Von Frey-Nociception Test

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Summary:

The test is used to assess mechanical/tactile sensitivity using the paw. It will indicate detection and processing of thermal sensory input. Von Frey filaments of different gauges or stiffness are used to determine the threshold that elicits a hind paw withdrawal response. The mechanical withdrawal threshold is defined as the minimum gauge Von Frey filament that elicits a withdrawal reflex.

Reagents and Materials:

Reagent/Material	Vendor	Stock Number
Electronic von Frey testing apparatus	IITC	
Electronic von Frey probes	IITC	
Lab coats/gloves/PPE		
Disinfectant Coverage Plus	Setris	

Protocol:

1. SET-UP (acclimation of mice, calibration of the electronic (e)-von Frey apparatus)

- **a.** Transfer the mouse from its home cage to one of the Plexiglas enclosure (testing unit) on the testing platform) and allow to acclimate for one hour.
- **b.** Ensure that the e-von Frey probe and cable are connected to the unit and turn the instrument on.
- **c.** Set the read out to zero (0.00 g) and place test weight (5.00 g) on the cone of the probe. Record readout. This calibration procedure is to be repeated at the end of each session. If there is a discrepancy greater than 0.2 g between the 2 readings, contact manufacturer.
- **d.** Switch the instrument from set-up to operation when ready to proceed.

2. PROCEDURES

- **a.** Place absorbent paper below to testing unit to collect waste.
- **b.** The apparatus should be elevated so that the technician can access the hind paw with the instrument comfortably.

c. Perform a set of baseline measurements 48 h prior administration of treatment. Mount the probe on the e-von Frey (rigid probe works well for hind paw). Zero the probe.

d. Holding the e-von Frey with both hands, raise to probe to stimulate the middle of the food pad (also use the same side hind paw for the duration of the test).

e. Increase pressure until a nociceptive response is noted (**responses may include**: retraction, licking or jumping). The observer notes the pressure corresponding to the nociceptive behaviors.

f. Repeat the process until all mice are tested once.

g. Repeat 4 more times so that each mouse has 5 measurements. Select the 3 values that deviate the least from the median of the 5 measurements. For a given mouse, if the average baseline withdrawal threshold is below 3.50 g exclude the mouse from the experiment. This ensures that all mice in the experiment have consistent baseline responses, and sufficiently high baseline withdrawal thresholds that allow observing lower thresholds following treatment.
NOTE: 2 baseline tests (at 48 and 24 h) can be done and if the average baseline measurements between the 2 baseline tests differ by more than 2g, exclude the mouse).

h. Perform experimental measurements according to investigator's protocol (after insults or to measure chronic pain, etc). Follow similar steps as for the baseline (ie, 5 measurements per mouse).

i. Experimental withdrawal thresholds are expressed as difference from baseline.

IMPORTANT NOTES:

- The procedure requires *2 technicians*, one doing the testing on mice and one recording the readout withdrawal thresholds.
- To ensure consistency, the same person should carry all testing and be blinded to the readings.
- The test should be performed in the same environment at same time of day.
- During measurements, mice can be distracted with a food pellet placed just outside the chamber or a very slight noise produced by tapping or running a pen along the wire mesh of the floor of the chamber before, but not during, the measurement. This induces the mouse to hold still enabling the experimenter to take measurements more easily.

3. CLEAN-UP

a. Return mice to their home cage.

b. Clean plexiglass chambers with soap and disinfect with Coverage Plus.