Functional Observational Battery

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Summary:

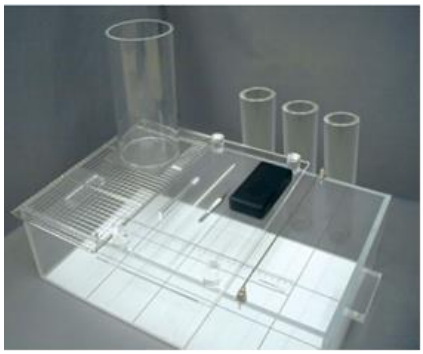
The modified SHIRPA protocol consists of a battery of functional observational measures of behaviors, and functional assessments. It is a first line screening that scores more than 40 specific unprovoked behaviors and morphological parameters. The set up includes a cylindrical viewing jar, a larger arena with clearly defined squares, a wire grid and click box. The protocol provides an assessment of muscle, cerebellar, sensory, and autonomic functions as well as physical and morphological characteristics.

Reagents and Materials:

Reagent/Material	Vendor	Stock Number
Perspex Viewing jar (20.25 cm x 15.25 cm)	Amuza	
Perspex tube (23 cm x 4 cm)	Amuza	
Plastic board (23 cm x 18 cm)		
Arena (550 cm x 330 cm, 15 squares)	Amuza	
Click box (20 KHz)	Amuza	
Timer		
Wire grid		
Scale	Mettler Toledo	
Lab coats/gloves/PPE		
Cleaning solution	10% Nolvasan	

Protocol:

1. SET-UP



a. Wipe down entire test area, including Arena, View Jar, and Contact righting tube with 10% Nolvasan prior to placement of first mouse. **NOTE: Make sure no Nolvasan residue remains as this may affect animals' behavior**.

b. Set viewing jar on top of plastic board on top of the scale on the far right side of the bench top (be sure to tare the scale), place the contact righting tube to the right of the arena. **NOTE: All**

equipment should be arranged in same order for each test and procedures done in same order.

2. PROCEDURE

a. Allow mice to acclimate to the phenotyping room for a period of 30 minutes prior to testing.

b. Open program on laptop, record the performer, the recorder, the temperature in degrees Fahrenheit, the date, location and arena square size, and any additional comments that apply to the entire project. Click on submit meta-data and proceed to Shirpa assessments.

c. Carefully grab a mouse to be tested, click the correct tab for the mouse and place mouse in the viewing jar (on scale).
NOTE: Throughout the test note any vocalization, aggression, unexpected behaviors or dysmorphological characteristics.

d. Record the weight of the mouse, the number of mice in the cage currently, and the days since cage change.

e. Proceed with SHIRPA:

A. Observations in Viewing Jar

Place mouse in View Jar for 30s and record activity body position, tremor, and head bobbing that most appropriately describes the animal's behavior.

B. Behavior Recorded in Arena

a. Click the timer with 32 seconds on it. Drop the mouse from the View Jar by removing the plastic board underneath at around 30 cm over the center of the SHIRPA arena.

b. Record the transfer arousal. An immediate transfer is when the mouse begins to walk within the first 0-1 second. A normal transfer is when the mouse pauses for 2-5 seconds before beginning to walk. An extended transfer is anything over 5 seconds. If the mouse moves a step to gain a better footing after being dropped, then proceeds to pause for 3 seconds, this is counted as a normal transfer arousal.

c. Record the number of 10cm squares the mouse moves in the first 30s in the arena. A square is counted by the distance the head and forelimbs move e.g., if the mouse begins in the center of one square and moves to the center of another square, this would be 1. Small shuffles within the square due to rearing are not counted. Movements that are diagonally are still counted.

d. Allow the mouse to move freely around the arena and observe gait fluidity and tail elevation.

e. Approach the mouse with index finger pointed and record initial response. (Try to move deliberately to the location of the mouse). Record the initial response: if the mouse tries to flee but is backed into a corner, you would call this response: "flees prior to touch"; if the mouse is touched before it flees you would call this "response to touch".

f. Hold the click box approximately 10cm behind the mouse with the speaker facing the mouse and press the button. Record the startle response of the mouse.

C. Observations above Arena

Pick up the mouse by the tip of the tail. Observe for positional passivity, trunk curl, limb grasping, and skin color.

Limb grasp is a clutching of the paws for at least 1 second. This applies for either forepaws, hind paws or both. Batting at the paws does not count; it must be a full grasp.
Trunk curl is an extended "sit-up" where the back is arched like an angry cat. **NOTE: If you are unsure if the mouse did a trunk curl, test it after the 20 second suspension is over. Holding the mouse by the tail, set it on the table and immediately pick it up again**. **If the trunk curl was present the mouse will do it again within the first few seconds.**

D. Contact Righting Reflex

Place the animal into the clear plastic tube and roll the tube slowly until the mouse is upside down, inside the tube. Observe the righting reflex five times. The mouse should right itself within 10 seconds of being turned over. If the mouse takes more than 10 seconds, the tester must determine if the mouse does not have the reflex or if extenuating circumstances caused the mouse to delay. One possible reason for a delay could be that the mouse barely fits in the tube.

E. Physical Exam

Scruff the mouse and observe tail, coat color and pattern. Examine the limbs, head, derma, genitalia, and teeth. Record anything that differs from the normal as expected mouse.
There are comment sections for any additional comments or clarifications to the drop down options.

F. Submitting and Clean-up

Click on submit to save the data for that mouse. Return the mouse to its home cage. Clean the viewing jar, arena and contact righting tube with Nolvasan. Clear and reset the timer before grabbing the next mouse. Change gloves between mice. Make sure no Nolvasan residue remains since it may affect animals' behavior. When the entire cohort is done clean the bench top with Coverage Plus.

IMPORTANT NOTES:

- To ensure reliability, score should be obtained by same examiner for a given study.
- It is important that individuals carrying out this procedure are proficient with mouse handling to avoid potential confounding effects of increased anxiety in the mouse.
- The validity of results obtained from behavioral tests is largely dependent on animal husbandry methods and familiarity with the animal being assessed. Environmental factors may contribute to results obtained therefore temperature, humidity, ventilation, background noise and light intensity must be maintained at appropriate and comparable levels prior to and throughout assessment.
- Behavioral parameters may be influenced by circadian rhythm therefore testing should be routinely carried out around the same time of day to obtain balanced and valid results. Avoid testing immediately after light-dark transition as behavioral outcome may be affected. Differences in behaviors may also be dependent age/sex/strain effects.