

## Protocol Behavior

### Key people

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For each pedigree, four mice will go through the behavioral screen. In addition, from each pedigree, one mouse will undergo whole-body imaging by microCT, two mice will undergo hematology analysis (Cell-dyne 3500), gametes will be cryopreserved from two males, and one male/one female will be dissected for the cryopreservation of representative tissues.

### Gross Physical and Neurological Screen at Weaning and P548-560

Test	Age	Method
Righting reflex	P21-25	Pup placed on back, observed for regaining of ventral-down posture
Reaching reflex	P21-25	Mice are lowered by the base of their tail from a height of 15 cm above the edge of a table. Extension of forelimbs is noted
Forelimb grasp	P21-25	Wire hang, 1 minute by forepaws
Pinna reflex	P21-25	the auditory meatus is lightly touched with the tip of a wire probe. Ear retraction and head movement are noted
Vibrissae Reflex	P21-25	Vibrissae are stimulated Head turn toward gentle whisker stimulation whisker mobility, palpebral closure, and head-turning are noted
Negative geotaxis	P21-25	Pup placed head down on inclined plan, should rotate to face uphill.
Visible	P21-25, P548-560	Observation by trained staff for Body size Coat color and texture Ear size Skeletal abnormalities (head, back, or extremities) Limbs and digits (Webbing and number) Tail (kinks, length, skin condition) Mottling spotting, striations Whiskers (barbering)
Pupillary light reflex	P21-25, P548-560	An ophthalmoscope is used to determine the pupillary reaction to light. Prior to the test mice are placed in a dark area for 30 minutes. This test is done in conjunction with the light dark test
Preyer reflex	P21-25, P548-560	a 90dB click is presented 30cm above the mouse. Pinna reflex and head movement are noted
Trunk curl	P21-25, P548-560	Mice are lifted about 30cm in the air by the tail presence or absence of a trunk curl is noted.

Spontaneous behavior	P21-25, P548-560	Mice are observed in an empty cage for atypical behaviors including circling, head bobbing, abnormal gait, perseveration, excessive grooming etc.
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## Behavioral Screen P50-60 and aging 18 months

### Day 1

#### 1. MOTOR ACTIVITY, REARING, EXPLORATION

Hamilton-Kinder Smart-frame Photobeam System computerized recording of animal activity over a designated period of time

Exploratory locomotor activity is recorded in an open field measuring 27 X 27 cm over a 20-30 minute period. Infrared beams automatically record horizontal movements and rearings in the open field. The task provides measures of not only locomotor activity, but of anxiety in response to a novel environment, and habituation by including number of beams broken in the periphery vs the corners, and the mid zone areas every five minutes.

#### 2. LEARNING AND MEMORY Y-MAZE

Columbus Instruments computerized recording of animal activity is used for the Y-maze test.

The Y-Maze is a gross test for spatial memory. It tests to see if the mouse remembers the arm it has just explored and will therefore enter one of the other arms of the maze.

Columbus Instruments Y-maze spontaneous alternation. Mice are placed at the bottom of the Y (middle arm) in the Y-maze and are allowed to explore freely all three arms for an 8 minute session. The first 2 minutes are for habituation and for the last 6 minutes the alternation between arms is recorded via photo beam breaks. (Test started July 2002)

#### 3. WEIGHT

Mettler computerized balance is used to accurately determine the weight of each mouse

#### 4. OLFACTORY

Latency to find buried food by fasted mouse (odor). A piece of corn chip is hidden in bedding of the home cage. Mice are exposed to a corn chip one week prior to testing to prevent food neophobia. Latency to find the food is recorded

### Day 2

#### 1. PUPILLARY RESPONSE TO LIGHT

An ophthalmoscope is used to determine the pupillary reaction to light. Prior to the test mice are placed in a dark area for 30 minutes. This test is done in conjunction with the light dark test

#### 2. LIGHT DARK EXPLORATION.

The light vs dark preference trial, a gross measure of anxiety, is performed using a computerized test and equipment from the H. Gershenfeld lab at Univ. of Texas Southwestern. A polypropylene animal cage 44X21X21 cm has 1/3 of the area dark and 2/3 light. Florescent lights above the cages provides the illumination. Thirty minutes prior to the test the cages of the mice to be tested are covered with a dark cloth. To start the session, a mouse is placed into the light side of the box and left to wander freely for 10 minutes. There is a small opening between the light and dark parts of the cage. The software program gives the number

of transitions across the partition from the light to the dark side and the amount of time spent in the light and dark compartments. (Test started in December 2002)

3. STARTLE RESPONSE/PRE-PULSE INHIBITION

Hamilton-Kinder Startle Boxes computerized recording system for Sensorimotor gating  
Acoustic Startle

A test to determine the ability of the mice to “gate” or inhibit the effect of environmental information. Each prepulse inhibition session consists of 50 trials over a 20 minute period. The prepulse stimuli are 20msec bursts of 70-85dB white noise presented before the startle stimulus of 120dB. The prepulse stimuli are pseudo-randomly ordered.

4. LEARNING and MEMORY FEAR CONDITIONING (Learning Phase)

Hamilton-Kinder Smart-frame Photobeam System a Computerized recording of animal activity.

Animals are placed in the enclosure. They are given 5 minutes to habituate. At the end of the 5 minutes recorded movement is started. At 180 seconds mice are presented with an 85dB tone for 30 seconds and then receive a 2 second 0.36-0.40mA footshock. The mouse is removed to its home cage 120 seconds after the footshock (with food) for 24 hours.

Day 3

1. LEARNING and MEMORY (Cue Phase)

Hamilton-Kinder Smart-frame Photobeam System computerized recording of animal activity.

Twenty four hours after the learning phase animals are placed in an enclosure that has a different context (white wall insert with black floor over grids) from the enclosure that they received the shock in on the previous day. They are presented with the identical 85dB tone from the previous day. Movement is recorded by the number of photocell beam breaks for 5 minutes prior to during and after the presentation of the tone. This is to assess the ability of the animal to remember the tone and pair it with the shock from the previous day.

2. LEARNING and MEMORY (Context Phase)

Hamilton-Kinder Smart-frame Photobeam System computerized recording of the animal's activity.

Approximately one hour after the cue phase, inserts are removed and animals are placed back into the identical enclosure that they received the shock in on the previous day.

Movement is recorded for 5 minutes to assess the ability of the animal to remember the context and pair it with the shock from the previous day.

3. TAIL SUSPENSION (Depression/helplessness)

The Med Associates computerized tail suspension system is used, in which immobility is a determinant of helplessness.

The mouse is suspended by the tail for 6 minutes. Body and limb posture as well as immobility and amount of movement are assessed at 30 second intervals over the 6 minute period.

Day 4.

1. HOT-PLATE TEST assesses pain and temperature pathways and receptor function. Reaction to an unpleasant sensory heat stimulus is measured by confining the mouse in a chamber atop a hot plate heated to 55 degrees Centigrade, and timing the latency to paw-licking, squeaking, or

distressful behavior. The mouse is removed immediately upon the first paw lick or sign of distress or within 20 seconds if it does not appear to perceive the heat.

Any retesters or left over tail suspension tests are completed on this day

The Behavior Screen at 18 months will be the same as for tests performed at 7-8 weeks

Monthly weights are taken for the 8 mice from each pedigree assigned to the aging colony beginning when the mice are one month of age.

Anatomy/morphology	P50-60, P548-560	MicroCT scanner
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