Behavourial assessment of neurological deficits in rats post-stroke

SCOPE

When addressing the development of brain injury following stroke or traumatic brain injury it is important to measure any functional deficit that occurs as a result of neuronal death. Groups of rats can be routinely scored on a number of commonly used behavioural assessments and each rat acts as its own control pre-injury. It is important to use a variety of different tests in order to pick up what are often quite subtle changes in function in rats following injury to the brain. In addition, it has been reported that rats often appear to have no deficits in one test (they overcome their disabilities) but will still show deficits in others. Behavioural tests described here will determine limb function, motor control and sensorimotor function.

AUTHORISATION TO UNDERTAKE PROCEDURE

Authorisation must be given by AEC for persons to undertake this procedure. The investigator must be named in an AEC approved protocol and if training is required it must be provided by a person experienced in this technique.

EQUIPMENT

Neurological Deficit Score:
- Running beam (3 cm wide x 70 cm long, approx. 5mm thick).

Sensorimotor Hemi-neglect (Sticky label test):
- Timer
- Small 1cm round sticky labels in colours other than white or black
- Clear plexiglass box (approx. height 30cm, width 20 cm, length 40cm)

Rota-Rod performance:
- Rat accelerating Rota rod

Cylinder test:
- Plexiglass cylinder (height 30cm, diameter 20cm, thickness 7mm) open top
- Mirror (50cm x 50cm)
- Video camera

Adjusting Step Test:
- Smooth surface 100cm x 30 cm
- Start and finish bands of coloured tape (1cm) across width of the surface, 90 cm apart.
PROCEDURES

Neurological Deficit Score
Neurological abnormalities are evaluated with the use of a neurological deficit score based on detection of abnormal posture and hemiplegia, as described by Yamamoto and colleagues (1988) and De Ryck and colleagues (1989).

1. Suspend the rat by the tail 10 cm above the bench top or home cage floor for ~5 seconds and observe any twisting of the thorax defined by the rat reaching up towards its tail. In normal rats with no deficit there will be no twisting observed and the rat will reach for the ground (score 0). In rats with damage to their striatum or cortex there may be a wobble to contralateral side (score 1), some twisting up towards the tail (score 2), or twisting all the way up to touch the tail (score 3).

2. In addition to twisting, observe forelimb flexion. A normal rat will extend both forepaws towards the ground as if reaching for it (score 0). After damage to the brain as described above the contralateral forepaw may not reach to the ground but flex to the contralateral side. Slight flexion (score 1), 45° flexion (score 2) and pronounce 90° flexion (score 3). Often severe flexion is accompanied by obvious twisting of the thorax.

3. Set up the running beam so that it is raised approximately 20 cm above the bench. Limb dysfunction is tested by placing the rat on the narrow beam (3 cm wide x 70 cm long). Limb paralysis is scored by ability to grip and keep all limbs on the beam. Loss of grip and occasional slipping (score 1), no grip and limb resting over edge of beam (score 2), no grip and limb dangling from beam with an inability to move along the beam (score 3).

Scoring
1. Add all scores from the above tests together for a total neurological deficit score with a maximum score of 9.
2. Compare neurological deficit scores post-stroke to pre-stroke scores such that each rat acts as its own control.

Sensorimotor Hemi-neglect (Sticky label test)
Sensory hemineglect is evaluated by a test developed by Schallert and Whishaw (1984) that measures sensitivity to simultaneous forelimb stimulation. This test is based on observations of behaviour in humans with unilateral brain damage. If 2 stimuli are presented simultaneously, 1 on each side of the body, the contralateral stimulus appears to be masked (“extinguished”) and either remains undetected until the ipsilateral stimulus is removed or feels subjectively weaker. In rats, the test consists of placing adhesive tapes (Avery adhesive labels, 1-cm circles) on the distal-radial region of each wrist.

1. Place the rat in a clear plexiglass box and allow that rat to explore the new environment for 2-3 minutes.
2. Gently restrain the rat and place a small (1 cm diameter) adhesive label (any colour except white or black) on the inside surface of each forelimb just above the thumb, on the wrist. Placement of the first tape should be randomized between contralateral and ipsilateral limbs.
3. Place the rat back into the clear plexiglass box and time how long it takes for the rat to first touch each label and to remove each label. Each trial should only last a maximum of 3 min and should be conducted twice in training and then once only on subsequent days.
Scoring
1. The maximum score if tape is not removed is 180 seconds.
2. Compare the time to touch the tape from the contralateral forepaw to that of the ipsilateral forepaw, and plot against pre-stroke scores.
3. Compare the time to remove the tape from the contralateral forepaw to that of the ipsilateral forepaw, and plot against pre-stroke scores.

Rota-Rod performance
Rats are pre-trained to remain on the Rota-Rod for 3 min. Each rat will be given 2 training sessions of 3 trials each, 1 h apart on an accelerating Rota-Rod (spinning wheel). Rats will be scored by timing how long they are able to remain on the Rota-Rod compared with pre-stroke scores. When a rat falls off the Rota-Rod, it lands 20 cm below on a plastic plate which trips and stops the timer. Bubble-wrap is used as a cushioning device under the Rota Rod so that the rat does not harm itself when falling off.

Scoring
1. Compare the time to fall off post-stroke to pre-stroke scores such that each rat acts as its own control.

Cylinder test
To determine post-stroke bias or post-treatment correction of voluntary forelimb movements in long-term recovery studies post-ET-1 stroke (Soleman S, 2010). Rats that are placed in the cylinder will voluntarily rear and explore the walls using their forelimbs. Pre-stroke rats will use both forelimbs equally. Post-stroke rats will no longer use their impaired forelimb, most often the contralateral forelimb.

Note: for accuracy in scoring, tests should be videotaped.

1. Stand cylinder on smooth clean surface
2. Lean mirror against wall behind cylinder
3. Angle the video camera in front of the cylinder so that movements by the rats on all sides of the cylinder can be seen (recommended position: 100 cm in front; 75 cm to side).
4. Start video camera before placing rat in cylinder as many movements can occur during the initial exploration
5. Place the rat in the cylinder. The rat will rear and use forelimbs to explore the walls of the cylinder. Rats will typically explore walls with at least three paw touches before landing, therefore ten landings should be counted in order to be confident of obtaining at least thirty vertical wall touches.
6. Repeat test 24 hours after stroke, 72 hours and then at 7 days with 7-day intervals thereafter.

Note: This test is not sensitive to the time period, thus exploratory movements may be encouraged by such stimulants as momentarily turning lights off or sliding the cylinder over a minute distance. Caution: Do not overstimulate as rat may freeze or become agitated.

Scoring
1. Analyse the videotaped movements in slow motion
2. Count only vertical wall touches where the paw is flat on the wall with the digits spread apart.
3. Score the first thirty vertical wall touches as wither a left forelimb touch, or a right forelimb touch. Right and left forelimb movements are counted independently; if one
forelimb remains stationary on the wall while the other moves, the first scores only 1 until it moves again.
4. Do not score ambiguous movements.
5. Compare post-stroke and post-treatment scores to a pre-stroke baseline such that each rat acts as its own control.

Note: Additional analysis could include horizontal landings scored as right, left, or both. The number of vertical movements per horizontal landing could then be assessed in order to determine the amount of movement the rat can accomplish per exploration.

**Adjusting Step Test**
To determine ability to perform pre-trained forelimb movements according the methods of Barnéoud and colleagues (2000) animals are held so that one forelimb touches a flat board, and will step with that forelimb as they are moved along the board by the handler. Steps are scored in both the forehand and backhand directions for the left and right forelimb. Pre-stroke rats will take an equal number of steps with both forelimbs. Post-stroke rats will take fewer steps with their impaired forelimb, most often the contralateral forelimb. Note: To ensure consistency in repeated measures, testing should always be executed by the same researcher.

1. Position the rat on the rectangular bench top so length of the animal’s body is perpendicular to length of the bench top, with head facing away from researcher. Hold the animals body with two hands, so forelimbs on bench top and hindquarters are raised and supported by the researcher. The researchers right hand should cradle the ribs, pinning the right forelimb against the animals body, whilst the left hand supports the hindquarters and secures hind-limbs from moving. All the rat’s left forelimb to rest on the bench. When testing the opposite forepaw reverse the handholds.
2. Begin with the animal at the left end of the bench. With the rats left forelimb touching the board, move the animal in the forehand direction to the right end of the board. Following this, move the rat in the backhand direction towards the left end of the bench. Repeat for a total of three moves in each direction for the left forelimb, followed by three moves in each direction for the right forelimb. The rat should be moved 90cm in approximately 5 seconds.
3. When moving in the forehand direction, the rat will step along the bench so the paw travels laterally from the periphery of the body to the median. This is termed forehand stepping. When moving in the backhand direction, the rat will step laterally moving the paw away from the median of the body. This is termed backhand stepping.
4. The rat should be moved with a consistent speed along the bench, keeping the rat at a height where it can comfortably reach the bench with the paw being tested. To ensure the body remains perpendicular to the researcher, the researcher should move with the rat along the length of the bench.
5. Begin with the paw resting on the tape. Move the rat along the full extent of the bench between tape and count the number of steps taken between the two lines of tape. In order to minimise fatigue effects, alternate scoring in the forehand and backhand directions for each forelimb.

**Scoring**

*Ensure the same researcher scores each test.*

1. Steps are scored between the start and the finish tape. A step that is initiated prior to crossing the finish tape is to be counted. A step that is initiated on or after crossing the finish tape is not counted.
2. Score in the forehand direction and the backhand direction three times each. Allow the rat to rest for 30 minutes and repeat for a total of 6 scores in each direction. Repeat of
the next two days for a total score of 18 scores in each direction. *The test should be performed at the same time each day.*

3. Compare post-stroke and post-treatment scores to a pre-stroke baseline.

**Staircase test:**

A novel reaching test for the rat has been developed to assess the independent use of forelimbs in skilled reaching and grasping tasks. The apparatus is a plexiglass box with a removable baited double staircase. Food pellets are placed on the staircase and presented bilaterally at 7 graded stages of reaching difficulty to provide objective measures of side bias, maximum forelimb extension and grasping skill.

Animals should be well handled prior to training. The time required to train the animals for the task will take two weeks, with training conducted twice daily at the same time each day, taking 10-15 minutes each session.

**Materials:**
- Staircase apparatus
- Bio-Serve Sugar pellets (45mg each; Able Scientific, Australia)
- Forceps for pellet positioning during training sessions.

The apparatus into which the animal is placed consists of a clear Perspex chamber (203mm long x 108 mm high x 60 mm wide) with a hinged lid. A narrower compartment (165 mm long x 108 mm high x 60 mm wide) with a central raised platform running along its length, creating a 19 mm wide trough on either side, connected to the change. The narrowness of the side compartment prevents the animal from turning around, so that it can only use its left paw for reaching into the left trough and right paw reaching into the right trough.

A removable double staircase is inserted into the end of the box, sliding into the troughs.

**Pellet positioning:**
Each of the seven steps of the staircase contains a small 3mm deep well into which 3 food pellets are placed. Therefore 21 pellets are placed into the staircase. The highest step to the staircase is 13 mm below the central platform. A trained animal can collect pellets by reaching into the trough: the number of steps from which pellets have been removed provides an index of how far the rat can reach, and the number of pellets remaining at the end of the test indicates the rat’s success in grasping and retrieving pellets.

**Training:**
On the first day, animals are familiarised to the experimental apparatus by placing them into the test box for 15 to 20 minutes. On the second and third trial during the training period, the experimenter helps the rat into the narrower compartment. For this purpose some pellets are first distributed along the platform to attract the rats into the narrower compartment. Once in the compartment more pellets are then presented to each well with forceps in order to help the rat localise them. Rats will begin to learn to reach food by the 4-5th training session.
Wipe the floor and walls of the starting chamber between rats, but not the staircase chamber to encourage the next rats to explore this area. Clean the test box more thoroughly at the end of each day.
If rats struggle to locate pellets in the wells try raising the staircase by holding the silver handle up so that the uppermost stair is almost equal with the platform. If rats show no interest in going on to the platform try tapping the end of the staircase chamber or running a finger along the ceiling of this chamber. Poking extra pellets through the gap at the end of the staircase chamber may also encourage uncooperative rats.

By the fifth day commence recording pellet retrieval.
1. Loading each step with 3 pellets for a total of 21 pellets on either side and place the rats in the test box for 15 minutes.
2. Count how many pellets are retrieved from each side over the 15 minute trial period.
3. The final 4 tests will be used to determine baseline scores.
4. Only rats that can collect a minimum of 12 pellets from each side will be included in analysis of the staircase test.
5. Results are expressed as a percentage of forepaw performance compared to pre-stroke scores.

References:

**MONITORING REQUIREMENTS**

None of these tests require additional monitoring outside of the test period described for each.

**EXPECTED RISKS**

There are no expected risks to the animal’s welfare during the test periods.

**ASSOCIATED PROCEDURE/S**

Many rats who undergo these tests will have had, or will be having, an endothelin-1 induced stroke described in SOP 45.
REFERENCES

Australian code for the care and use of animals for scientific purposes (8th Edition 2013)


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