 Standard Operating Procedure	Title: Modified SHIRPA	
	Doc. Number: ESLIM_008_001 Rev No.	Date Issued: 26/06/08

Change Record

Date	Responsible Person	Description of Change
26 June 2008	S. Wells	Revised according to clinic management phone conference May 2008

1.0 Purpose:

- 1.1 Use modified SHIRPA for a simple first-line phenotyping screen.

2.0 Scope:


- 2.1 Individuals who have been trained and are competent in performing the procedures described herein must follow this procedure.
- 2.2 Any queries, comments or suggestions, either relating to this SOP in general or to a specific problem encountered during a procedure, should be addressed to the EUMODIC coordinator.
- 2.3 Any deviances from this protocol must be reported to the EUMODIC coordinator.r.

3.0 Safety Requirements:

- 3.1 General laboratory procedures should be followed, which include: no eating, no chewing gum, no drinking, and no applying of cosmetics in the work area. Laboratory coats and gloves must be worn at all times in the work area, unless the protocol specifically describes the appropriate attire for the procedure.

4.0 Associated Documents:

- 4.1 Appendix: Modified SHIRPA scoresheet

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4.2 SHIRPA Movie (.mov)


- a) Tremor
- b) Gait
- c) Transfer Arousal
- d) Tail Elevation
- e) Touch Escape

4.3 SHIRPA Movie (.mp4)

5.0 Notes:

- 5.1 The validity of results obtained from behavioural phenotyping is largely dependent on methods of animal husbandry and animal handling. It is important that individuals following this procedure are experienced and aware of the animal's welfare, and be familiar with the animal being tested, in order to reduce the anxiety levels of the animal prior to testing.
- 5.2 The majority of mouse behavioural studies are age/sex/strain dependent. It is important to keep these parameters comparable throughout a single experiment.
- 5.3 Environmental factors may contribute to the levels of mouse anxiety. The temperature, humidity, ventilation, noise intensity and light intensity must be maintained at levels appropriate for mice. It is essential that the mice be kept in a uniform environment before and after testing to avoid anomalous results being obtained.
- 5.4 It is recommended that all phenotyping experimentation is conducted at approximately the same time of day as physiological and biochemical parameters can change throughout the day.
- 5.5 Metadata should include whether mice are tested within a laminar flow hood or on an open bench.
- 5.6 Metadata should include how many days prior to this protocol the mice were cleaned out.

6.0 Quality Control:

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7.0 Equipment:


- 7.1 Clear Perspex cylindrical viewing jar (14 cm diameter, 18 cm height)
- 7.2 Tripod (7.5 cm height).
- 7.3 Plastic sheet [sandpapered down to create a surface that is not smooth] (20 cm x 20 cm)
- 7.4 Clear Perspex arena (60 cm length, 37 cm width and 18 cm height). In the floor of the arena is a Perspex sheet marked with 15 squares (11 cm). A grid (40 cm x 20 cm) with 12 mm mesh (approximate) is secured across the top of the width of the box
- 7.5 IHR click-box (generates a brief 20 KHz tone at 90 dB)
- 7.6 Clear Perspex tube (3 cm diameter, 20 cm length)
- 7.7 Stopwatch
- 7.8 A dowel rod (14 cm) with a fine cotton probe

8.0 Supplies:

- 8.1 Tissues
- 8.2 Alcohol wipes

9.0 Procedure:

- 9.1 Refer to the SHIRIPA score sheet for details on how to score the responses in the tests described in this procedure.
- 9.2 Behaviour recorded in Viewing Jar:
 - 9.2.1 Wipe the area clean with alcohol and allow it to dry before use.

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9.2.2 Place the viewing jar upright on top of the plastic sheet. Remove a mouse from its home cage, gripping the tail between the thumb and the forefinger and place into the viewing jar.

9.2.3 Record the following highlighted behaviours without disturbing the mouse. Record any incidents of bizarre behaviour, stereotypy and convulsions separately.

9.2.3.1 Body Position

Observe the mouse and identify whether it appears to be inactive, active or excessively active.

9.2.3.2 Tremor

Make a note of whether or not the mouse appears to tremble. The movement should be a more exaggerated movement than that seen as a consequence of the mouse breathing.

9.2.3.3 Palpebral Closure

Study the mouse for palpebral closure.

9.2.3.4 Lacrimation

Make a note of the presence or absence of lacrimation.

9.2.3.5 Defecation


Make a note of whether or not the mouse defecates within one minute of being placed in the viewing jar.

9.3 Behaviour recorded in the Arena:

9.3.1 Wipe the arena clean with alcohol and allow it to dry before use.

9.3.2 Transfer the mouse to the centre of the arena inside the viewing jar, using the plastic sheet underneath the jar to contain the mouse. Carefully remove the plastic sheet from underneath the viewing jar at approximately 25 cm above the arena floor.

9.3.3 Transfer Arousal

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Observe the immediate reaction of the mouse to the new environment and make a note of whether it appears to freeze, or makes an immediate reaction to its surrounding e.g. moves immediately, moves its head, looks around.

9.3.4 Locomotor Activity

Start the stopwatch and make a note of the total number of squares the mouse enters with all four feet in 30 seconds.

9.3.5 Gait

Study the gait of the mouse and determine how fluid its movement, e.g. whether there is retropulsion or uncoordinated movement, etc.

PELVIC ELEVATION

iStudy the position of the pelvis and the extent to which it is elevated..

9.3.6 Tail Elevation

During the locomotor activity tests, observe the tail making a note on its position for the majority of this time: whether it is dragging, is horizontally extended or is elevated

9.3.7 Startle Response

Hold the IHR click-box approximately 30 cm above the arena and emit 90 dB tone. Observe the response of the mouse.

9.3.8 Touch Escape


Approach the mouse from the front with a bent index finger before stroking on the back of the neck. Observe the response of the mouse.

9.4 Behaviour recorded above the Arena:

9.4.1 Remove the mouse from the arena gripping the tail between the thumb and forefinger and observe the following:

9.4.2 Positional passivity

Measure the immediate response to a sequence of handling positions. Once a response is recorded the sequence is terminated and no more positions are used. Use the following sequence of holds:

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9.4.2.1 Identify whether the mouse struggles when held by its tail. Move on to 9.4.3 trunk curl if it struggles, if it does not struggle move on to 9.4.2.2 holding by the scruff.

9.4.2.2 Identify whether the mouse struggles when held by the neck, a loose scruff between the forefinger and thumb Move on to 9.4.3 trunk curl if it struggles, if it does not struggle move on to 9.4.2.3 hold the mouse in a supine position

9.4.2.3 Identify whether the mouse struggles when lying supine.

9.4.3 Trunk Curl

Make a note of *forward* curling (i.e. head to abdomen) of the mouse when held by the tail.

9.4.4 Limb Grasping

Make a note of the mouse grasping its limbs when held by the tail.

9.4.5 Pinna Reflex

Place the mouse gently on to a grid and touch the proximal part of the inner canthus lightly with the tip of a fine cotton probe, observing ear retraction.

9.4.6 Corneal Reflex

Whilst the mouse is on the grid, touch the cornea lightly with the tip of a fine cotton probe, observing the eye-blink response.

9.4.7 Contact Righting Reflex


Place the animal into the clear Perspex plastic tube and roll the tube slowly until the mouse inside it is upside down. Observe the righting reflex.

9.4.8 Evidence of Biting

Make a note of whether or not the mouse makes an attempt at biting the handler or any equipment at any time during the entire screen.

9.4.9 Vocalisation

Make a note of whether or not the mouse is vocal at any time during the the entire screen.

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10.0 Supporting information:

11.0 History Review:

12.0 Emergency Procedures: