Standard Operating Procedure
The Jackson Laboratory Mouse Neurobehavioral Phenotyping Facility (JAX-MNBF)

von Frey Test
[VFT]

Area:	JAX-MNBF

Controls:	
Author	Stacey J Sukoff Rizzo, PhD Director, MNBF
Effective Date Reason for Revision	July 17, 2014 (original); Revised January 28, 2018 Revision refines protocol to incorporate larger range of stimuli presentations to accommodate for strains with different sensitivities beyond C57BL/6J

1. PURPOSE

This SOP addresses the routine procedures used for conducting the von Frey test for the assessment of mechanical nociception in mice including methods for analysis of data, and quality monitoring procedures.

2. SCOPE

The SOP applies to The Jackson Laboratory Center for Biometric Analysis and Mouse Neurobehavioral Phenotyping Facility (JAX).

3. RESPONSIBILITIES

3.1. Laboratory Staff

3.1.1. Remain up to date in training with this SOP

3.1.2. Comply with this SOP

3.2. Director of the JAX-MNBF

3.2.1. Ensures that all personnel involved running this SOP are trained to comply with this SOP

4. GLOSSARY/DEFINITIONS

Item	**Definition**
von frey Filaments	Individually calibrated plastic filaments (hairs) applied to the plantar surface of the paw that require force to induce bending motion
withdrawal response	The observation of the mouse's response to the presentation of a filament(s) indicated by a flick or lick of the stimulated paw
withdrawal threshold	The minimum amount of force (g) required to induce a withdrawal response

5. MATERIALS

5.1 Instrumentation

5.1.1. Wire floor grid. Stoelting product #57816 (Stoelting Co., Wood Dale, IL, USA; Figure 1).

Figure 1. Set up of von Frey test. Note that the picture on the left illustrates the observation arenas with individual subjects placed atop the wire floor grid. The picture on the right is a close up illustrating the grid floor and a representative filament on approach to the plantar surface of the hindpaw.

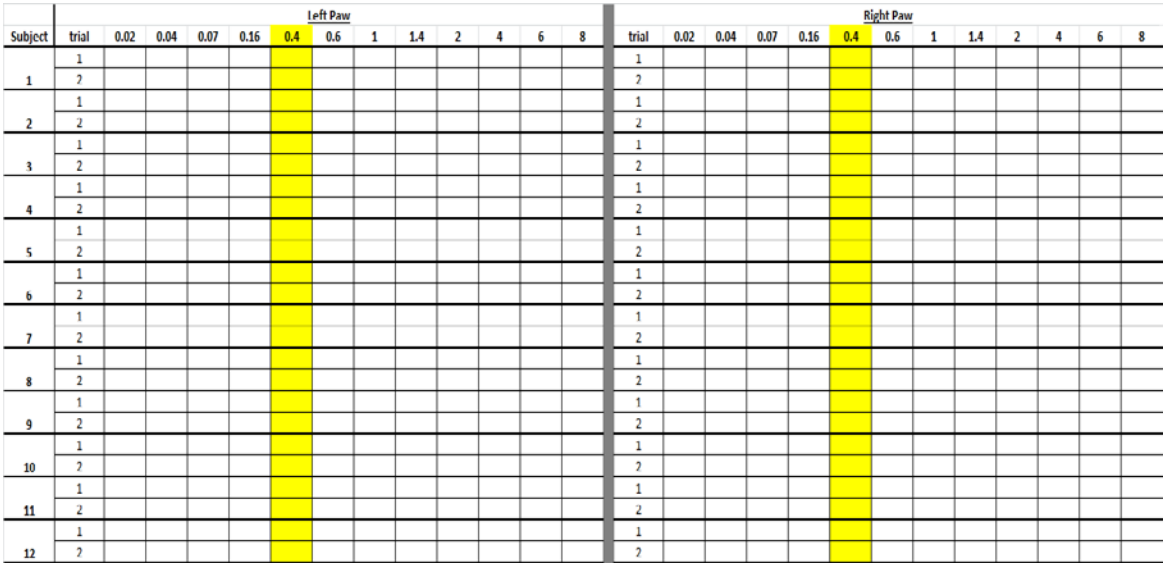
5.1.2. Observation arenas. Clear observation arenas with an open floor that is placed atop the wire floor grid to allow access to the plantar surface of the subject's feet. (Stoelting product # 57823; Multiple Configuration Animal Enclosure for 12 mice with aerated lids; Stoelting Co., Wood Dale, IL, USA; Figure 1)

5.1.3. von Frey filaments. Set of monofilaments based on the Semmes-Weinstein monofilament set that range in force from 0.02 grams to 8 grams when properly applied to the plantar surface of the hindpaw (Product #58011; Set of 20 monofilaments: Stoelting Co., Wood Dale, IL, USA; Table 1)

5.1.4. Recording template (Figure 2).

5.1.5. A clock or timer (without audible stimuli), required for studies with pretreatment time (e.g. compound testing).

Figure 2. Recording template example. Each mouse is subjected to 2 trials per paw. An "X" is placed in the recording template in the associated cell if no response is observed and the next highest filament in the series is then presented. An "O" in the template would be indicative of a withdrawal response.



	Left Paw													Right Paw											
Subject	trial	0.02	0.04	0.07	0.16	0.4	0.6	1	1.4	2	4	6	8	trial	0.02	0.04	0.07	0.16	0.4	0.6	1	1.4	2	4	6	8
1	1													1												
	2													2												
2	1													1												
	2													2												
3	1													1												
	2													2												
4	1													1												
	2													2												
5	1													1												
	2													2												
6	1													1												
	2													2												
7	1													1												
	2													2												
8	1													1												
	2													2												
9	1													1												
	2													2												
10	1													1												
	2													2												
11	1													1												
	2													2												
12	1													1												
	2													2												

Table 1. Individual filaments listed in target force (grams) and correlating Force in milliNewtons. During testing the 0.4g filament is the initial force presented and subsequent presentations of filaments are presented consistent with the "up-down" method (Chaplan et al. 1994) where as a no response would result in a presentation of the next highest microfilament in the series while a response would result in the presentation of the next lowest microfilament in the series.

Target Force (grams)	Target Force (milliNewtons)
0.008	0.078
0.02	0.196
0.04	0.392
0.07	0.686
0.16	0.1569
0.4	3.922
0.6	5.882
1	9.804
1.4	13.725
2	19.608
4	39.216
6	58.824
8	78.431

5.2. Consumables

5.2.1. 70% ethanol (ETOH) in water solution: used to sanitize the grid and observation cages

5.2.2. Paper towels

5.2.3. Non-toxic markers: Sharpie brand (red and blue preferred) for labeling of the tail with the ID number as in 6.2.8 below.

5.2.4. Small post-it notes, tape, or other means of visual identification to visually identify mouse IDs on the observation chambers during testing. Optional.

6. PROCEDURE

6.1. Environment

6.1.1. Procedure Room. The procedure room is set up with the von frey platform elevated above eye level and placed in the center of the room in order to allow a technician sitting in a chair to move around all sides of the platform without impeded access.

6.1.2. Temperature. The temperature range in the testing room is 71± 3 F.

6.1.3. Humidity. The humidity range in the procedure room is 50 ± 20%.

6.1.4. Lighting. Room lighting in the testing room are wall mounted LED sconces illuminated to the highest setting which results in an approximate lux range at the level of the arena of ~ 400-500 lux.

6.1.5. Noise. The background noise levels in the procedure room are 55-70dB. No additional or ancillary noise (white noise) is provided. Audible timers are not used during this test.

6.1.6. Visual Cues. No intended visual cues are provided for this test

6.1.7. Time of day. The test is conducted during the light phase of the circadian cycle; beginning at least 30 min after the lights on and concluding at least 30 min prior to lights off.

6.2. Subjects

6.2.1. Species. Mice

6.2.1.1. Study specific animals ordered and documented

6.2.1.2. Receipt of animals logged

6.2.2. Sex. Males or females

6.2.3. Age. The test is validated for mice ≥ 4 weeks of age.

6.2.4. Housing. Subjects may be group housed or individually housed for this test. Subjects are housed in the MNBF housing room for a minimum of 5 days prior to behavioral testing.

6.2.5. Husbandry. The test should not be conducted on the same day after a cage change.

6.2.6. Transport to Procedure Room. Subjects are transported in their home cages from the housing room to the procedure room on a wheeled transport rack. The procedure room is located ~ 10 feet from the housing room on the same floor.

6.2.7. Body Weight. If required such as for dosing, body weights are recorded to the nearest 0.1 gram.

6.2.8. Subject Identification. The tail of each test subject is identified with an ID number for the test day, written on each subject's tail using a non-toxic marker (Sharpie® brand marker in red or blue color).

6.2.9. Acclimation. Prior to the start of testing, upon transport to the procedure room, subjects are briefly handled and assessed for welfare concerns that may result in exclusion from testing (e.g., fight wounds or bite marks), weighed (if required as for dosing), identified by a tail label (see 5.2.8 above) and then left undisturbed to acclimate to the procedure room environment for a minimum 60 min prior to dosing or testing.

6.2.10. Randomization, blinding, and counterbalancing. The technician should be blind to treatment/genotype. Testing order of subjects with respect to genotype or treatment should be counterbalanced across time of day and testing order and randomized for order of testing such as to avoid all of one genotype/treatment being tested in the first part of the day. For the assessment of a test compound, where possible, an entire cage is randomized with representative samples from each drug treatment group or dose level. An excel spreadsheet is created that lists the assigned subject ID to each test session, observation arena, treatment group (blinded), the pretreatment time, the test time, the details of the environment, and a comments section to document any unexpected events (e.g. mis-injection).

6.3. Test Compound (if required)

6.3.1. Test compound preparation. Test compounds are prepared with dosing frequency, route of administration, pretreatment time, and vehicle excipient formulation documented.

6.3.2. Test compound blinding. The technician conducting the test and data analysis is blind to treatments. Following compound preparation,

information on the test compound vials are concealed and re-labeled with a code (e.g. A, B, C, D). The code is maintained until after the data is collected and analyzed.

6.4. Testing

6.4.1. Sanitization. Prior to the first mouse placed into the observation chambers atop the wire grid, and between subjects, the observation arenas and grid are thoroughly sanitized with 70% ETOH solution (in water), gross urine and feces are removed, and the grid and observation arenas are wiped dry with clean paper towels.

6.4.2. Acclimation to the observation arenas. Subjects are individually placed into the observation arenas and an aerated lid is placed atop the arena. Subjects are left undisturbed for a minimum of 60 minutes to acclimate to the wire grid and observation area.

6.4.2.1. Prior to starting the acclimation period as in 6.4.2 above, a small piece of tape or post-it with the subject's ID number may be placed on the side of the grid to provide visual ID of the test subject in its observation arena (optional).

6.4.2.2. It is critical that activity levels are low during the assessment of this test. If after the 60 minute acclimation period, to the observation arenas the mice are still active, then the acclimation period should be extended for an additional 30-60 minutes.

6.4.3. Presentation of stimulus filaments. Testing always begins with the left hindpaw. Once the withdrawal threshold for the left hindpaw is determined then the right hindpaw is tested immediately thereafter. The filament is placed onto the plantar surface of the foot with increasing pressure until it bends. Typical time required to complete both hindpaws is approximately 5 minutes but varies depending on activity level of subject and sensitivity of stimuli.

6.4.3.1. Wait for the mouse to be still with no walking or rearing. Locate the left mid-plantar surface of the hind paw of the mouse and identify the middle of the paw (application site)

6.4.3.2. Start with the presentation of the 0.4g filament and place onto the plantar surface until it bends (~ 2-3 seconds).

6.4.3.3. Record an “X” on the recording template for a non-response or an “O” when a withdrawal response (flicking, licking of the stimulated paw) is observed.

6.4.3.3.1. If no response is observed, move to the next highest filament in the series and record as in 6.4.3.3. above

6.4.3.3.1.1. Continue moving through the series in order until a withdrawal response is observed or the 8g (maximum) filament is presented.

6.4.3.3.1.2. Once a withdrawal response is observed repeat the same filament and record in the trial 2 associated cell for the left hindpaw.

6.4.3.3.1.3. Continue the presentation of the next lowest filament in the series until at least 3 consecutive presentations of filaments in the series with no responses observed.

6.4.3.3.2. If a response to the initial 0.4g presentation is observed, move to the next lowest presentation in the series and record as in 6.4.3.3.

6.4.3.3.2.1. Continue moving through the low end of the series in order until at least 3 no response presentations are observed or the lowest filament is presented (0.02g).

6.4.3.3.2.2. Once 3 consecutive lower force filaments are presented, without response, initiate trial 2 on the same paw with the presentation of filaments of increasing force in the series until a withdrawal response is observed as in 6.4.3.3.1.1. above and record in the trial 2 associated cells.

6.4.3.4. Once the thresholds are determined for trial 1 and 2 for the left paw, immediately proceed with the right paw as in 6.4.3.2 through 6.4.3.3.2.2. above.

6.5. Data Analysis and QC

6.5.1. Prior to unblinding for genotype/treatment, exclude data for subjects in which presentations to the hindpaw were erroneously switched (e.g. the technician accidently stimulated the right paw while conducting the left paw series of presentations).

6.5.2. The minimum threshold required to induce a paw withdrawal is averaged across left and right paws within each subject

6.5.2.1. If the model system includes inflammation or lesion of one side, then the unaffected paw is used for comparison.