LAB_080 Simplified SHIRPA for Mice

I. OBJECTIVE

To describe the procedure for detecting phenotypes of neurological disease or of newly developed transgenic strains. The protocols are designed to provides a behavioral and functional profile by observational assessment of mice. This test will indicate defects in gait or posture, motor control and coordination, changes in excitability and aggression, salivation, lacrimation, piloerection, defecation, muscle tone, and temperature. All parameters are scored to provide a quantitative assessment that enables comparison of results.

NB: **S**mithKline Beecham, **H**arwell, **I**mperial College, **R**oyal London Hospital, **p**henotype **a**ssessment (**SHIRPA**)

II. COMMENTS / RECOMMENDATIONS

- Behavioural assessments are ideally performed in a dedicated behavioural suite.
- The environment should be free from uncontrolled external stimuli that may influence the animal's behaviour such as human traffic, unnecessary noise, and intense lighting.
- Male and female rodents should be tested separately, with one sex in the room at a time. Where possible males should be tested first, preferably on separate days but with at least thorough cleaning between the sexes. This is unless rodents are already housed within wire top cages or equivalent and both sexes are present in the home room.
- Only experienced researchers should be performing the SHIRPA assessment because it is easy to miss something if unsure or not experienced enough.

III. EQUIPMENT

- PPE

Minimum PPE is gloves and gown, additional PPE may be required based on facility or additional risk e.g. working with infectious animals.

- Appropriate trolley for transporting cages.
- Disinfectant, little brush, and paper towel for cleaning equipment.
- SHIRPA Kit - Set of equipment listed below.

Perspex viewing jar (15 cm diameter x 11 cm height)

Scale

Clear Perspex arena (55 x 33 x 18 cm , the floor is marked with grid of 15 squares)

Steel rod (0.4 cm diameter x 20 - 25 cm length)

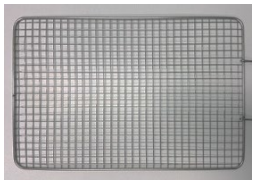
Clear Perspex tube (3 - 5 cm diameter x 15-20cm length)

Single end cotton tips (disposable)

Conditions:

- Investigators named in an animal ethics application, relative to this SOP, must be competent to implement the SOP
- Any variation to this SOP must be described in the relevant animal ethics application
- If this SOP has not been reviewed and approved by a UQ AEC within the last three years it is no longer valid and cannot be used in animal ethics applications until reapproved (see "AEC Reviewed/Approved" date in this document's header).

Metal grid
(40 cm length x 20 cm width with ~1.2 cm mesh)



Fine Forceps

Timer, Pens, Rulers

IV. PREPARATION

- Check AEC approvals to ensure that the correct procedure and personnel are approved for the planned work.
- Prepare equipment items including disinfecting prior to first use.
- Bring rodents into the room (with lighting levels pre-set at the level required for the experiment) for at least 30 mins prior to start of experiment.

Length of habituation time in the testing room should be consistent for all rodents within an experiment.

V. PROCEDURE

1. Record light levels in the middle of the arena, for reproducibility and consistency.

Lux range should be between 80-100 LUX and should remain the same for all rodents within an experiment.

2. Handling of rodents as per: LAB_006 Handling and Restraint in Mice and Neonates

Observations in a viewing Jar – 1st block of observations

3. Put the animal into the Perspex viewing jar to weigh the animal and observe undisturbed behaviours for 1 min. Score the 1st block of observations on the score sheet (attached at the end).

Behaviour in a Perspex arena – 2nd block of observations

4. Tip the animal into the middle of the Perspex arena in a single swift motion to observer motor behaviour. Immediately start a 30-second count-down timer. Score the "Transfer arousal" state and count the number of squares the animal enters with all four feet within 30 seconds.
5. Score the rest of the 2nd block of observations on the score sheet.
6. For "positional passivity", hold the animal by tail for ten seconds to observe whether there are any struggles to the handling. If not, hold the animal by neck to observe whether there are any struggles to the handling. If struggles are still not observed, go ahead with laying the animal on back and further holding the animal by hind legs. Mark it as "No struggle" if no struggles are observed at all.

Behaviour on or above a wire grid – 3rd block of observations

7. Place a wire grid on the top of the arena for the 3rd block of observations. When holding the animal by tail, observe the presence of the "Trunk Curl" and "Limb Grasping". Then lower the animal from a height of approximately 15 cm above the wire grid and score the "Visual Placing".
8. Restrain the animal on the wire grid and score the rest of the 3rd block of observations.

Behaviour when held in a supine restraint – 4th block of observations

9. Firmly grab the animal by the scruff of the neck and hold the animal in a supine restraint and score the 4th block of observations.

Other behaviours – 5th block of observations

10. Place the animal into a clear Perspex tube and record the Contact Righting Reflex.

11. Remove the mouse from the tube and hold the animal by the tail and flick it backwards through the air such that it performs a backward somersault when released. Observe the landing position to score the "Righting Reflex".

Note that this step needs to be excluded for fragile animals.

12. Place the animal on a horizontal wire grid and then rotate the grid to make it vertical with the animal's nose facing the floor. Start the stopwatch and observe for 30 seconds to score the "Negative Geotaxis".
13. The animal is held above the wire by tail suspension and lowered to allow the forelimbs to grip a horizontal wire. The animal is then held in extension and rotated around to the horizontal and released. Observe and score the "Wire Manoeuvre" according to the score sheet.
14. Return the animal back to the home cage. Remove scat and thoroughly disinfect the apparatus and allow to dry completely before proceeding to the next animal.

NOTES:

This behavioural assessment is subjective - ie: it is the individual researcher's judgement on what is normal and therefore it is CRITICAL that the same person perform the task for experimental and control mice. Furthermore, because it is subjective - the person should be blinded to the experimental groups.

VI. ANALYSIS

Once the scores are recorded on the score sheet, mouse groups can be compared with parametric or nonparametric tests. For quantitative measures such as fecal bolus counts and arena activity, two groups can be compared with the unpaired t-test and multiple groups with an analysis of variance (ANOVA). When SHIRPA is used on two or more occasions, parametric measures can be analyzed with a repeated-measure ANOVA. For nonparametric measures of the prevalence of specific behaviors, tests such as chi-square or Mann-Whitney tests can be used. When judging whether the experimental group exhibits normal behavior or not, experimenters should take into account the mouse background strain because normal mice on different backgrounds differ on certain SHIRPA subtests.

VII. REFERENCES

1. Rogers DC, Fisher EM, Brown SD, Peters J, Hunter AJ, Martin JE. Behavioral and functional analysis of mouse phenotype: SHIRPA, a proposed protocol for comprehensive phenotype assessment. Mammalian Genome. 1997; 8(10): 711-3, doi: 10.1007/s003359900551.
https://pubmed.ncbi.nlm.nih.gov/9321461/

2. Lalonde R, Filali M, Strazielle C. SHIRPA as a Neurological Screening Battery in Mice. Current Protocols. 2021 ; 1(5): e135**,** doi: 10.1002/cpz1.135.
https://pubmed.ncbi.nlm.nih.gov/34000103/

Version #	Reviewing AEC (note: all other relevant AECs ratify the approval)	AEC Review Date	Approval To Date
1	LBM	06/10/2022	06/10/2025

Attachment: Score sheet

Ex: Name:_____ Date:_____ Time:_____ Mouse ID:

		0	1	2	3	4	5	6
1st - Viewing Jar - 1 min	1. Body Position (BP) *When initially dropped into the jar*	Completely flat	Lying on side	Lying prone	Sitting or standing	Rearing on hind legs	Repeated vertical leaping	
	2. Spontaneous Activity (SA) *During the first 30 seconds*	None, resting	Casual scratch, groom, slow movement	Vigorous scratch, groom, moderate movement	Vigorous, rapid/dart movement	Extremely vigorous, rapid/dart movement		
	3. Respiration Rate (RRa)	Gasping, irregular	Slow, shallow	Normal	Hyperventilation			
	4. Tremor (T)	Important	Mild	None				
	x Urine / faeces *At the end of 1 minute*	U=	F=	Weight	g			

2nd - Behaviour recorded in Arena	1. Transfer arousal (TA)	Coma	Prolonged freeze, slight movement	Extended freeze, moderate movement	Brief freeze (<2 seconds), active movement	Momentary freeze, swift movement	No freeze, immediate movement	Extremely excited ("manic")
	2. Locomotor Activity (LA)	# squares entered with all 4 feet in 30 sec:						
	3. Palpebral Closure (PC)	Eyes closed	Eyes 1/2 closed	Eyes wide open				
	4. Piloerection (Pi)	Coat stood on end	None					
	5. Gait (G) *Observe from the side*	Incapacity	Limited movement only	Fluid but abnormal	Normal			
	6. Pelvic Elevation (PE) *Observe from the side*	Markedly flattened	Barely touches	Normal (3mm elevation)	Elevated (> 3mm elevation)			

Conditions:

- Investigators named in an animal ethics application, relative to this SOP, must be competent to implement the SOP
- Any variation to this SOP must be described in the relevant animal ethics application
- If this SOP has not been reviewed and approved by a UQ AEC within the last three years it is no longer valid and cannot be used in animal ethics applications until reapproved (see "AEC Reviewed/Approved" date in this document's header).

	7. Tail Elevation (TE) *During forward motion*	Dragging	Horizontally extended	Elevated / Straub Tail			
	8. Touch Escape (TEs) *Finger stroke from above*	No response	Mild (escape response to firm stroke)	Moderate (rapid response to light stroke)	Vigorous (escape response to approach)		
	9. Positional Passivity (PP)	No struggle	Struggles - held by hind legs	Struggles - laid supine (on back)	Struggles - held by neck (not scruff)	Struggles - held by tail	

3rd - Behaviour on or above arena	1. Trunk Curl (TC)	Absent	Present				
	2. Limb Grasping (LG)	Absent	Present				
	3. Visual Placing (VP) *The height the mouse extends fore limbs to grid*	None	Upon nose contact	Upon vibrasse contact	Before vibrasse contact (18mm)	Early vigorous extension (25mm)	
	4. Grip Strength (GS) *A gentle horizontal backwards pull*	None	Slight grip, semi-effective	Moderate grip, effective	Active grip, effective	Unusually effective	
	5. Body Tone (BT) *Compress sides of the mouse between thumb & index finger*	Flaccid, no return of cavity to normal	Slight resistance	Extreme resistance, board like			
	6. Pinna Reflex (PR) *Touch the inner pinna with the tip of the fine wire probe*	None	Active retraction, noderately brisk flick	Hyperactive, repetitive flick			
	7. Corneal Reflex (CR) *Touch the cornea with the side of the*	None	Active single eye blink	Multiple eye blink			

Conditions:

- Investigators named in an animal ethics application, relative to this SOP, must be competent to implement the SOP
- Any variation to this SOP must be described in the relevant animal ethics application
- If this SOP has not been reviewed and approved by a UQ AEC within the last three years it is no longer valid and cannot be used in animal ethics applications until reapproved (see "AEC Reviewed/Approved" date in this document's header).

	fine wire probe						
	8. Toe Pinch (TP) Gentle lateral *Compression of mid digit of hind foot with fine forceps*	None	Slight withdrawal	Moderate withdrawal, not brisk	Brisk, rapid withdrawal	Very brisk repeated extension and flexion	

		0	1	2	3	4	5	6
4th - Behaviour recorded while scruffed	1. Skin colour (SC) *Plantar surface and digits of hindlimbs*	Blanched	Pink	Bright, deep red flush				
	2. Heart Rate (HR) *Felt by palpation below sternum*	Slow, bradycardia	Normal (~700 beats per minute)	Fast, tachycardia				
	3. Limb Tone (LT) *Resistance to gentle fingertip pressure on plantar surface of the hind paw*	No resistance	Slight resistance	Moderate resistance	Marked resistance	Extreme resistance		
	4. Abdominal Tone (AT) *Palpation of abdomen*	Flaccid, no return of cavity to normal	Slight resistance	Extreme resistance, board like				
	5. Lacrimation (L)	Present	None					
	6. Salivation (S) *Gently insert the wooden end of the cotton tips between the teeth at the side of the animal's mouth*	Wet zone entire sub-maxillary area	Slight margin of sub-maxillary area	None				
	7. Provoked Biting (PB)	Present	Absent					

Conditions:

- Investigators named in an animal ethics application, relative to this SOP, must be competent to implement the SOP
- Any variation to this SOP must be described in the relevant animal ethics application
- If this SOP has not been reviewed and approved by a UQ AEC within the last three years it is no longer valid and cannot be used in animal ethics applications until reapproved (see "AEC Reviewed/Approved" date in this document's header).

5th - Other	1. Contact Righting Reflex (CRR)	Absent	Present				
	2. Righting Reflex (RR)	Fails to right when placed on back	Lands on back	Lands on side	No impairment		
	3. Negative Geotaxis (NG)	Falls off	Does not move within 30 seconds	Moves, but fails to turn	Turns but then freezes	Turns and climbs the grid	
	4. Wire Manoeuvre (WM)	Falls immediately	Unable to lift hindlegs, falls within seconds	Unable to grasp with hindlegs	Difficulty to grasp with hindlegs	Active grip with hindlegs	

Additional measures	1. Fear (F)	Freezes during transfer arousal	None	
	2. Irritability (I)	None	Struggle during supine restraint	
	3. Aggression (A)	None	Provoked biting or attack	
	4. Vocalization (V)	None	Provoked during handling	
	Other:			

Notes:

Conditions:

- Investigators named in an animal ethics application, relative to this SOP, must be competent to implement the SOP
- Any variation to this SOP must be described in the relevant animal ethics application
- If this SOP has not been reviewed and approved by a UQ AEC within the last three years it is no longer valid and cannot be used in animal ethics applications until reapproved (see “AEC Reviewed/Approved” date in this document’s header).