WELLCOME SANGER INSTITUTE

STANDARD OPERATING PROCEDURE PACKET

SANGER INSTITUTE
STANDARD OPERATING PROCEDURE

SUBJECT: Mod-SHIRPA & Hair Analysis

INTRODUCTION

This procedure is used for gross motor and neurological observation of mice and to check the condition of their hair.

HEALTH & SAFETY

Do not undertake this role unless cleared by Occupational Health to work with laboratory animals.

1. Risk of being bitten.
2. Risk of allergens.

Refer to the Risk assessment and safe working practices relating to Laboratory Animal Allergy and handling of animals.

RESPONSIBILITIES

All staff performing this procedure are responsible for ensuring that this SOP has been read, understood and is followed. It is the duty of the Senior Managers to ensure that all staff is aware of this responsibility and that they are trained and competent to perform the procedure.

1. The procedure is to be performed inside the room in a functioning change station or down flow table.
2. The procedure is to be carried out by a trained, competent licensed individual.

RESOURCES

Equipment:

1. Two clear Perspex cylinders of 14 cm diameter, one of 18 cm height and one of 7.5 cm height
2. Clear Perspex sheet of 20 by 20 cm
3. Clear Perspex arena of 55 by 33 cm and 18 cm height with 15 squares of 11 cm drawn onto the bottom of it
4. Metal lid for the arena with 12 mm mess grid work in the centre
5. Clear 20 cm Perspex tube with a 30 mm diameter
6. Two Timers *OR* 1 timer with multiple settings
7. Click-box generating a 19.3 kHz tone at 90 dB
8. Fine cotton probe
9. Disinfectants & cleaning agents: 70% ethanol, alcohol wipes & hand spray
10. Paper towels
11. Mobile IVC rack
12. Interactive cage change station

Staff:

This test requires two phenotypers to be performed optimally. One phenotyper to perform most of the mouse handling and one to fill in the database and to aid the first phenotyper.

Additional SOPs:

1. *Use of the Tecniplast Interactive Cage Change Station*
2. *Grip Strength*

METHOD

1. Find the mice needed for the test and **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**.
2. Move the mice from the animal holding room a minimum of 15 minutes before the start of the test and place them on the mobile rack to let them acclimatize.
3. Prepare the change station for use (see SOP *Use of the Tecniplast Interactive Cage Change Station*), opening both sides.

Mod-SHIRPA:

4. Put the Perspex equipment and metal lid in the change station and clean with 70% ethanol.
5. Place the arena at one end of the change station and lean the metal lid against the glass beside it. Place the small Perspex cylinder next to the arena and the sheet on top of it, with the large Perspex cylinder on top of that to create the viewing jar. Make sure there is enough room between the viewing jar and the other side of the change station for a cage. Put the fine cotton probe, timer(s), 30 mm Perspex tube and click-box on the side of the change station away from the computer.
6. Set the first timer/setting to 1 minute and the second to 30 seconds.

7. Place the first cage in the change station, with the front facing the side closest to the computer.
8. Person A should retrieve a mouse from the cage and place it into the viewing jar. Person B should start the one minute timer when the mouse is in the jar.
9. Note whether the mouse bites, or tries to bite, at any point during the test.
10. Note whether the mouse squeaks at any point during the test.
11. Note whether the mouse displays headbobbing and/or circling behaviour at any point during the test.
12. Note whether the mouse convulses at any point during the test.
13. Person A should observe the mouse for one minute and call out the responses to person B to enter into the database. The behaviours/characteristics to observe are:
    - Body Position: active, inactive or excessively active
    - Palpebral Closure: eyes open or eyes closed
    - Lacrimation: present or absent
    - Tremors: present *for the majority* of the time or absent
    - Defecation: present or absent
    - Urination: present or absent
14. At the end of the minute, person A should lift the Perspex sheet with the mouse and top cylinder still on it and move it above the uncovered arena before slowly sliding the top cylinder off the sheet so that the mouse is manoeuvred towards the edge of the sheet and falls into the centre of the arena from a height of about 25 cm. As this is done, person B should start the 30 sec timer.
15. Person A should watch the mouse and count the number of squares the mouse enters *with all four paws* during the 30 seconds. The square(s) the mouse lands in do not count, instead 1 is the first square it enters (forwards or backwards) with all four paws after being dropped into the arena.
16. Person B should note what the mouse does immediately upon landing in the arena. Whether it freezes (either briefly or for more than 5 seconds) or moves immediately. Movement is defined as steps forward or backwards.
17. During the 30 seconds, person B should also note whether the mouse's gait is fluid or non-fluid (includes retropulsion of more than one square) and make any necessary comments regarding the gait in the corresponding *Gait related comment* box.
18. Person B should check the elevation and position of the pelvis.
19. Then Person B should check whether the tail elevation is:
    - Dragging
    - Horizontal extension
    - Elevated/straub tail

    If the mouse isn't moving, wait until after step 23 and have person A gently prod the mouse into moving. If the mouse still refuses to move, use the *Fail* option.
20. Person B should then clean the viewing jar with 70% ethanol and a paper towel.
21. At the end of the 30 seconds, person A should call out the number of squares the mouse entered.
22. Person A should now lower their hand into the arena with their forefinger bent. They should approach the mouse from the front and see whether it

flees before being touched, reacts to be touched or has no response to being touched.

23. Person A should hold the click-box about 30 cm above the mouse before pressing the button. The mouse should be observed to see whether it has a Preyer reflex (flicking of the ears), a Preyer reflex with movement or no response at all. It is best to wait until the mouse is sitting still so that movement can clearly be detected if it is present. If it is necessary to repeat this measurement, wait 20 seconds to prevent habituation to the stimulus. *Do not* repeat more than necessary.
24. Next, person A should pick up the mouse near the base of the tail and lift it above the arena while placing the metal grid on top of the arena. *This step should **not** be performed if the mouse has a tail welfare issue* (eg: malformed or fight wounds). If this is the case, skip step 25 and move straight to step 26 instead.
25. While holding the mouse by the tail for about 5 seconds, person A should look for the following behaviours:
    - Struggling
    - Trunk Curl: present or absent
    - Limb Grasping: present or absent

    When done, place the mouse on the metal grid. If it struggles when held by its tail, proceed to step 28. If not, perform step 26.
26. Loosely scruff the mouse and see if it struggles when held by its neck. If it does, skip step 27. If it doesn't, perform step 27.
27. Retaining the mouse in a loose scruff, hold the mouse supine and see if it struggles.
28. With the fine cotton probe, person A should gently touch the inside of the mouse's ear to see if the pinna reflex is present or absent.
29. Person A should gently touch the fine cotton probe to the mouse's eye to see if the corneal reflex is present or absent.
30. Person A should take the 30 mm Perspex tube and manoeuvre the mouse into it. Once the mouse is entirely in the tube, rotate the tube 180^{o} so the mouse is upside-down and see if the mouse is able to right itself within 10 seconds.
31. This is the end of the Mod-SHIRPA portion of the SOP. Any biting, attempts to bite, squeaking, headbobbing, circling and convulsions beyond this point do not count and should not be recorded *as part of this SOP*. Relevant health records should still be created for the mouse in question if appropriate.

Hair Analysis:

32. Person A should hold the mouse on the back of their hand to check for guard hairs. This is most easily visible on the mouse's back (see image 1 in the Appendix).
33. Then Person A should observe the dorsal coat and see whether it looks normal or not (long, short, rough, sparse, coarse, fine and/or skin visible) (see image 2 in the Appendix).
34. Person A should then observe the ventral coat of the mouse to see whether it looks normal or not (long, short, rough, sparse, coarse, fine and/or skin visible) (see image 3 in the Appendix).
35. Next, Person A should examine the area behind mouse's ears for the presence or absence of hair (see image 4 in the Appendix).

36. Person A should then examine the tail to see if there are hairs present along the length of it or not (see diagram 1 in the Appendix).
37. Then Person A needs to examine the tip of the tail to see if there are hairs present there or not (see diagram 1 & image 5 in the Appendix).
38. Person A should then examine the whiskers to see whether they are normal or not (short, long, curly, disorientated, and/or absent) (see image 6 in the Appendix).
39. Place the mouse on the cage grid if more mice from the cage are to be run or back into the cage if not.
40. Clean the remaining Perspex equipment and metal grid with 70% ethanol and a paper towel.
41. Repeat steps 8-40 for the rest of the mice in the cage.
42. If more than one cage is to be tested, clean hands with the hand spray and repeat steps 7-41 for all of the remaining cages.
43. Clean the Perspex equipment and metal grid. Give the click box a careful wipe as well. Make sure all of the equipment is dry before returning it to the cupboard.
44. Clean the change station (see SOP *Use of the Tecniplast Interactive Cage Change Station*).
45. Continue on to the Grip Strength test (see SOP *Grip Strength*).

APPENDIX

Image 1: Guard hairs present & absent.

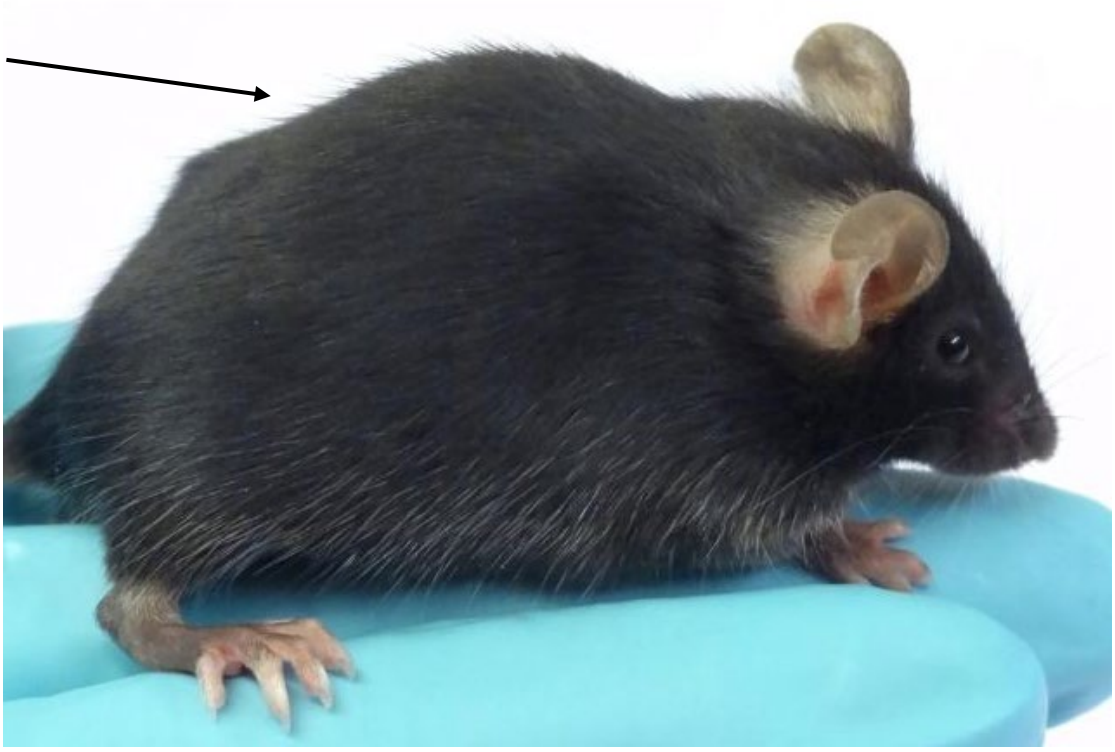
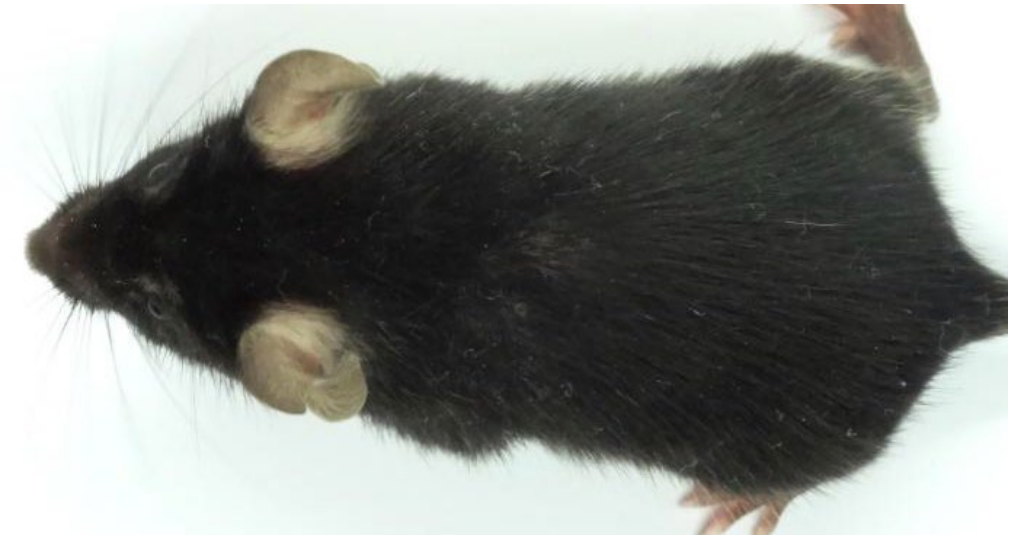
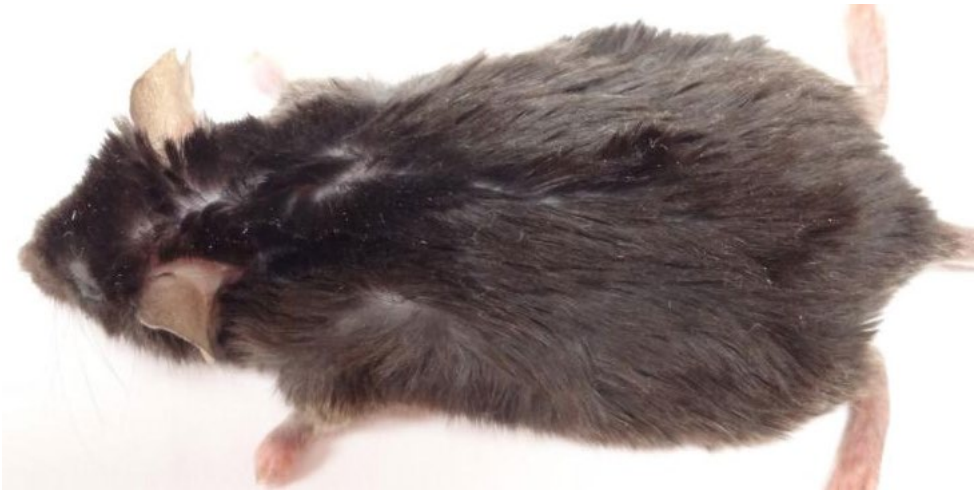


Image 2: Dorsal coat: normal in the 1st photo, greasy in the 2nd photo and sparse hair with skin visible in the 3rd photo.





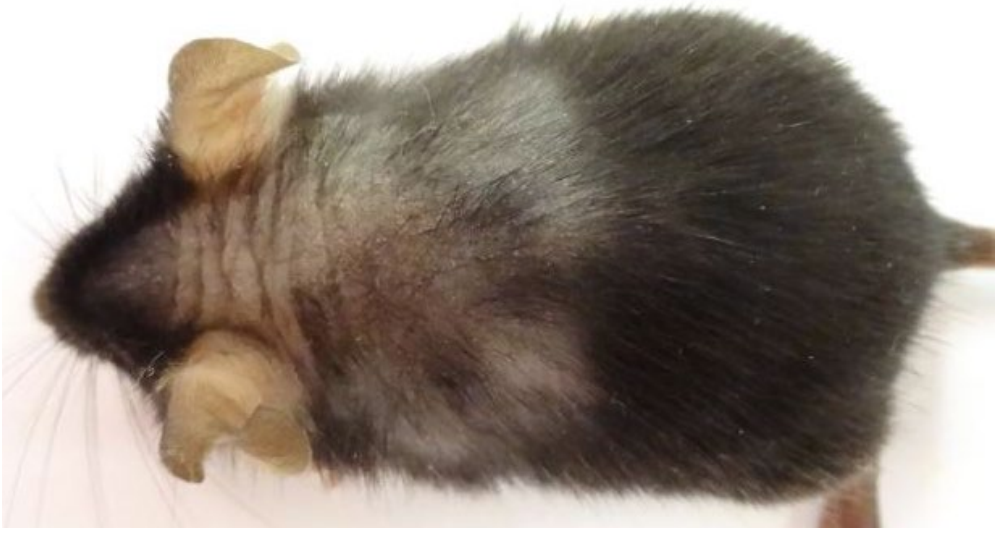


Image 3: The mouse on the left has a normal coat, while the mouse on the right has a sparse coat with skin visible.

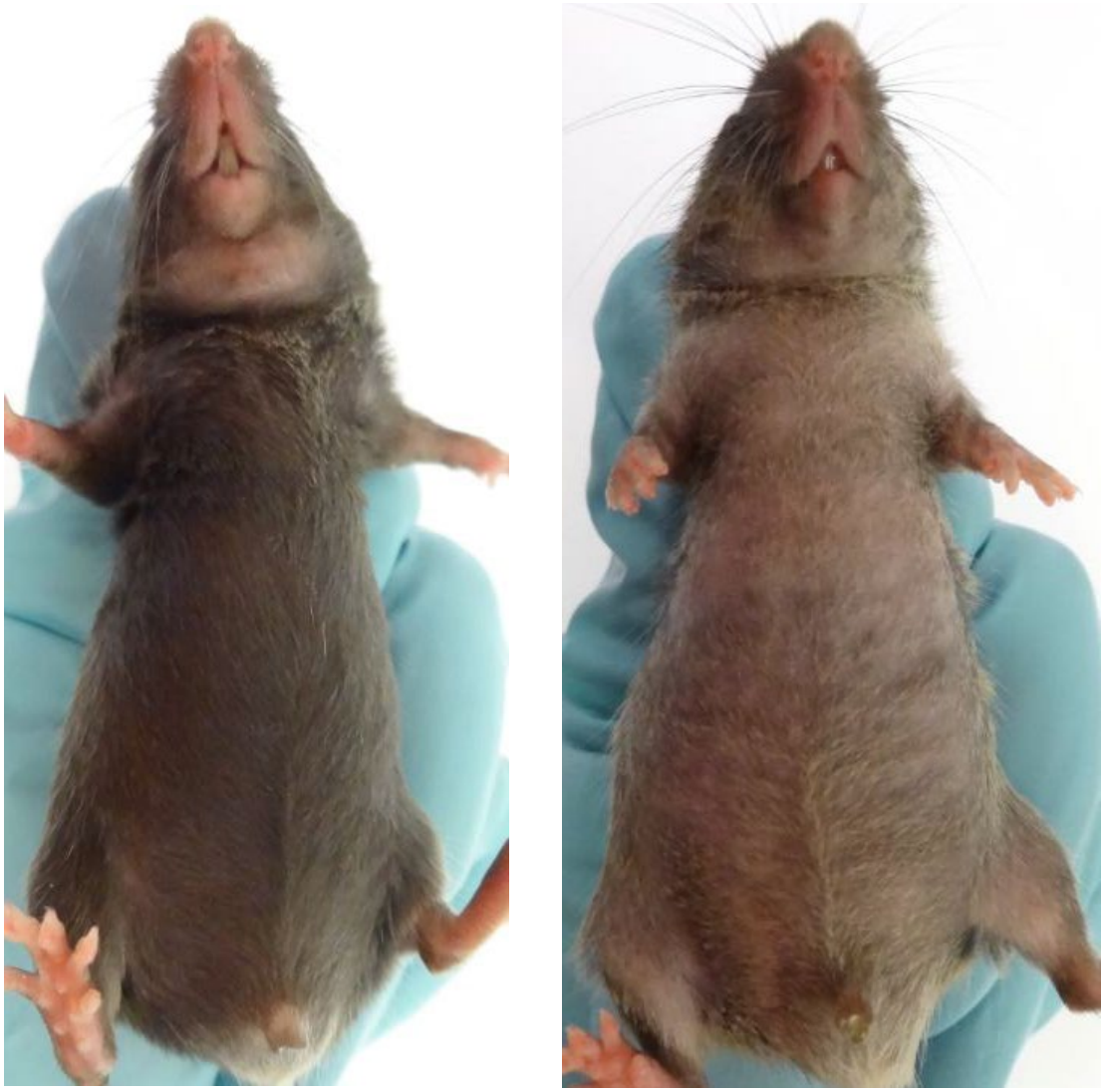


Image 4: The mouse on the left has hair present behind its ears as normal, while the mouse on the right doesn't.

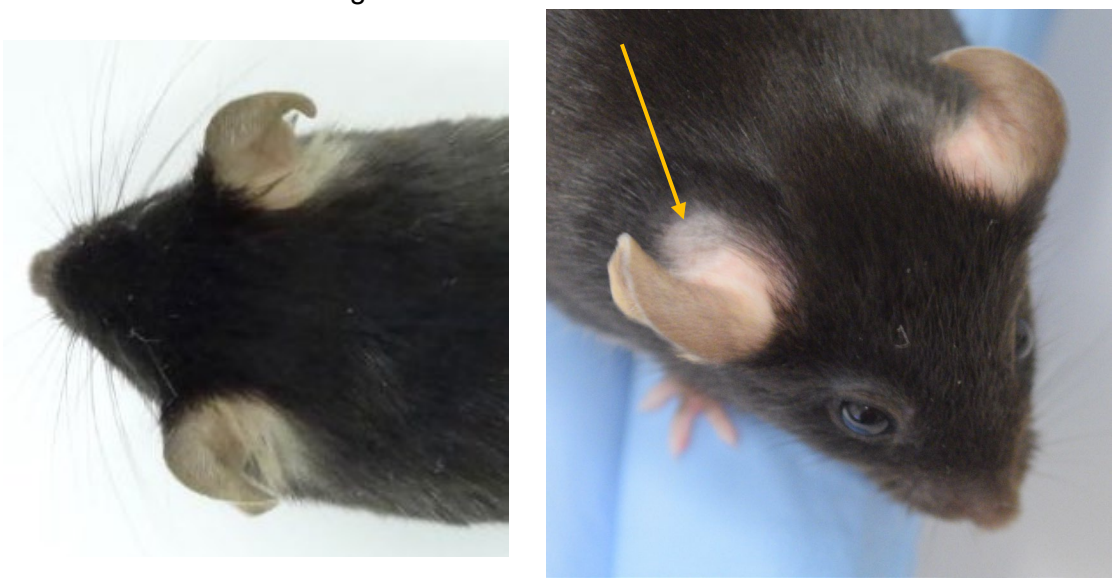


Diagram 1: The difference between having hair on the tail and on the tip of the tail. Both examples would be marked as *present* for having *hair on tail*, but the second would be marked as *absent* for having *tail tip hairs*.

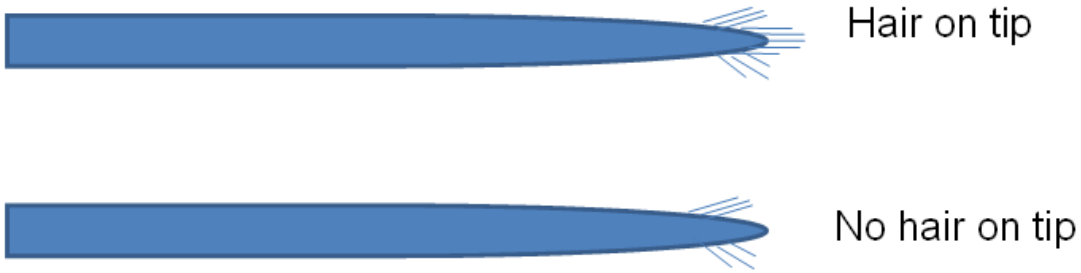


Image 5: This is a normal tail with hairs present at the tip.

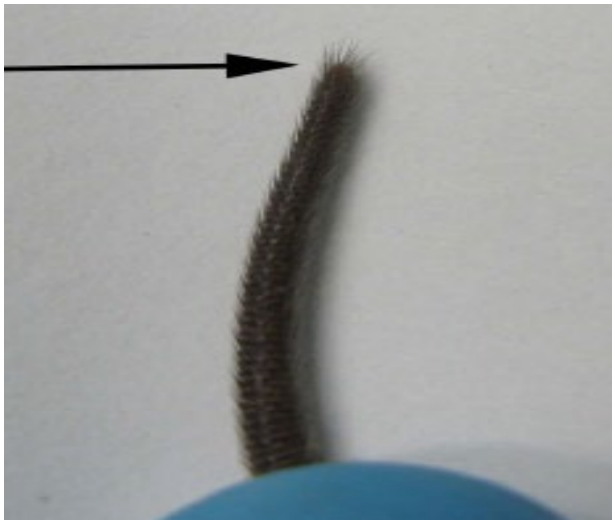
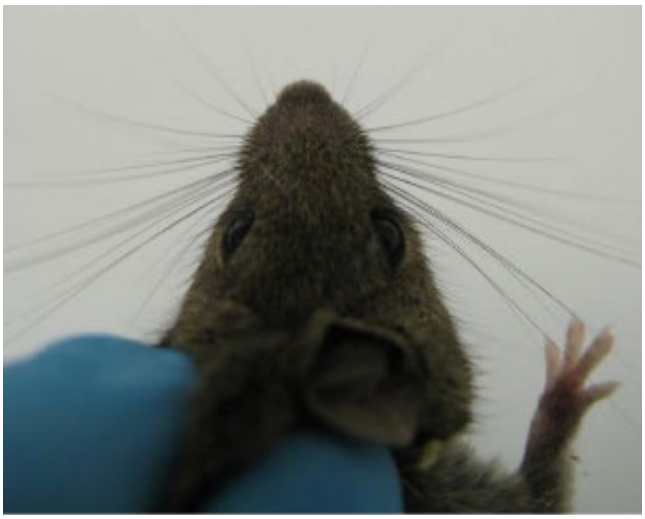
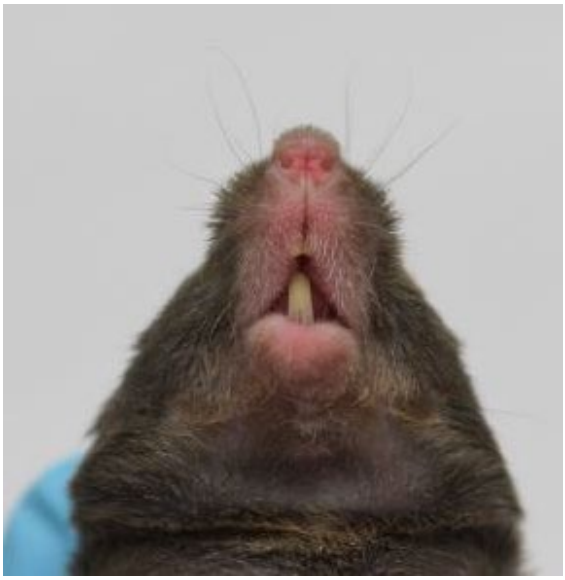
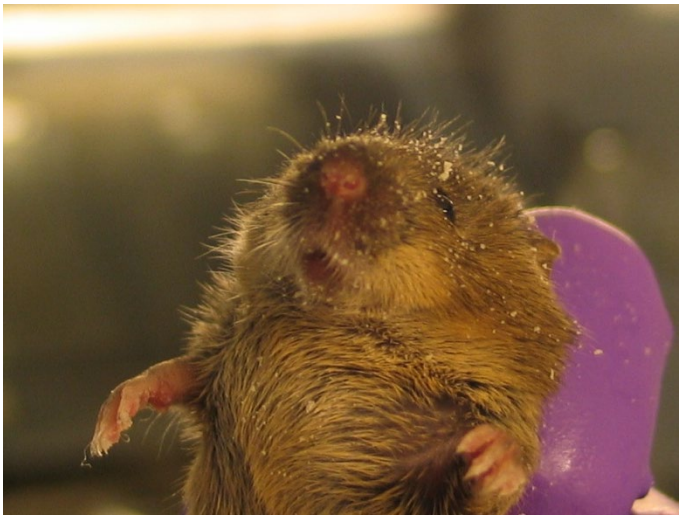


Image 6: The 1st photo shows normal whiskers, the 2nd short whiskers and the 3rd absent whiskers.







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.

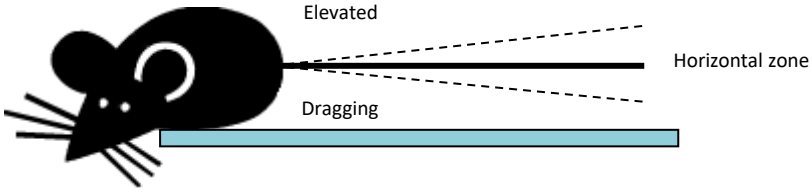
Headbobbing/Circling
Present – the mouse exhibits head movements and/or circling behaviour which are indicative of balance deficiencies. This can include repeated tilting the head to look upwards, repeated shaking of the head, running in circles, etc.
Absent – the mouse does not exhibit head movements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

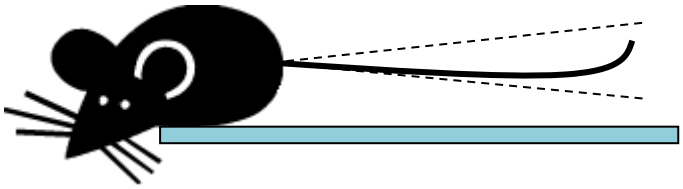
Convulsions
Present – The mouse shows signs of an involuntary seizure at any point during the experiment. This can include a minor seizure where the mouse gasps and blinks repeatedly whilst remaining stationary, 'excited running' where the mouse seems to run in random directions and bumps into objects 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.

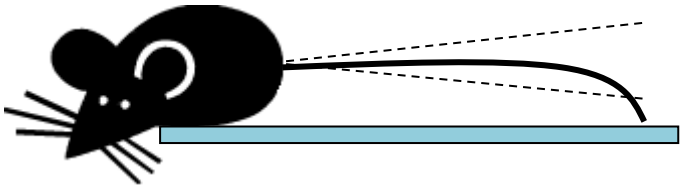
Appendix: Tail Elevation

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.

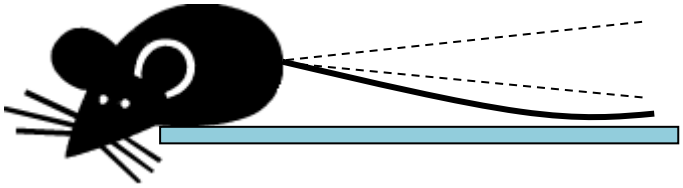




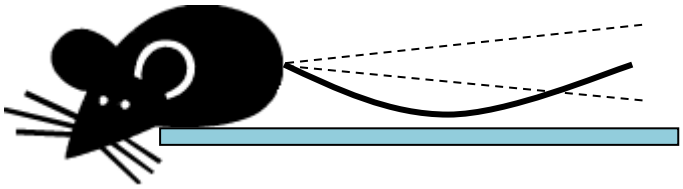
Horizontal tail – middle third of the tail within the horizontal zone.



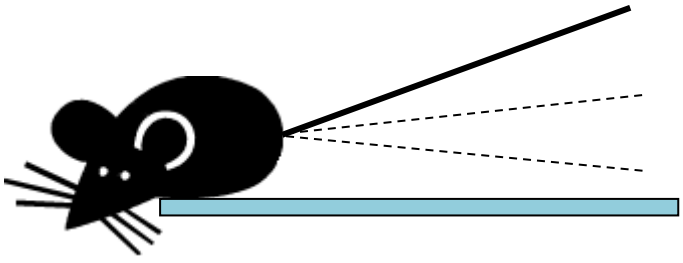
Horizontal tail – middle third of the tail within the horizontal zone.



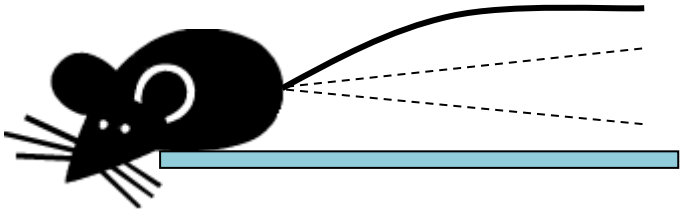
Dragging – middle third of the tail below the horizontal zone.



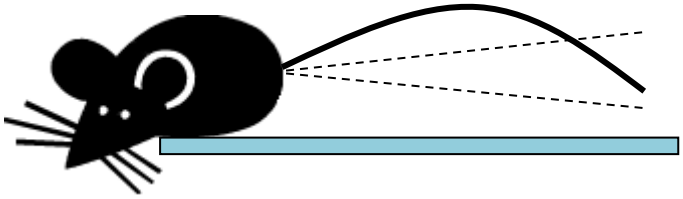
Dragging – middle third of the tail below the horizontal zone.



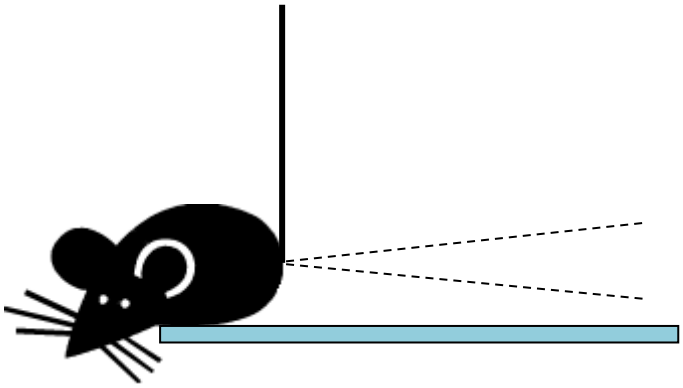
Elevated tail – middle third of the tail above the horizontal zone.



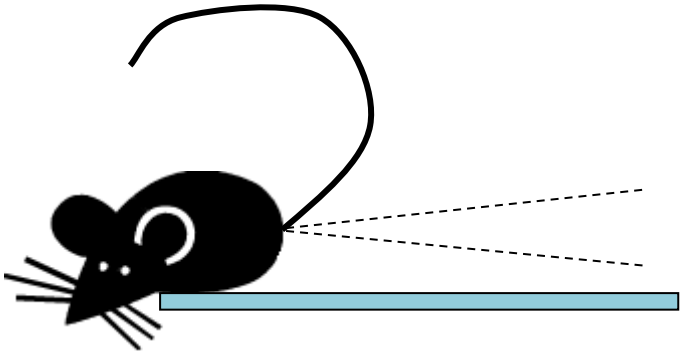
Elevated tail – middle third of the tail above the horizontal zone.



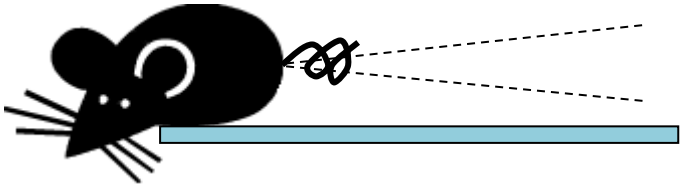
Elevated tail – middle third of the tail above the horizontal zone.



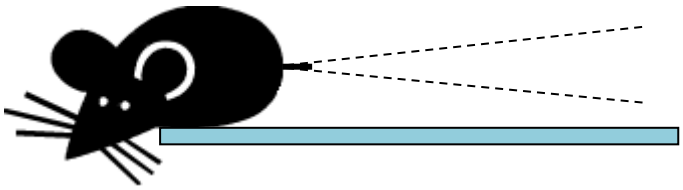
Straub tail – middle third of the tail is raised at an angle greater than 90 degrees from horizontal.



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.