Murine Neurobehavioral (Irwin) Screen

Page 1. Physical factors and gross appearance

A. Coat color (if more than one, list all separated by a slash)
- A = Albino
- Ag = Agouti
- Bl = Black

B. Rectal temperature (°C; rectal probe lubricated with peanut oil is inserted for 5-10 seconds to obtain a stable reading)

C. Body weight (g)

D. Presence of whiskers (important not to strictly compare to wild-types but to mice in general)
- 0 = None
- 1 = A few
- 2 = Most, but not a full set
- 3 = A full set

E. Appearance of fur (not counting patches of missing fur)
- 0 = Ungroomed and disheveled
- 1 = Somewhat disheveled
- 2 = Well-groomed (normal)

F. Piloerection
- 0 = None
- 1 = Most hairs standing on end

G. Patches of missing fur on face
- 0 = None
- 1 = Some
- 2 = Extensive

H. Patches of missing fur on body
- 0 = None
- 1 = Some
- 2 = Extensive

I. Wounds
- 0 = None
- 1 = Signs of previous wounding
- 2 = Slight wounds present
- 3 = Moderate wounds present
- 4 = Extensive wounds present

Page 2. Observation of behavior in a novel environment

Place the mouse in a clean tub cage for 3 minutes. Record the following behaviors without disturbing the mouse. Incidents of bizarre or stereotyped behavior and convulsions should be noted in the comments column, using the codes listed on the last page.

A. Transfer behavior
- 0 = Coma
- 1 = Prolonged freeze (>10 sec.), then slight movement
- 2 = Extended freeze, then moderate movement
- 3 = Brief freeze (a few seconds), then active movement
- 4 = Momentary freeze, then swift movement
- 5 = No freeze, immediate movement
- 6 = Extremely excited ("manic")

B. Body position
- 0 = Completely flat (on stomach)
- 1 = Lying on side
- 2 = Lying on back
- 3 = Sitting or standing
- 4 = Rearing on hind legs
- 5 = Repeated vertical leaping

C. Spontaneous activity
- 0 = None, resting
- 1 = Casual scratch, groom, slow movement
- 2 = Vigorous scratch, groom, moderate movement
- 3 = Vigorous, rapid/dart movement
- 4 = Extremely vigorous, rapid/dart movement

D. Respiration rate
- 0 = Gasping, irregular
- 1 = Slow, shallow
- 2 = Normal
- 3 = Hyperventilation

E. Tremor
- 0 = None
- 1 = Mild
- 2 = Marked

F. Palpebral closure
- 0 = Eyes wide open
- 1 = Eyes 1/2 closed
- 2 = Eyes closed

G. Piloerection
- 0 = None
- 1 = Coat stood on end

H. Gait
- 0 = Normal
- 1 = Fluid but abnormal
- 2 = Limited movement only
- 3 = Incapacity

I. Pelvic elevation
- 0 = Markedly flattened

1 = Barely touches
2 = Normal (3mm elevation)
3 = Elevated (more than 3mm elevation)

J. Tail elevation (during forward motion)
- 0 = Dragging
- 1 = Horizontally extended
- 2 = Elevated (Straub tail)

K. Urination
- 0 = None
- 1 = Little
- 2 = Moderate amount
- 3 = Extensive

L. Defecation (count the number of fecal boli emitted during the 3-min. period)

Page 3. Reflexes and reactions to simple stimuli

A. Touch escape (finger stroke from above, starting light and slowly getting firmer)
- 0 = No response
- 1 = Mild (escape response to firm stroke)
- 2 = Moderate (rapid response to light stroke)
- 3 = Vigorous (escape response to approach)

B. Positional passivity (struggle response to sequential handling)
- 0 = Struggles when restrained by tail
- 1 = Struggles when restrained by neck (finger grip, not scruffed)
- 2 = Struggles when held supine (on back)
- 3 = Struggles when restrained by hind legs
- 4 = Does not struggle

C. Trunk curl (grip tail between thumb and forefinger and lift about 30 cm)
- 0 = Absent
- 1 = Present

D. Reaching reflex (extension of forelimbs when the mouse is lowered by the base of its tail from a height of 15cm above the edge of a table)
- 0 = None
- 1 = Upon nose contact
- 2 = Upon vibrasse contact
- 3 = Before vibrasse contact (18mm)
- 4 = Early vigorous extension (25mm)

E. Body tone (compress both sides of the mouse between thumb and index finger)
- 0 = Flaccid, no return of cavity to normal
- 1 = Slight resistance
- 2 = Extreme resistance, board like

F. Pinna reflex (while the mouse is gently restrained, touch each auditory meatus lightly with the tip of a 31-ga. stainless-steel wire probe; watch for either ear retraction or head movement)
- 0 = None
- 1 = Active retraction, moderately brisk flick
- 2 = Hyperactive, repetitive flick

G. Preyer reflex (90 dB click 30 cm above mouse; watch for pinna reflex or head twitch)
- 0 = None
- 1 = Active retraction, moderately brisk flick

2 = Hyperactive, repetitive flick

H. Toe pinch (first lift the mouse by its tail so the hind limbs are clear of the table; then apply a gentle lateral compression of the middle digit of the hind foot with fine forceps)
0 = None
1 = Slight withdrawal
2 = Moderate withdrawal, not brisk
3 = Brisk, rapid withdrawal
4 = Very brisk repeated extension and flexion

Page 4. Measures recorded during supine restraint

A. Skin color (color gradations of plantar surface and digits of forelimbs)
0 = Blanched
1 = Pink
2 = Bright, deep red flush

B. Heart Rate (felt by palpation below sternum)
0 = Slow, bradycardia
1 = Normal
2 = Fast, tachycardia

C. Limb Tone (resistance to gentle finger tip pressure on plantar surface of hind paw)
0 = No resistance
1 = Slight resistance
2 = Moderate resistance
3 = Marked resistance
4 = Extreme resistance

D. Abdominal Tone (palpation of abdomen)
0 = Flaccid, no return of cavity to normal
1 = Slight resistance
2 = Extreme resistance, board like

E. Righting Reflex (10 sec. maximum)
0 = No impairment
1-10 = Number of seconds required to right

F. Air Righting Reflex (drop the mouse from a supine position, 30 cm above a tub cage with bedding)
0 = No impairment
1-10 = Number of seconds required to right

Provoked Salivation and biting (gently insert a dowel between the teeth at the side of the mouse's mouth; record provoked biting and salivation)

G. Salivation (the area of wetness in the sub-maxillary area)
0 = None
1 = Slight margin of sub-maxillary area
2 = Wet zone entire sub-maxillary area

H. Provoked Biting
0 = Absent
1 = Present

Page 5. Grip strength, motor coordination, and locomotor activity

A. Grip strength (allow the mouse to grip a grid then apply a gentle horizontal backwards pull)

0 = None
1 = Slight grip, semi-effective
2 = Moderate grip, effective
3 = Active grip, effective
4 = Unusually effective

B. Wire maneuver (hold the mouse above the wire and lower it, allowing the forelimbs to grip the horizontal wire; holding the mouse in extension, rotate to the horizontal and release)
0 = Active grip with hindlegs
1 = Difficulty to grasp with hindlegs
2 = Unable to grasp with hindlegs
3 = Unable to lift hindlegs, falls within seconds
4 = Falls immediately

C. Wire hang (similar to wire maneuver but have the mouse hang vertically from the wire from the start)
0 = Active grip with hindlegs
1 = Difficulty to grasp with hindlegs
2 = Unable to grasp with hindlegs
3 = Unable to lift hindlegs, falls within seconds
4 = Falls immediately

D. Inverted screen (place the mouse on a grid screen, wave lightly in the air, then invert 60 cm over tub cage with soft bedding material; 60 sec. maximum)
0-60 = Number of seconds before falling

E. Pole climb (place mouse on a dowel covered with cloth tape; turn vertical so that mouse is facing down; on top of the dowel place a placard that is isoluminant with the table top)
0 = Climbs down within 30 sec.
1 = Turns and climbs up the pole
2 = Turns but then freezes
3 = Does not move within 30 seconds or climbs down but not off the pole
4 = Falls off

Irwin S. Comprehensive observational assessment: Ia. A systematic, quantitative procedure for assessing the behavioral and physiologic state of the mouse. Psychopharmacologia 1968; 13: 222-57.

Additional comments on Neurobehavioral Screen

Bizarre Behavior

HF	Head flicking or head shaking
HS	Head searching—repetitive
H	“Hallucinating”—mouse appears to respond to objects not present (may be “boxing”)
B	Compulsive biting
L	Compulsive licking
SB	Self destructive biting—usually of toes with bleeding
P	Prancing forelimbs—shifting from one forelimb to another
UW	Upright walking
AW	Aimless wandering—slow plodding around with no apparent purpose
C	Circling
W	Waltzing—rapid turning in circles
R	Retropulsion—animal walks backwards
D	Spatial Disorientation—walking or stumbling into objects
HB	Head bobbing

Convulsions

Enter data in the comments area, using the following codes [e.g., Clonic (C); Miscellaneous (Pr)].

Clonic type: alternative contraction and relaxation of the voluntary muscles

C	Clonic—coordinated, unsymmetrical convulsion and natural, purposeful like movements, e.g. running, sometimes preceded by a running excitement (Rn)
Cs	Clonic symmetrical—repetitive symmetrical jerks or twitches of the limbs
Rn	Running excitement—often accompanied by mild clonus or leading to a severe convulsion
Ch	Champing—clonus of the jaws only
P	Popcorn—seizure where animal repeatedly "pops" into the air
A	Asphyxia—a terminal clonic or clonic-tonic convulsion resulting from respiratory failure.

Tonic type: persistent contraction and spasm of a set of voluntary muscles.

T	Tonic—sustained extension of hindlimbs, usually preceded by tonic flexion. (Tf) is used if tonic flexation occurs without extension
Op	Opisthotonus—head, body and limbs are rigidly extended and arched backwards.
Em	Emprosthonus—opposite of Op i.e. extended forward.

Miscellaneous Type

Rr	Rock & roll—the mouse is prostrate on its back and rocks from side to side apparently trying to right itself, sometimes rolling over (overshooting) and continuing to rock again.
Su	Sitting up—the mouse sits upright on hindlimbs during the seizure
Pr	Praying—The mouse is sitting upright and the forelimbs are held together or crossed in attitude resembling prayer. Extra points for speaking in tongues.