Supplementary Information

Behavioral Assessment Protocol

1. Place mice in separate, empty cages w/ lid 10 minutes prior to first observation
2. Set timer for 2 minutes
3. Start timer
4. Watch mouse #1
5. Tally "stereotypic behaviors" on paper
   Behaviors:

   a. Grooming (time/episodes): Self-cleaning and/or scratching behavior
   -Count each grooming episode and use a stopwatch to record total time grooming. Ex: If an animal grooms 3 different times within the 2 minute period for 3 seconds each, the score for grooming episodes would be 3, and the score for total time would be 9.0 seconds. Stop, but don't clear the stopwatch after the animal is done with a grooming episode.

   b. Head bobbing (tally): Involuntary head nods, usually at regular intervals. These are short, quick downward movements, then recovery.
   -Tally each "bob" and report total number of head bobs

   c. Myoclonus (tally) --These are rapid, irregular jerks/twitches.
   -Tally each twitch in the region of origin.
   -trunk- irregular movement originates in trunk region, usually will cause movement of the whole body
   -head/facial—myoclunus of *just* the head and/or face, these will be more sudden, jerky, and less rhythmic than head bobs,
   -limbs- myoclonus of any limb region

6. At the end of the 2 minutes, gently pick up the mouse to note its resistance to handling (for motor-impairment score)
7. Assign **"Drowsiness"** and **"motor-impairment"** scores

Drowsiness

0	Fully alert
1	Eyes half closed at rest
2	Eyes closed at rest, but easily rousable
3	Eyes closed at rest, difficult to rouse

Motor impairment

0	Normal posture at rest, move normally in cage on handling
1	Mild splaying of legs at rest, movements slowed and jerky, mild reduction in struggle on handling
2	Moderate splaying of legs at rest, little movement in cage, moderate reduction in struggle on handling
3	Prominent splaying of legs at rest (sprawled), no movement in cage, minimal struggle on handling

Scale from Aitchison et al. (2000).

8. Report all data on provided score sheet.