

Modified-SHIRPA (43 items) for drug test 行為及外觀檢測 (43 項) 藥物測試

1. Purpose

1.1 The "SHIRPA" method is a package developed by the Medical Research Council (MRC) in the United Kingdom for comprehensive testing of mouse behaviors. The SHIRPA method allows primary screening for 40 test items by visual inspection and manual handling and can evaluate characteristic behaviors of mice in a relatively short period of time. RIKEN Japan Mouse Clinic focused on these primary screens, added items related to morphological characteristics of the mice, and named the modified procedure the "modified SHIRPA" method. This modified method is compatible with the original "SHIRPA" method. In this version, the times and testing criteria were improved and new items related to eye morphology were added. The skilled staff members can process the morphological and behavioral characteristics of a mouse using a package of tests comprising 43 items within 15 minutes.

2. Safety Requirements

- 2.1 Testing is carried out in accordance with common animal handling procedures.
- 2.2 If something unexpected happens during the primary screening, it must be quickly reported to the TMC director.

3. Notes

- 3.1 Considerations before the tests
Mice behavior can be affected by the rearing environment. Therefore, it is important that the environmental influences are consistent as possible.
- 3.2 Cleaning equipment
When a number of mice are tested continuously, residual odors from the equipment used in the preceding test may affect the test results. The floor and walls of the arena, ruler, and metal net should be wiped clean before introducing the next mouse. To prevent infection, the equipment should be washed with water at the completion of the day's tests.
- 3.3 Recording comments and phenotypes and the scoring system
Because test scores alone do not completely capture behavioral phenotypes, written records containing other comments should be kept.

4. Equipment

- 4.1 Viewing jar
The viewing jar is a transparent acrylate cylinders (25 cm high x 11 cm diameter).
- 4.2 Arena (clear Perspex arena)
The arena is a transparent acrylate box (internal dimensions: 55 x 33 x 18 cm). The bottom is a white plate composed of 11 x 11 cm square grids. A 40 x 12 cm metal net with a mesh size of 12 x 12 mm (the same mesh size as that of the grid floor) covers about a half of the top area (the metal net should be removable).
- 4.3 Grid floor
The grid floor is a 40 x 12 cm metal net with a mesh size of 12 x 12 mm.
- 4.4 Supports for grid floor
Four cylindrical acrylate supports (3 cm high x 2.5 cm diameter) to raise grids

off bench.

4.5 Wire attached to the end of a dowel

A wire (length: 15 mm; thickness: 0.15 mm) is attached to a dowel and used to examine the pinna and corneal reflexes.

4.6 Plastic rod

A plastic dowel rod sharpened to a pencil point to test salivation and biting

4.7 Forceps with bent tips

The forceps are used to deliver a toe pinch. The tip should be relatively fine but not so sharp that it can injure the mice.

4.8 Stopwatch

Use a stopwatch that is easy to handle and equipped with a number of separate timers.

4.9 IHR click box

The click box is used at a distance of 30 cm to test the auditory responses of the mice. It emits a 20-KHz sound at 90 dB.

4.10 Scale to weigh the mice

4.11 Ruler

The ruler is used to measure the body and tail lengths of the mice. A transparent ruler about 20 cm in length is easy to use.

4.12 Transparent tube

The tube is used to test the contact righting reflex. It is a transparent acrylate cylinder 30 cm in length. Tubes with various diameters (2.5-3.0 cm) should be used for mice of different sizes. A mouse should be able to enter the tube easily and stay close to the internal wall of the tube.

5. Supplies

5.1 Paper towels

5.2 Disinfecting ethanol

5.3 Ear muffs

Ear muffs should be used to protect the experimenter's ears from the IHR click box during the startle response test.

6. Procedures

6.1 Overview

The Arabic numeral of each test corresponds to number for the test in the modified SHIRPA method. In this chapter, the tests are listed according to the screening order.

6.2 Equipment installation

Install the pieces of equipment introduced in the previous section such that they are easy to use.

6.2.1 Arena

Place the arena in a stable position where it is easy to handle.

6.2.2 For convenience, other equipment can be placed within reach.

6.3 In the viewing jar

First, transfer a mouse from a rearing cage to the viewing jar. Take the utmost care that animal is not frightened (do not peek in). Allow the mouse to get used to the condition for five minutes.

6.3.1 Q1: respiration rate

- 0=gasping, irregular
- 1=slow, shallow
- 2=normal
- 3=hyperventilation

6.3.2 Q2: Tremor

Determine whether if the mouse shakes when it is placed in the arena.

- 0 = None
- 1 = Mild
- 2 = Marked

6.3.3 Q3: body position

Observe the body posture and position of the mouse in the viewing jar.

- 0= completely flat
- 1=lying on side
- 2=lying prone
- 3=sitting or standing
- 4=rearing on hind legs
- 5=repeated vertical leaping

6.3.4 Q4: spontaneous activity

Observe the frequency of the mouse movement in the viewing jar.

- 0=none, resting
- 1=casual scratch, groom, slow movement
- 2=vigorous scratch, groom, moderate
- 3= vigorous, rapid/dart movement
- 4=extremely vigorous, rapid/dart movement

6.3.5 Q5 defecation

Leave the mouse in the viewing jar for five minutes and record the number of feces.

6.3.6 Q6 urination

Leave the mouse in the viewing jar for five minutes and see if the mouse has urination or not.

- 0=none
- 1=done

6.4 In the arena section

Transfer a mouse from the viewing jar to the arena. After completing in the viewing jar section, gently insert the plastic plate between the viewing jar and the grid floor and place the mouse on the viewing jar onto the plastic plate (take care not to pinch the fingers of the mouse). Bring the viewing jar and the plastic plate right above the center of the arena to hold still at a height of 30 cm. turn the plastic plate downward to drop the mouse to the arena. Start the stopwatch simultaneously to begin the tests described below.

6.4.1 Q7: Elapsed time before the mouse starts to move (seconds).

6.4.2 Q8: locomotion activity.

Number of squares entered by all four feet in 30 seconds. The number of grids (take a count assuming the mouse turned at a right angle if it has moved in a diagonal direction).

6.4.3 Q9: transfer arousal.

- 0=coma

- 1=prolonged freeze, then slight movement
- 2=extended freeze, then moderate movement
- 3=brief freeze (few seconds), then active movement
- 4= momentary freeze, then swift movement
- 5=no freeze, immediate movement

6.4.4 Q10: piloerection.

- 0=none
- 1=coat stood on end

6.4.5 Q11: palpebral closure.

Observe both eyes and their closure.

- 0=eyes wide open
- 1=eyes 1/2 closed
- 2=eye closed

6.4.6 Q12: Startle Response.

Use the IHR click box placed 30 cm above the arena to present an auditory stimulus and evaluate how the mouse responds using the following scale:

- 0 = No response
- 1 = Preyer's reflex (backward flick of the pinna)
- 2 = Small jump (lower than 1 cm)
- 3 = Large jump (higher than 1 cm)

6.4.7 Q13: Gait.

Observe the gait of the mouse.

- 0 = Normal
- 1 = Fluid but abnormal
- 2 = Limited movement only
- 3 = Incapacitated

6.4.8 Q14: Pelvic elevation.

Visually measure the pelvis height of mouse in gait. Keep the eye level of the observer at the height of the mouse. Make the observation from the side of the arena.

- 0=markedly flattened
- 1=barely touches
- 2=normal (3mm elevation)
- 3=elevated (more than 3mm elevation)

6.4.9 Q15: Tail Elevation.

Determine how the mouse carries its tail.

- 0 = Dragging
- 1 = Horizontally extended
- 2 = Elevated/Straub tail (more than 45 degrees)

6.4.10 Q16: Touch Escape.

Approach the mouse from the side with a finger. Determine how close the finger is to the mouse when it performs an escape response.

- 0 = No response
- 1 = Mild (escape response to a firm stroke)
- 2= Moderate (escape response to a light stroke)
- 3 =Vigorous (escape response as the finger approaches)

6.5 Above the arena part I.

Place the metal net over the arena. Hold the mouse by the tail and place it on the metal net to make the observations.

6.5.1 Q17: Positional Passivity.

Hold the mouse by the tail on the arena to see if the mouse resists being held. If the mouse does not resist, hold the mouse by the neck, place it on its back, and then hold it by the hind legs. Determine whether the mouse resists handling at each step and stop when the mouse resists.

0 = Struggles when held by the tail

1 = Struggles when held by the neck (finger grip, not scuffed)

2 = Struggles when laid on its back

3 = Struggles when held by the hind legs

4 = Does not struggle

6.5.2 Q18: Trunk Curl.

Determine whether the mouse will raise its upper body up by lowering its ventral side and sitting up (trunk curl) when it is held up by the tail. Twisting the upper body to the side is not a trunk curl.

0 = Absent

1 = Present

6.5.3 Q19: Limb Grasping.

Determine whether the mouse will hold its forelimbs and hind legs together (limb grasping) when it is held up by the tail.

0 = Absent

1 = Present

6.6 Above the Arena part II.

Manipulate the mouse and observe the responses using the metal net mounted on the arena.

6.6.1 Q20: Visual Placing.

Hold the mouse by the tail and bring it down onto the metal net in the arena. Determine the distance between the mouse and the metal net when it extends its forelimbs to grasp the metal net. The eyes of the observer should be at the level of the metal net.

0 = None

1 = upon nose contact

2 = upon vibrissa contact

3 = before vibrissa contact (approximately 18 mm)

4 = Vigorous early extension (approximately 25 mm)

6.6.2 Q21: grip strength.

Hold the mouse by the tail and drag it to the fringe of the metal net on the arena. Evaluate the grip strength felt by the hand of the observer.

0=none

1=slight grip, semi-effective

2=moderate grip, effective

3=active grip, effective

4=unusually effective

6.6.3 Q22: body tone.

Pinch the mouse with the thumb and forefinger of the observer on the

dorsal and ventral sides while allowing the mouse to hold onto the metal net in the arena. Evaluate the resistance.

0=flaccid, no return of cavity to normal

1=slight resistance

2=extreme resistance, board like

6.6.4 Q23: Pinna Reflex.

Stimulate the base of the pinna with the wire attached to the dowel (do not prick the mouse). Record how the pinna moves. Only one of the two pinnae needs to be tested.

0 = None

1 = Active retraction, moderately brisk flicking

2 = Hyperactive, repetitive flicking

6.6.7 Q24: Corneal Reflex.

Stimulate the cornea of the mouse with the body (not the tip) of the wire attached to the dowel and determine whether the mouse closes its eyelids.

0 = None

1 = Active single eye blink

2 = Multiple eye blinks

6.6.8 Q25: Toe Pinch.

Use the forceps with the bent tips to stimulate the hind legs (do not pinch strongly enough to injure the mouse) while allowing the mouse to hold onto the metal net on the arena. Observe the response.

0 = None

1 = Slight withdrawal

2 = Moderate withdrawal, not brisk

3 = Brisk, rapid withdrawal

4 = Very brisk repeated extension and flexion of the hind legs

6.7 On the arena.

The athletic ability of the mouse is tested in the arena.

6.7.1 Q26: Lacrimation.

Restrain the mouse and determine whether lacrimation fluid is secreted

0 = None

1 = Lacrimal fluid

2 = Other

6.7.2 Q27: Provoked Biting.

Approach the mouth of the mouse with the plastic rod held sideways to determine whether the mouse will bite the rod.

0 = Absent

1 = Present

6.7.3 Q28: Salivation.

Examine the plastic rod that was bitten in Q38 (place the plastic rod into the mouth of the mouse if it did not bite the rod) to determine how much saliva adhered to the rod.

0 = None

1 = Saliva in an area that represents the margin of the submaxillary area

2 = Wet zone represents the entire submaxillary area

6.7.4 Q29: Heart Rate.

The observer places their forefinger on the chest of the mouse to feel the heart beat. Determine the overall relative rate because the heart rate of a mouse is too fast to count.

0 = Slow, bradycardia

1 = Normal

2 = Fast, tachycardia

6.7.5 Q30: Abdominal Tone.

The observer gently presses the abdomen of the mouse with their forefinger to evaluate how hard the abdomen is.

0 = Flaccid, cavity does not return to normal

1 = Slight resistance

2 = Severe resistance, board-like

6.7.6 Q31: Skin Color.

Examine the color of the ventral sides of the limbs (palms and soles).

0 = Blanche

1 = Pink

2 = Bright, deep red flush

3 = Mild pigmentation

4 = Dark footpad, pigmentation

6.7.7 Q32: Limb Tone.

The observer presses the rear of the mouse's hind legs with their forefinger and middle finger to evaluate how violently the mouse kicks back against the tactile stimulus.

0 = No resistance

1 = Slight resistance

2 = Moderate resistance

3 = Marked resistance

4 = Extreme resistance

6.7.8 Q33: wire maneuver.

Hold the mouse by the tail and follow the mouse to hold onto the wire of the arena with the forelimbs. Keep the body of the mouse parallel to the arena floor and then release to see if the mouse can grasp the wire.

0=Active grip with hindlegs (5sec)

1=Difficulty to grip with hindlegs (the mouse can't hold onto the wire for five seconds)

2= unable to grip with hindlegs (the mouse does not fall for four seconds or longer)

3=unable to lift hindlegs, falls within seconds

4=falls immediately

6.7.9 Q34: Righting reflex.

Hold the mouse by the tail and place with the limbs on the arena floor and the head toward the observer. Then, gently flip the mouse to make a full turn and fall down to the arena. Observe how the mouse lands.

0=no, impairment

1=lands on side

2=lands on back

3=fails to right when placed on back

6.7.10 Q35: Contact Righting Reflex.

When a transparent tube approaches the head of the mouse held up by its tail, the mouse will enter the tube. Turn the tube so that the mouse is on its back, and determine whether the mouse tries to turn over onto its abdomen.

0 = Absent

1 = Present

6.7.11 Q36: Negative Geotaxis.

Place the metal net from the arena onto the arena floor. Hold the mouse by the tail (tail toward the observer) and allow the mouse to hold onto the metal net. When released, the mouse should move forward. Turn the metal net 90 degrees so that the mouse is oriented with its head facing down. Observe how the mouse moves in response to this orientation.

0 = Turns and climbs the grid

1 = Turns but then freezes

2 = Moves, but fails to turn

3 = Does not move within 30 seconds

4 = Falls off the metal net

6.8 Additional Comments and Body Weight.

A scale is used to measure the body weight of the mouse. Additionally, it is advisable to record as many written comments as possible.

6.8.1 Q37: Fear Behavior.

Determine if the mouse freezes (complete lack of movement, including the whiskers), whenever it is transferred from one place to another.

0 = None

1 = Freezes during transfers

6.8.2 Q38: Irritability Behavior.

Determine whether the mouse violently resists being restrained.

0 = None

1 = Struggled in response to spine restraint

6.8.3 Q39: Aggression.

Determine whether the mouse defied and bit the handler.

0 = None

1 = Provoked biting or attack

6.8.4 Q40: Vocalization.

Determine whether the mouse squeaked during the tests.

0 = None

1 = Provoked squeaking during handling

6.8.5 Q41: Bizarre behavior.

Record scores when mice showed bizarre behaviors as described below.

No phenotype

A) HF Head flicking or head shaking

B) HS Head searching- repetitive

C) H Hallucinating-like abnormal appears to respond to objects not

present, e.g. visual tracking or fear withdrawal

- D) B Compulsive biting
- E) L Compulsive licking
- F) SB Self destructive biting
- G) P Prancing forelimb
- H) UW Upright walking
- I) AW Aimless wandering
- J) C Circling
- K) W Waltzing
- L) R Retropulsion
- M) D Spatial Disorientation
- N) HB Head bobbing

6.8.6 Q42: Convulsions.

No phenotype.

- A) C Clonic
- B) Cs Clonic symmetrical
- C) Rn Running excitement
- D) Ch Champing
- E) P Popcorn
- F) A Asphyxia
- G) T Tonic
- H) Tf Tonic flexation occurs without extension
- I) Op Opisthotonus
- J) Em Emprosthonus
- K) Rr Rock and roll
- L) Su Sitting up
- M) Pr Praying

6.8.7 Q43: Body Weight.

Measure the body weight of the mouse with a scale (g).