert precise levels of force when they are pressed against the skin. Their use allows measurement of mechanical stimulation (Fig. 3). The commonly used

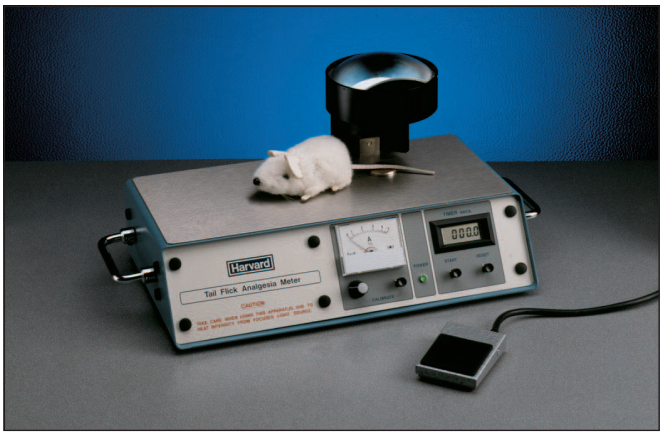

Figure 2. The tail flick test. The rat in the figure shows the placement of the tail under the light beam. When the tail moves out of the beam, a photocell is activated, and the light and timer both stop. The foot pedal is used to start the test. Photograph courtesy of Harvard Apparatus, Holliston, Mass.

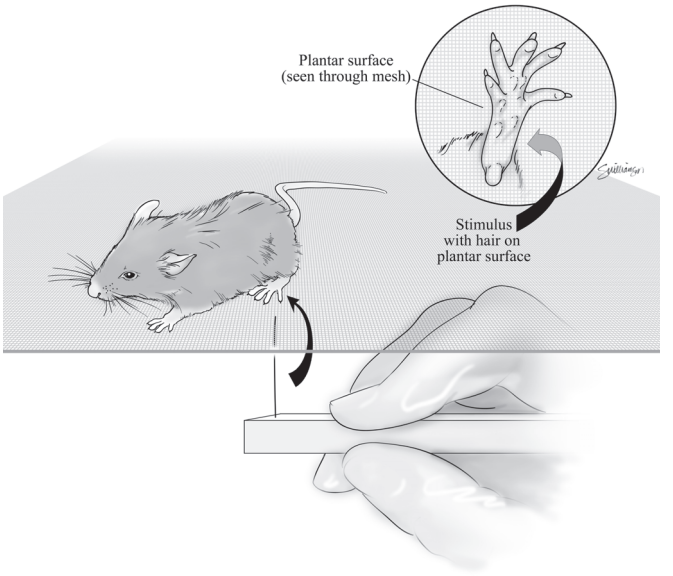

Figure 3. The Von Frey hairs. The illustration shows the proper use of the Von Frey hairs on the plantar surface of a mouse's foot.

set of hairs consists of 20 filaments. The test is performed by placing an animal on an elevated platform with a surface of wire mesh. The Von Frey hair is then inserted perpendicularly from below the platform, up through the wire mesh and into the plantar surface of the hind paw. Starting with the smallest gauge filaments, each filament is applied to the skin of the paw. The pressure is increased slowly, until the hair just begins to bend. The test is then repeated with the next largest gauge filament until the animal shows a withdrawal response by quickly flicking the paw away from the hair. This may also be followed by lifting the paw, licking it and possibly vocalizing. The outcome measure of the test is the withdrawal threshold, defined as the smallest gauge wire that produced a withdrawal response (2, 5).

Variations. Several variations have been described for the tail flick test. One variation involves immersing the tail in either hot or cold water. The hot water and cold water tail flick tests are performed by immersing the tail of a rodent into either hot (52-55°C) or ice water, respectively, and measuring the latency for the animal to remove the tail. Another variation on the tail-flick test is the Hargreaves test. This test is similar to the tail flick test described above, but the test is performed on an unrestrained animal. In this test, a beam of light is focused on the skin of the hind paw rather than the tail, and latency to remove the paw from the light spot is measured (6).

Animal welfare considerations. In any analgesiometry testing, great care must be taken to prevent the animals from inadvertent harm. Administration of analgesic substances may result in longer latencies for withdrawal, and subsequent tissue damage. Instruments should be calibrated so that tissue damage is avoided in both control and experimental groups. Multiple exposures to heat may stress the animal and influence subsequent testing latencies. As with any other behavioral testing apparatus, analgesiometry devices are often difficult or impossible to disassemble and thoroughly disinfect due to their complexity, or the materials used, and this must be taken into consideration when planning tests. The use of shared equipment involving animals of different health statuses may expose "clean" animals to unwanted pathogens.

Acknowledgments

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