STANDARD OPERATING PROCEDURE

TITLE: 28-point Neuroscore Test
CATEGORY: Behavioral Assay

Introduction

Goal: This document aims to provide the reader information on how to conduct the 28-point Neuroscore Test. The test is used to assess post-ischemic motor and behavioral deficits in rats and may be run simultaneously with the Garcia Neurological Test. Scores for the following 11 tests are summed to give a maximum total score of 28 points:

1. Circling (max score 4)
2. Motility (max score 3)
3. General condition (max score 3)
4. Righting reflex (max score 1)
5. Paw placement (max score 4)
6. Behavior on a horizontal bar (max score 3)
7. Behavior on an inclined platform (max score 3)
8. Grip strength (max score 2)
9. Contralateral reflex (max score 1)
10. Visual forepaw reaching (max score 2)
11. Contralateral rotation (max score 2)

As this is a general description of standard materials, test settings, and procedures, variations may be made to fit specific needs.

Materials

- *Subjects*: any strain of rats. No prior training is required, though subjects should be acclimated to testing environment and experimenter before testing.
- *Ribbed bar*: used in horizontal bar test.
- *Grid plane*: used in inclined platform and grip strength tests.
- *Virkon*: used between trials to eliminate visual and olfactory residue in arena.

Test Settings
- *Test area*: clean tabletop with sufficient space for animal and two home cages.
- *Lighting*: well-lit.

Detailed Standard Operating Procedure

Before testing:
- *Acclimation*: subjects in home cage are placed in testing room for at least 1hr before testing to minimize effects of stress on behavior during testing.
- *Subject training*: none required.

Testing procedures:
- Animal is subjected to a number of tests, with sequence of testing unimportant. Each test is scored according to score systems presented in Appendix A.
- Animal is returned to home cage and test area is cleaned with Virkon between trials.

Data Analysis
- Scores for each test are summed to determine the overall test score.

Appendix A: Scoring

1. Circling
(Circling is observed when animal is placed on floor.)

4	Non-circling, normal movement
3	Circles on one side
2	Circles when lifted from the tail
1	Circles when pulled from the tail, or spontaneously spins in medium-sized circles (20-50cm radius)
0	Spontaneously spins in tight circles (<20cm radius)

2. Motility
(Motility is observed when animal is placed on floor.)

3	Normal motility
2	Lively but circling
1	Unsteady
0	Reluctant to move

3. General condition

3	Normal (good coat condition, alert, moving about, normal weight gain)
2	Very good but weight gain is less than normal
1	Good

0	Fair (dirty coat, limited grooming, hunched posture, weak muscle tone)

4. Paw placement
(Paw retraction, gripping, and placement on bench are observed when animal is held lengthwise along edge of bench, with two paws hung over edge at a time.)
     1pt for each successful paw placement (max score 4)

5. Righting reflex
(Righting is observed when animal is grasped firmly, rotated until lying on back in palm of experimenter, and grip is released.)

1	Successful righting
0	Unsuccessful righting

6. Horizontal bar
(Grip and hindlimb raising are observed when animal is placed with forepaws on a ribbed bar and allowed to hang.)

3	Raises both hindlimbs onto bar
2	Raises one hindlimb onto bar
1	Hangs at least 5s, hindlimbs do not reach bar
0	Falls off due to weak/absent grip in less than 5s

7. Inclined platform
(Grip and rotation are observed when animal is placed facing 'downhill' on 45° grid plane.)

3	Rotates to face 'uphill' within 20s
2	Rotates in 20-40s
1	Rotates after 40s
0	Falls off grid due to weak/absent grip or remains facing 'downhill'

8. Grip strength
(Grip is observed when animal is dragged back by tail with only forepaws contacting grid.)

2	Normal grip strength
1	Weakened grip strength
0	No grip

9. Contralateral reflex
(Reflex is observed when animal is held vertically by base of tail.)

0	Reflex (curling)
1	No reflex

10. Visual forepaw reaching
(Forepaw reaching and placement on bench are observed when animal is held by base of tail with head just below level of bench top. Vibrissae should almost touch bench and animal should arch and attempt to place forepaws on bench surface.)

      1pt for each successful paw placement (max score 2)

11. Contralateral rotation
(Ability to swivel contralaterally to direction of rotation is observed when animal is held vertically by base of tail and rotated animal either clockwise and then counterclockwise.)

      1pt for each side (max score 2)

Example 28-Point Neuroscore Test Score Sheet

Study #:	**28-Point Neuroscore Test Group:**	Date:
Test #:		Initials:

Animal ID#	Circling 0-4	Motility 0-3	Gen. Cond. 0-3	Paw Place. 0-4	Right. Reflex 0-1	Horiz. Bar 0-3	Inclined Platform 0-3	Grip Strength 0-2	Contralat Reflex 0-1	Forepaw Reach 0-2	Contralat Rotation 0-2	Sum