Motor and coordination phenotypes

1st ISBS Summer School

St. Petersburg, Russia

May 9th -15th, 2008

Quantifying behavioral responses

- Scoring customized for specific subjects/test
- Experimenters should be:
  1)“blind” to treatment
  2) high in inter/intra rater reliability
  3) consistent (time, season, place)

Pletnikov, 2006

SHIRPA

- **S**mithKline Beecham Pharmaceuticals
- **H**arwell, MRC Mouse Genome Centre
- **I**mperial College School of Medicine
- **R**oyal London Hospital, St. Batholomew's
- **P**henotype
- **A**ssessment

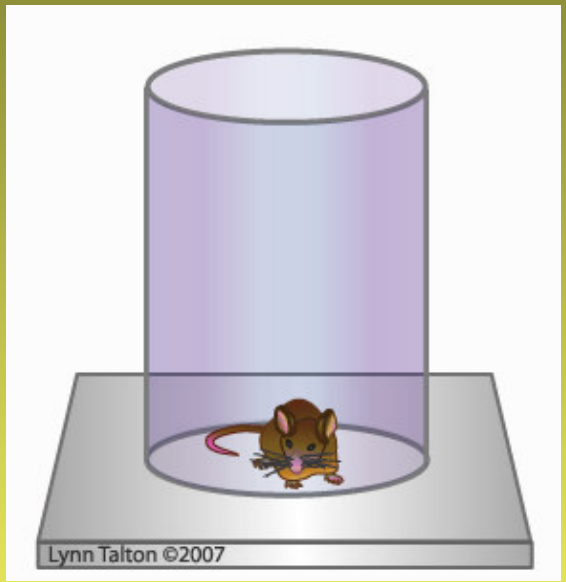
Assessing motor phenotypes

- SHIRPA battery: a widely-accepted neurological battery involving a three-stage protocol
- It is very basic, and includes measures of muscle function, cerebellar function, sensory function, neuropsychiatric function, and autonomic function

http://btc.bol.ucla.edu/shirpa.htm

Behavior recorded in Viewing Jar:

- **Body Position**
  0 = Inactive
  1 = Active
  2 = Excessive Activity
- **Tremor**
  0 = Absent
  1 = Present
- **Palpebral Closure**
  0 = Eyes open
  1 = Eyes closed
- **Coat Appearance**
  0 = Tidy and well groomed coat
  1 = Irregularities such as piloerection
- **Whiskers**
  0 = Present
  1 = Absent (include any further comments



Behavior recorded in Viewing Jar:

- **Lacrimation**
  0 = Absent
  1 = Present
- **Defecation**
  0 = Present
  1 = Absent
- **Behaviour recorded in the Arena:**
- **Tansfer Arousal**
  0 = Extended freeze (over 5 seconds)
  1 = Brief freeze followed by movement
  2 = Immediate movement
- **Locomotor Activity**
  The total number of squares the animal enters with all four feet in 30 seconds.
- **Gait**
  0 = Fluid movement and approximately 3mm pelvic elevation
  1 = Lack of fluidity in movement (include comments eg. retropulsion, more than 3 mm pelvic elevation)

Behavior recorded in Viewing Jar:

- **Tail Elevation**
  0 = Dragging
  1 = Horizontal extension
  2 = Elevated/straub tail
- **Startle Response**
  0 = None
  1 = Preyer reflex (backwards flick of the pinnae)
  2 = Reaction in addition to the Preyer reflex (eg. Startled response)
- **Touch Escape**
  0 = No response
  1 = Response to touch
  2 = Flees prior to touch

Behaviour recorded above the Arena:

- **Positional passivity**
  0 = Struggles when held by the tail
  1 = Struggles when held by the neck
- 2 = Struggles when laid supine
  3 = No struggle

Behavior recorded in Viewing Jar:

- **Skin Color**
  0 = Blanched
  1 = Pink
  2 = Bright, deep red flush
- **Trunk Curl**
  0 = Absent
  1 = Present
- **Limb Grasping**
  0 = Absent
  1 = Present
- **Pinna Reflex**
  0 = Present
  1 = Absent
- **Corneal Reflex**
  0 = Present
  1 = Absent
- **Contact Righting Reflex**
  0 = Present
  1 = Absent
- **Evidence of Biting**
  0 = None
  1 = Biting in response to handling
- **Vocalisation**
  0 = None
  1 = Vocal

Summary: SHIRPA battery

- A battery of tests that can be completed within a few minutes
- Observation for normal and abnormal spontaneous behaviors, and measurements of activity levels, arousal, respiration, gait, muscle tone, reflexes, aggression, etc.
- If a subject group shows unusual behavior or function, further testing can be done in that domain

Gait assessment

- Trained mice run down a corridor that with dye or ink on their feet, leaving a trail
- Parameters measured: stride length, width of gate, accuracy of foot placement
- Also utilizes computer analysis equipment

Gait patterns

- Detects walking abnormalities
- Easy to perform: place non-toxic paint on mouse's feet
- Sensitive to atypical patterns due to genetic alterations (see below)

A) Wild type
B) Mutant mice

(Photo: Peter J. Detloff)

- Problems: May be sensitive to procedure-evoked anxiety/stress

Swimming

- Assess ability to swim
- Abnormal patterns (vertical vs. horizontal)
- Circling
- Diving
- Sinking

Normal horizontal swimming
www.umt.edu/urelations/rview/summer06/mice.htm

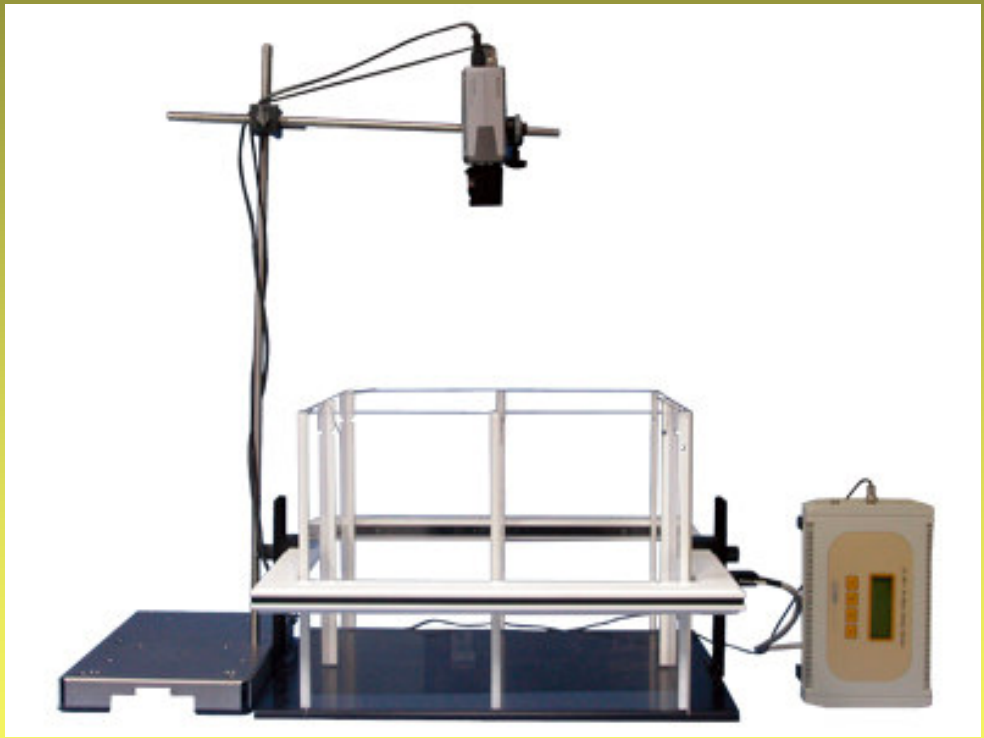
Abnormal vertical swimming
Kalueff et al., 2006

Motor skills tests

- Drug-induced turning (Rotation Test)
- Forelimb asymmetry (Cylinder Test)
- Beam walking
- Grip strength
- Grid walking
- Placing test
- Rotorod
- Landing Foot Spread Test
- Skilled reaching (forelimb motor control)

Neurodetective International, 2008

Homecage activity chambers



www.med-associates.com

Homecage observation

Normal behaviors to assess:

- Digging
- Grooming
- Thigmotaxis
- Rearing
- Exploration

Homecage observation

Abnormal behaviors to assess:

- Hyperactive running
- Stereotypes (jumping, circling, somersault)
- Seizures
- Freezing/ inactivity
- Overactive itching
- Overgrooming and self-damage
- Impulsivity

Motor problems

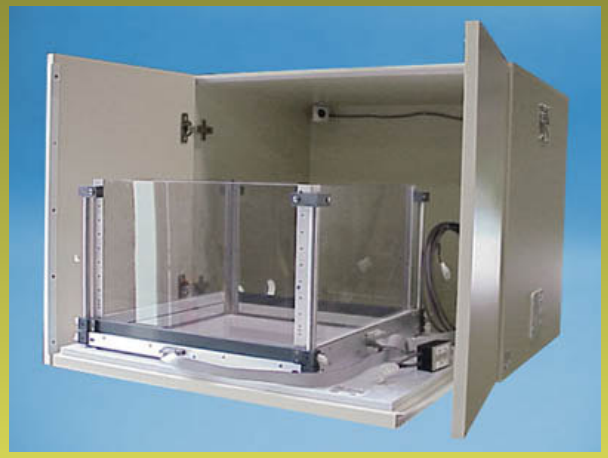
Disorders may have both peripheral and central origins:

- Cerebellum
- Brain stem
- Striatum
- Basal ganglia
- Motor cortex
- Spinal cord
- Peripheral nervous system
- Musculoskeletal deficits

Animal tests for motor ability

- Balance (e.g. Rotorod)
- Reflex testing
- Strength testing
- Gross activity levels (GAL)
- Fine motor analysis (FMA)
- Straight observation

Open field test (OFT)



www.med-associates.com

- High/low activity level
- Body posture
- Movement coordination
- Rearing, exploring
- Additional movements (e.g. head twitches)
- Thigmotaxis (avoidance of open central areas)

Open-field test

- Open square or circular arena
- Typical parameters:

1. zones entered
2. time spent in periphery vs. center
3. grooming time
4. Rears
5. defecation

- Often use videotracking software (e.g. Ethovision, HVS Image) providing distance traveled, speed, etc.
- Measures both locomotor activity and anxiety

Rating scales

Scoring technique using a number to represent the degree of behavioral severity

0	1	2
Normal behavior	Intermediate motor disturbances	Consistent abnormal motor coordination

Potential concerns:

- Statistics are non-parametric
- More “quantitative” than “qualitative”

Pletnikov, 2006

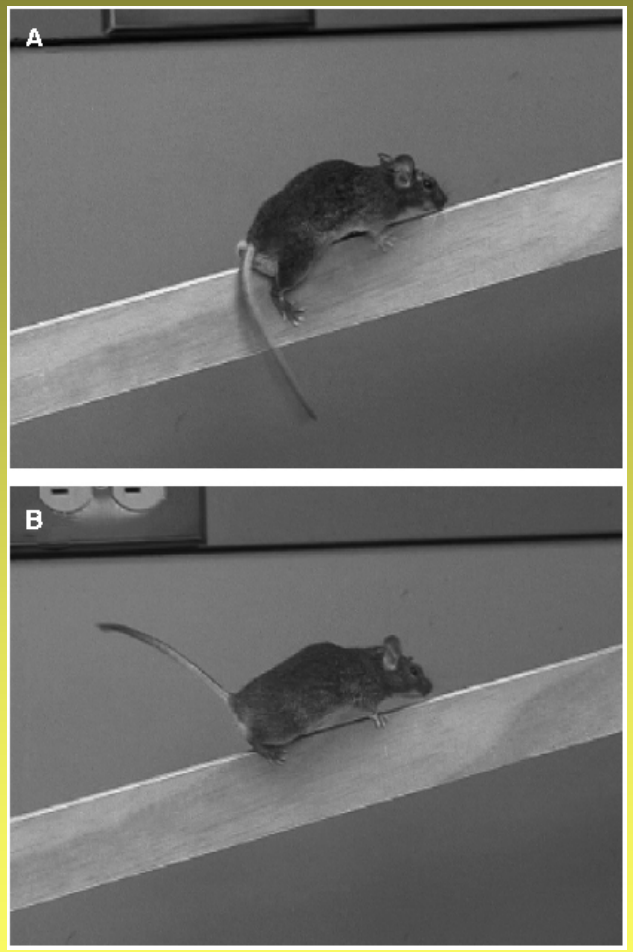
Locomotion

Many potential confounds:

- Habituation problems (too much/little)
- Testing time (consider circadian rhythms)
- Variability (e.g. interstrain)
- Problems with housing (e.g. multi-species odors, sex pheromones)
- Effect of sound
- Floor/ceiling effects

Pletnikov, 2006

Beam walking test



Chang et al., 2005

Use food reward or dark "escape" area as incentive

↓ beam width = ↑ difficulty

Endpoints recorded:

- Time to cross beam
- Falls
- Hind-leg slips

Problems:

- Often requires pre-training
- May involve motivational factors

Rotorod (Rotarod)

- A rotating bar, revolves at constant or increasing speeds
- Latency to fall is primary endpoint
- Typically, mouse performance ↑ as number of trials ↑

Van Meer and Raber, 2005

Rotorod

Motor abnormalities:

- Coordination
- Weakness
- Muscle tonicity
- Involuntary movements

Other domains:

- Sensory function
- Cognitive ability
- Anxiety
- Non-motor seizures
- Chronic/systemic problems
- Problems: Cognitive phenotypes (e.g. habituation) may affect motor performance

Pletnikov, 2006

Measuring strength

Hanger test:

Time latency to fall from an upside-down screen

phenome.jax.org/.../Lake3_Protocol

Rope climbing test

- Ability to climb rope
- Latency to reach a 20 cm mark

Kalueff et al., 2007

Normal grip strength

Chimney test

- Consists of a hollow tube large enough for a mouse or rat to fit inside comfortably
- The animal is placed in the tube, and then the tube is positioned vertically, with the animal's snout oriented downwards
- The animal will attempt to keep itself from falling and will slowly walk backwards up to the top of the "chimney"
- This measures the animal's motor ability and coordination

Hind-leg clasping reflex

Normal reaction
in a normal mouse

This mouse will appear normal in the cage but with you pick it up, it exhibits "clasping" rather than the normal plantar reaction

Ansorge et al. 2006

Clasping indicates neurological/motor impairments in animals

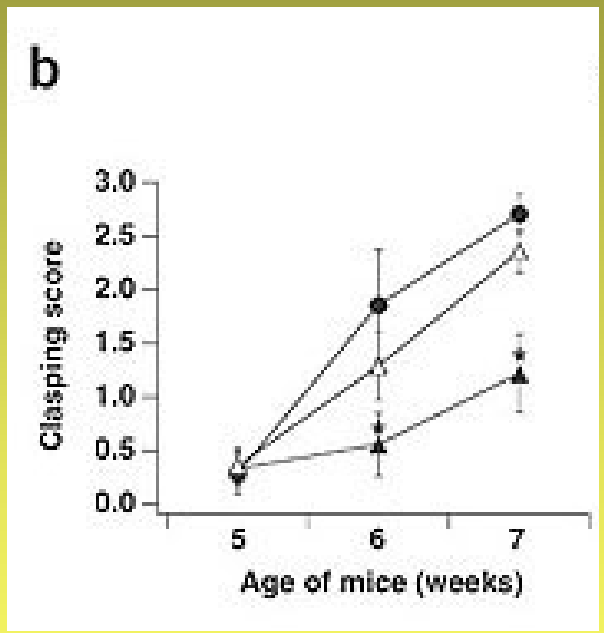
Herzing, 2008

Davis, 2000

Davis, 2000

Foot-clasping

Example of a foot-clasping phenotype



Tanaka et al., 2004

Assessing other motor reflexes

Righting reflex

- Mice right themselves onto feet after put on their backs, or dropped from some height (e.g. 20 cm) on a cushioned surface
- Generally normal unless movement/vestibular disorders are present
- Tail suspension test (abnormal spinning if vestibular problems)

Digging behaviors

- A very common behavior in rodents
- Sensitive to stress, and anxiolytic/ anxiogenic pharmaceutical compounds
- Marble-burying test often used to measure this behavior

Marble burying/digging

- To kick sand in someone's face is an archetypal agonistic interaction between humans
- Rodents have been filmed kicking earth toward an approaching snake in their burrows. They also bury noxious objects such as shock probes; rats also bury non-noxious objects such as marbles and food
- Most behavioral scientists would assume that marbles are non-aversive to mice
- Mice are probably not deliberately burying the marbles; they simply fall through the displaced bedding. The present view, therefore, is that marble burying simply measures digging behavior

Deacon, 2006

Digging Test Protocol

Digging is defined as coordinated movements of fore- or hind limbs that displace the substrate

- Fill the cage 5 cm deep with wood chips
- Several test cages can be run simultaneously
- Place a mouse in each cage and start the test timer. Test duration is 3 min.
- The latency to start digging, the number of digging bouts and the total duration of digging are recorded

Deacon, 2006

Another version of the Digging Test

1. Place a fixed amount of bedding (200-300 ml) concentrated in the corner of a transparent cage
2. Place the mouse into the cage and leave undisturbed for thirty minutes
3. Remove the mouse, and place the cage over a “grid” diagram to calculate the number of squares that are covered by bedding

This test is highly sensitive to motor phenotypes, pharmacological treatments, genetic manipulations, and brain legions

Kalueff et al., 2006

Marble Burying Protocol

1. Fill the cage approximately 5 cm deep with wood chip bedding
2. Place a regular pattern of glass marbles on the surface, evenly spaced, each about 4 cm apart
3. Place one animal in each cage and leave for 30 min
4. Count the number of marbles buried (to 2/3 their depth) with bedding
5. Alternatively, count the number of marbles buried fully, partially (2/3 their depths), and non-buried

Deacon, 2006
Kalueff et al., 2006

Unusual escape attempts

- When animal demonstrates abnormally active escape attempts
- E.g. immediately after being placed on a surface the animal will jump/run away, rather than freezing
- Could indicate hyperactivity, very high overall anxiety, hyperexcitability, or other phenotypes
- If animal shows these abnormal behaviors, it needs further examination before being tested in other paradigms

Reflexes and postural reactions

Common tests:

- Trunk curl
- Rear-limb withdrawal
- Low/flat body
- Tremor
- Hind-leg abduction
- Forelimb positioning

Diagnostic physical exam

Physical features to note:

- Exposed skin or bald patches – indicates fighting, dermatitus, barbering or obsessive self-or hetero-grooming
- Injuries on eyes, legs, tail – indicates fighting or congenital/genetic defects, inflammation
- Matted, ungroomed hair – indicates illness (sickness)

Pletnikov, 2006

Phenotyping Tourette-like behavior

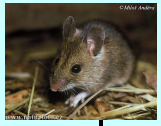
Tourette syndrome (TS) is a neurological disorder with motor or vocal tics [1% - severe TS, 6-10% - mild TS]

Genetic component: 11q, 2p, rare variants: 7q, and 13q (SLITRK1)

Co-morbid with psychiatric disorders (ADHD, depression, OCD)

Stress exacerbates TS symptoms

Importance of new animal models of TS



Genetic models (DAT-/-)

"Co-morbidity" models

Stress-related models

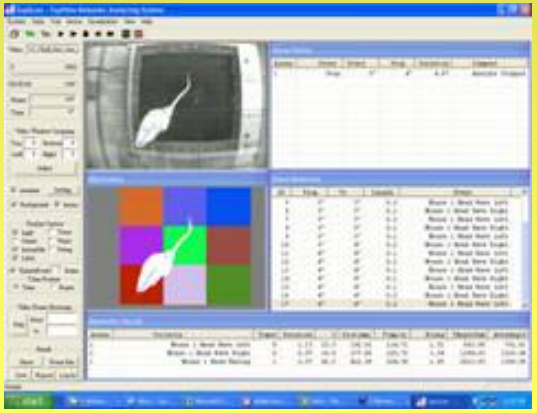
Several experimental (including genetic) models of TS have been reported

Q: Can behavioral phenotyping of TS can be improved?

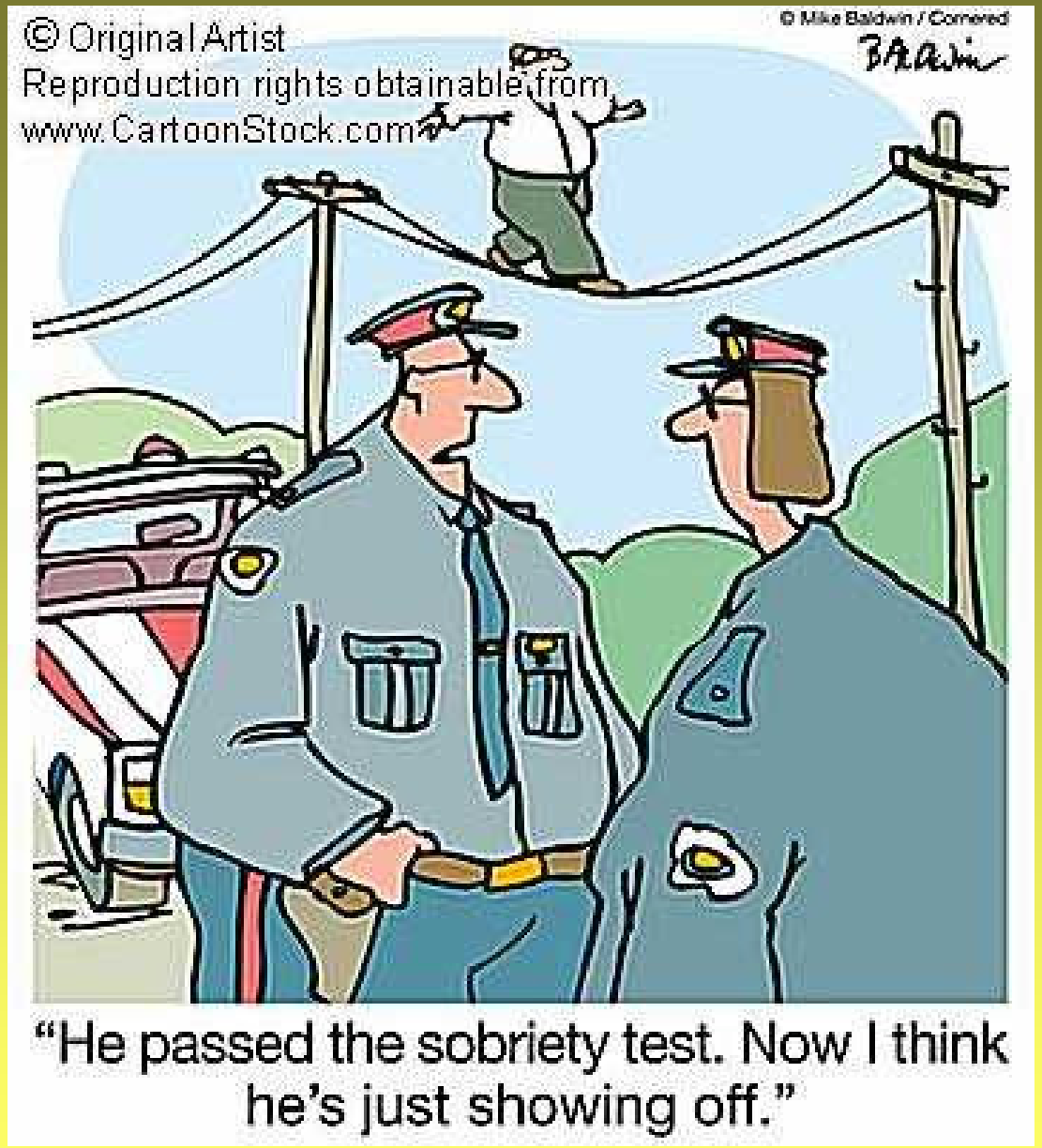
Recent behavior-recognition technologies create the possibility for high-throughput video-tracking systems

These systems are already able to recognize some rodent phenotypes (e.g., head twitching, head weaving and tics) that are relevant to TS

This system can promote the discovery of future animal models of TS, also enabling high-throughput screening of TS-active drugs



www.CleverSysInc.com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"He passed the sobriety test. Now I think he's just showing off."