WELLCOME SANGER INSTITUTE

STANDARD OPERATING PROCEDURE PACKET

SANGER INSTITUTE
STANDARD OPERATING PROCEDURE

SUBJECT: Modified SHIRPA -V1

SOP Number: SOP0063	To be reviewed:
Author(s): Signed:	Date:
Editor: Signed:	Date:
Date of Implementation:	

INTRODUCTION:

The purpose of this procedure is to assess and record gross motor and neurological behaviours in wild-type and genetically altered mice.

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HEALTH & SAFETY:

- **RA003** – Hazardous Substances; *Section RA003.2*
- **RA004** – Physical Hazards; *Section RA004.6*

RESPONSIBILITIES:

All staff performing this procedure are responsible for ensuring that this SOP has been read, understood and where applicable is followed in accordance with the relevant PPL. All staff should be trained and competent to perform the procedure, where applicable they should also be licensed to perform the procedure.

RESOURCES:

Equipment

1. Techniplast Interactive Cage Change Station
2. 70% Ethanol and tissues
3. Techniplast Mobile IVC Rack
4. Mobile transport rack
5. Clean cages as required by pipeline
6. Diet (as defined by pipeline).
7. Two clear Perspex cylinders of 14 cm diameter, one of 18 cm height and one of 7.5 cm height
8. Clear Perspex sheet of 20 by 20 cm
9. Clear Perspex arena of 55 by 33 cm and 18 cm height with 15 squares of 11 cm drawn onto the bottom of it
10. Metal lid for the arena with 12 mm mess grid work in the centre
11. Clear 20 cm Perspex tubes with the following internal diameters: 20, 26, 30, 34 & 36mm
12. Two Timers (set to 1min and 30 secs)
13. Click-box generating a 19.3 kHz tone at 90 dB
14. Fine cotton probe
15. *P2_QC_tool* spreadsheet
16. Cirrus decibel meter (CR:821c)
17. Measurement ruler

Associated Documents & SOPs:

- **SOP0045** – Weigh Mice
- **SOP0062** – Grip Strength
- **SOP0064** – Use of Change Station
- SHIRPA Training Aid

Staff Required: This test can be completed by two phenotypers.

NOTE:

This procedure should be performed in conjunction with Grip Strength as part of both Mouse GP and Pipeline 2. This procedure should also be performed in conjunction with hair analysis as part of Pipeline 2.

PROCEDURE:

Before performing the procedure, verify that this is the correct procedure at this point in the pipeline by consulting the cage card(s) and confirming that the procedure has not already been performed on the mice.

1. Collect scheduled mice from the animal room and transport to the holding equipment in the test area.

2. Prepare change station for use (see SOP Use of the Techniplast Interactive Cage Change Station), opening both sides.

3. Place all equipment in the change station and clean with 70% ethanol and paper towels. Place the arena at one end of the change station with the metal lid leaning against it. Place the small Perspex cylinder next to the arena with the sheet on it and larger Perspex cylinder on top to form the viewing jar.

4. Open the pipeline 2 QC tool to see if the click box meter QC is required. If so, perform it.

Viewing Jar

5. Identify mouse to be tested by ear mark and place in the viewing jar, start the 1 min timer.

6. Score according to the current DCF (see training aid for guidance on scoring parameters). Any abnormal behaviours or observations that are not included on the DCF should be noted in the comment section, indicating where they occurred.

Arena

7. At the end of the 1 minute, transfer the mouse from the viewing jar to the centre of the arena from a height of ~30cm starting the 30 secs timer when the mouse lands on the arena floor.

8. Score according to the current DCF (see training aid for guidance on scoring parameters). Any abnormal behaviours or observations that are not included

on the DCF should be noted in the comment section, indicating where they occurred.

- If the mouse does not move during the 30 secs complete the rest of the arena scores and then gently encourage the mouse into moving. If the mouse still refuses to move, fail the relevant parameters.
- To perform the startle response, hold the click box 30cm above the mouse and wait for it to stop moving before administering the stimulus. Record the response.

Above the Arena

9. **Positional Passivity:** Lift the mouse from the arena by the tail and suspend briefly, if the mouse does not struggle when being held by the tail, hold the mouse in a loose scruff. If the mouse still does not struggle, lay the mouse supine without altering the scruff. Score the positive response according to the DCF.
    - If the mouse has a tail welfare issue **do not** lift this way and mark the parameter 'Tail welfare issue' as '*Yes*'. QC fail the parameters that cannot be measured and add a comment at the bottom of the SOP as to the condition of the tail.

10. Transfer the mouse to the metal grid placed on top of the arena and score according to the current DCF. Any abnormal behaviours or observations that are not included on the DCF should be noted in the comment section, indicating where they occurred.

11. **Contact Righting Reflex:** Allow the mouse to enter the 30 mm Perspex tube, do not force this as this may induce behaviours. Gently rotate the tube by 180º and observe whether or not the mouse attempts a righting reflex. Allow 10 seconds for the mouse to react.
    - If it is believed that the response has been affected due to the size of the tube, repeat with a tube of a different size. Record the positive response and the tube size it occurred in.

12. Perform Hair Analysis and/or Grip Strength as defined by pipeline (Refer to SOP0044 - Hair Analysis and High Fat Diet or SOP0062 – Grip Strength as necessary).

13. Clean all equipment with 70% ethanol.

14. Repeat steps 5-11 for all mice to be tested and perform a cage clean as defined by pipeline.

15. Clean all equipment and surfaces. Transfer all waste to a yellow offensive waste bag or clearly labeled waste container.

16. Ensure all cages display updated cage cards and return mice to animal room. Place red observation cards on cages when returned.

Modified SHIRPA Training Aid

Body Position

The activity level of the mouse whilst in the viewing jar.

Active – The mouse moves around the viewing jar for over 10% of the time in the viewing jar. Supported and/or unsupported rears, grooming, changes of orientation or exploratory behaviours can be seen.

Inactive – The mouse fails to move around the viewing jar and remains stationary for about 90% of the time. No rears or grooming can be seen.

Excessive activity – The mouse continually moves at an increased speed for about 90% of the time in the viewing jar. If the mouse rears excessively, it is not scored as 'Excessive activity'.

Palpebral Closure

Eyes open – at least one of the eyes is open in the viewing jar. If one eye is closed or one is partially open, note this in the comments section, but score this as 'Eyes Open'.

Eyes closed – both eyes are closed.

Lacrimation

Present – excess lacrimal fluid is present on the eye and/or the surrounding fur whilst in the viewing jar.

Absent – the mouse does not have excess lacrimal fluid in the viewing jar.

Tremor

Involuntary shaking of the mouse's body.

Present – the mouse shudders for a large proportion of the time spent in the viewing jar. Tremors are sometimes seen for short periods of time in conjunction with urination or defecation, but should only be scored when the tremors are present throughout the duration of the time in the viewing jar. Tremors are most obviously seen in the hind quarters of the mouse when stationary.

Absent – the mouse does not show any of the above signs in the viewing jar.

Defecation

Present – the mouse starts to defecate within 60 seconds of entering the viewing jar. If the mouse starts to defecate whilst being transferred to the viewing jar, this event is not counted.

Absent – the mouse does not start to defecate within 60 seconds of entering the viewing jar.

Urination

Present – the mouse urinates within 60 seconds of entering the viewing jar. Any sign of urine in the viewing jar is counted as urination as long as it is present before the end of the 60 seconds.

Absent – there is no sign of urine within 60 seconds of entering the viewing jar.

Transfer Arousal

The reaction of the mouse to being 'dropped' into the arena. Head movements are disregarded, but exploratory behaviours (where the fore paws are extended forwards, but the hind paws remain stationary, known as a 'stretch attend') count as locomotion.

Extended freeze (over 5 seconds) – all the mouse's paws remains stationary for more than approximately 5 seconds after it lands in the arena.

Brief freeze followed by movement – all the mouse's paws remain stationary for less than approximately 5 seconds after it lands in the arena, before locomotion occurs.

Immediate movement – Locomotion occurs immediately as the mouse lands in the arena.

Gait (inc. Ataxia)

The way in which the mouse walks, including ataxia (coordination of the muscles in the limbs/body resulting in a 'wobbly walk', this can be identified by the base of the tail moving from side-to-side whilst the mouse is walking in a straight line), morphological abnormalities and voluntary/involuntary behaviours.

Fluid movement – there are no major defects in the way in which the mouse walks in the arena. Any 'slight limps', 'intermittent hops', 'mild ataxia' or any other minor defects should be noted in the 'gait related comment' section, but still be scored as fluid movement.

Lack of fluidity in movement – there are major defects (including severe limps, severe ataxia and retropulsion) in the way in which the mouse walks in the arena. All observations should be noted in the 'gait related comment' section.

Gait related comment
A list of the observations made regarding the movement of the mouse in the arena. This should include which paw(s) are involved and any visually apparent morphological causes.

Pelvic Elevation
The distance between the bottom of the pelvis and the floor of the arena.
Less than 5 mm – the pelvis appears lower than expected (for mice on that genetic background) or dragging during locomotion in the arena.
More than 5 mm – the pelvis appears to be at a normal height (for mice on that genetic background) or elevated during locomotion in the arena.

Tail Elevation
The way in which the mouse holds its tail during locomotion in the arena. This should be scored by considering the angle of the middle third of the tail and its elevation above the floor of the arena. See appendix for diagrams.
Dragging – the tail drags along the floor or the middle third of the tail is held at a low position or angle for a large proportion of the time that the mouse moves in the arena.
Horizontal extension – the middle third of the tail is held in an approximately horizontal position for a large proportion of the time that the mouse moves in the arena.
Elevated/straub tail – the middle third of the tail is held in an elevated position or angle for a large proportion of the time that the mouse moves in the arena.

Locomotor Activity
This is scored by counting the number of squares that the mouse enters (with all 4 paws at the same time and excluding any square in which it lands) in the 30 seconds after being 'dropped' into the arena.

Touch Escape
The response of the mouse to being approached from the front (and subsequently touched if it does not flee) by a bent finger whilst in the arena.
No response – the mouse shows no reaction and appears to be unaware of the stimulus.
Response to touch – the mouse reacts by either making contact with the finger prior to the touch or responds to the physical stimulus by trying to avoid the finger.
Flees prior to touch – the mouse flees whilst being approached before any contact is made.

Startle Response
The reaction of the mouse to a loud auditory stimulus made by a click box above the arena.
None – the sound from the click box does not elicit a reaction and the mouse appears to be deaf (does not show any of the behaviours below).
Preyer reflex – the sound from the click box elicits the preyer reflex (flicking of the ears), but with no other reaction or movement such as a whole body flinch, flick of the tail, jump or trying to flee. If a mouse shows signs of hearing the click, but does not show the preyer reflex, the reflex is late, performed slowly or only a slight twitch of the ears is seen, record this in the comments section
Reaction in addition to the Preyer reflex – the sound from the click box elicits the preyer reflex (flicking of the ears) in conjunction with another reaction or movement such as a whole body flinch, flick of the tail, jump or trying to flee.

Tail Welfare Issue
Yes – the mouse is unsuitable to be suspended by the tail for welfare reasons such as wounds or morphological defects.
No – the mouse is suitable to be suspended by the tail which seems normal.

Positional Passivity
The response of the mouse to being held in position(s) of restraint. The next position is only attempted if the mouse does not struggle in the previous position. Struggling is defined by the mouse moving any part of its body during the period of restraint. This is most often seen in the movement of the paws and curling of the trunk from side to side.
Struggles when held by the tail – the mouse struggles when suspended by the tail for more than 5 seconds.
Struggles when held by the neck – the mouse fails to struggle when suspended by the tail, but struggles when held vertically in a loose scruff.
Struggles when laid supine – the mouse fails to struggle when suspended by the tail or in a loose scruff, but struggles when laid in supine (held horizontally in a loose scruff).

No struggle - the mouse fails to struggle when suspended by the tail, in a loose scruff or when laid in supine.

Trunk Curl
Present – the mouse curls its torso, to bring its chin towards its stomach when held by the tail. Bending sideways does not count as a trunk curl.
Absent – the mouse shows no sign of a trunk curl for at least 5 seconds of being suspended by the tail.

Limb Grasping
Present – the mouse brings its fore limbs together and grips with the paw or brings its hind limbs together and grips with the paw. If no grip is achieved or if the hind paw grasps the fore limb (or vice versa), this is not scored as limb grasping.
Absent – the mouse shows no signs of limb grasping for at least 5 seconds of being suspended by the tail.

Evidence of Biting
None – the mouse makes no attempt to bite the equipment (probe, tunnel, grid etc) or the operator during the experiment.
Biting in response to handling - the mouse attempted to bite the equipment (probe, tunnel, grid etc) or the operator during the experiment. This should involve an aggressive attempt to bite and exploratory behaviours performed with the mouth open should not be considered.

Vocalisation
Present – audible vocalisation is heard during the experiment.
Absent – no audible vocalisation is heard during the experiment.

Pinna touch reflex
Present – the mouse reacts to the cotton thread placed in its ear by either flicking its ear or shaking its head.
Absent – the mouse fails to react to the cotton thread placed in its ear by flicking its ear or shaking its head.

Corneal touch reflex
Present – the mouse reacts to the cotton thread placed on the surface of its eye by blinking.
Absent – the mouse fails to react to the cotton thread placed on the surface of its eye by blinking.

Contact Righting Reflex
Present – the mouse reacts to being inverted by turning its head or moving its limbs to attempt to right itself within 10 seconds of the tube being rotated. Normal head movements should not be counted.
Absent – the mouse fails to react to being inverted by turning its head or moving its limbs to attempt to right itself within 10 seconds of the tube being rotated.

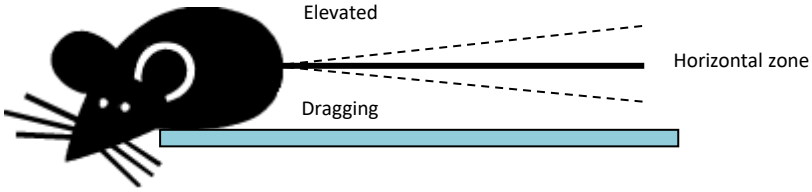
Headbobbing/Circling
Present – the mouse exhibits head movements and/or circling behaviour which are indicative of balance deficiencies. This can include repeated tilting the head to look upwards, repeated shaking of the head, running in circles, etc.
Absent – the mouse does not exhibit head movements or circling behaviour which are indicative of balance deficiencies.

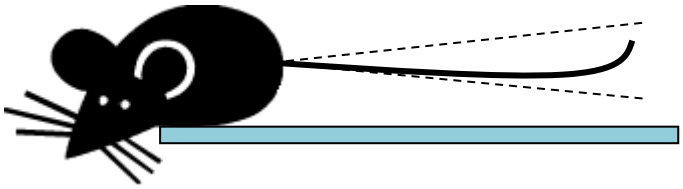
Convulsions
Present – The mouse shows signs of an involuntary seizure at any point during the experiment. This can include a minor seizure where the mouse gasps and blinks repeatedly whilst remaining stationary, 'excited running' where the mouse seems to run in random directions and bumps into objects or a full body seizure where the body of the mouse contracts and relaxes rapidly and repeatedly.
Absent – no sign of involuntary seizures are seen at any point during the experiment.

Comment section
Any unusual behaviours (head tilt, stereotypic behaviours, etc) that are observed throughout the entire experiment should be noted in this section. This includes any comments on the status of the eyes (closed, partially open), any excessive rearing or grooming, anything that could be called as a behavioural phenotype or affect the behaviour of the mouse.

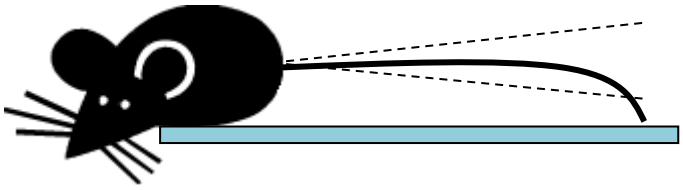
Appendix: Tail Elevation

The elevation of the tail is scored by observing the position and angle of the middle 3rd of the tail. The dashed lines indicate the horizontal zone (if the majority of the middle third of the tail is in the horizontal zone the tail is scored as horizontal), which is approximately 10 degrees above and below horizontal.

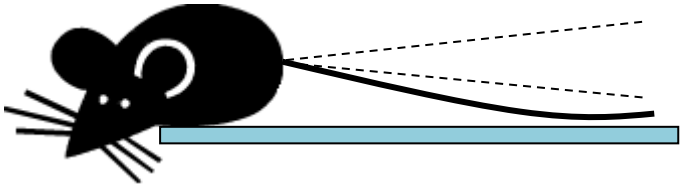




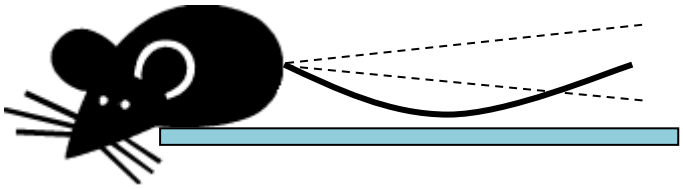
Horizontal tail – middle third of the tail within the horizontal zone.



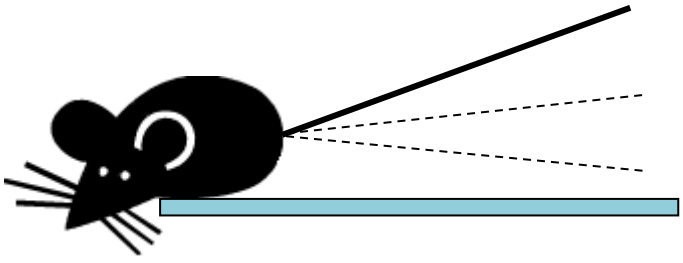
Horizontal tail – middle third of the tail within the horizontal zone.



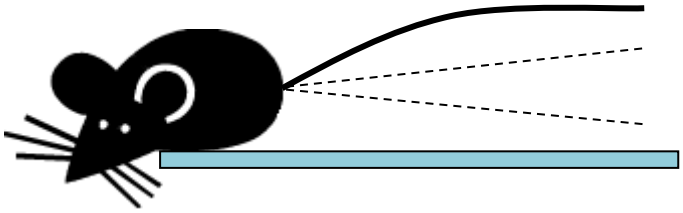
Dragging – middle third of the tail below the horizontal zone.



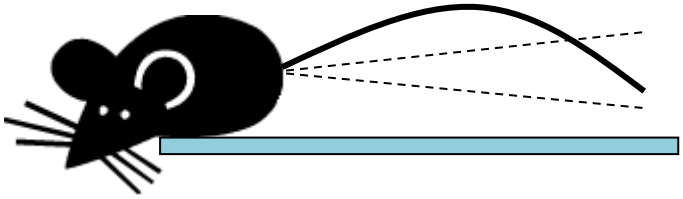
Dragging – middle third of the tail below the horizontal zone.



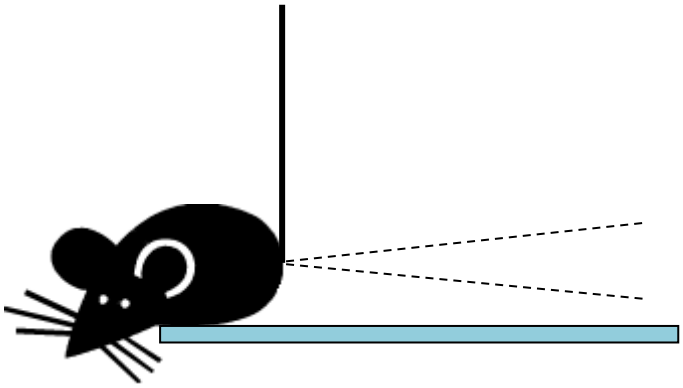
Elevated tail – middle third of the tail above the horizontal zone.



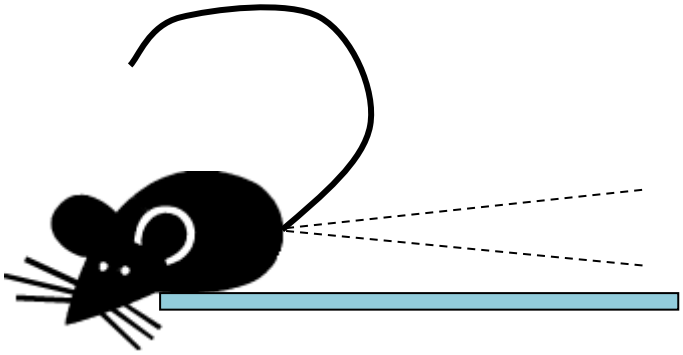
Elevated tail – middle third of the tail above the horizontal zone.



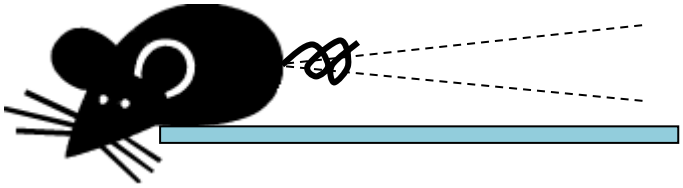
Elevated tail – middle third of the tail above the horizontal zone.



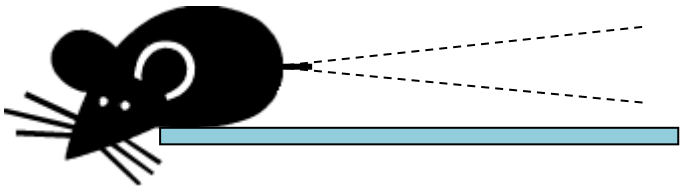
Straub tail – middle third of the tail is raised at an angle greater than 90 degrees from horizontal.



Straub tail – middle third of the tail is raised at an angle greater than 90 degrees from horizontal.



QC Fail – middle third of the tail is not identifiable and the parameter is not applicable.



QC Fail – middle third of the tail is not identifiable and the parameter is not applicable.