How to measure rodent behavior and perform a neurological screen.

Fiona Harrison, PhD
Department of Medicine
Vanderbilt University Medical Center
fiona.harrison@vanderbilt.edu
Introduction

• Mouse-centric lecture
• Apparatus for mice are typically the same as for rats but scaled down
• However, mice are not just little rats. Behavioral profiles differ
Why study behavior?

- Psychology - the basics of learning and memory. Fundamental rules apply to animals and humans
- Genetic manipulations - to assess phenotypes caused by genetic manipulations
- Experimental manipulations - to assess phenotypic changes caused by pharmacological, dietary or environmental manipulations
Colony Health

- Good practice
- Eliminate artefactual effects in your experiments (blindness, deafness, anosmic)
- e.g. Irwin screen or SHIRPA
- Appearance (grooming, fighting, coloration), weight, temperature, posture and gait, activity, neurological reflexes
Feeding behaviors

• Feeding = Survival
• Specialized circuits and mechanisms exist for hunger, thirst and satiation and all associated behaviors (e.g. activity and foraging)
  Insulin and glucose - gastrointestinal feedback - hindbrain - hypothalamus
• Many injected peptides can influence feeding
Feeding behaviors

- **24 hour consumption** of standard chow and water (weight and volume)
  - Easy but not sensitive
  - Spillage, meal size, meal frequency, diurnal rhythm
- **Restricted feeding** (4 hours per day)
  - Easier to detect effects of short acting drugs
- **Short access to highly palatable foods**
Feeding behaviors

- Continuous monitoring can be assessed in automated chambers
Control diets

**Purina**
Standard lab chow

**Research Diets Inc.**
Basic control diet

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Product Code D10001</th>
<th>kcal%</th>
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<tbody>
<tr>
<td>Protein</td>
<td>20.3</td>
<td>20.8</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>69.0</td>
<td>67.7</td>
</tr>
<tr>
<td>Fat</td>
<td>5.0</td>
<td>11.5</td>
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<tr>
<td>Total</td>
<td>94.3</td>
<td>100.0</td>
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<table>
<thead>
<tr>
<th>Ingredient</th>
<th>gm</th>
<th>kcal</th>
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<tbody>
<tr>
<td>Casein, 30 Mesh</td>
<td>200</td>
<td>800</td>
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<tr>
<td>DL-Methionine</td>
<td>3</td>
<td>12</td>
</tr>
<tr>
<td>Corn Starch</td>
<td>150</td>
<td>600</td>
</tr>
<tr>
<td>Sucrose</td>
<td>500</td>
<td>2000</td>
</tr>
<tr>
<td>Cellulose, BW200</td>
<td>50</td>
<td>0</td>
</tr>
<tr>
<td>Corn Oil</td>
<td>50</td>
<td>450</td>
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<tr>
<td>Mineral Mix S10001</td>
<td>35</td>
<td>0</td>
</tr>
<tr>
<td>Vitamin Mix V10001</td>
<td>10</td>
<td>40</td>
</tr>
<tr>
<td>Choline Bitartrate</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>1000</td>
<td>3902</td>
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</table>
Feeding behaviors

Hedonic control
Psychosocial factors
Environment

Blood glucose

Feeding behaviors/Energy intake

Digestive tract - Stomach; intestines; pancreas (ghrelin; GLP-1, PYY, CCK; insulin)

Adipose tissue (leptin, adiponectin)

Homeostatic feedback
Hunger/satiety
Feeding behaviors

- Novel taste neophobia
- Social transmission of food preference

Preference for novel food
Locomotor activity
Locomotor activity

- Automated measurement by beam breaks
- Computer scored from top-view video
- Hand-scored by square crossings
Locomotor activity

- Habituation - reduced exploration with repeated exposure
  - Within-trial and between-trials
- Activity response to drug e.g. scopolamine
Anxiety

- Using locomotor activity chambers
- Preferential exploration of dark, enclosed, wall/corner areas

Open field

Light-dark box
• Elevated Plus/Zero Maze (EPM/EZM)
• Time spent in Open versus closed arms
  Should show preference for closed (dark)arms
• Entries into each arm
• Distance traveled
• Stretch-attend postures
• EZM has same exploration conflicts minus the central square
In the EPM mice can choose between exploration in open and closed areas.
Depressive-like behaviors

- **Porsolt or forced-swim**
  - “Behavioral despair” indicated by time spent swimming
  - Be aware: Mice float, but rats sink

- **Tail suspension test**
  - “Behavioral despair” indicated by time spent immobile

- Behaviors improved by anxiolytic drugs
Abnormal behavior in db/db mouse

<table>
<thead>
<tr>
<th>Mouse strain</th>
<th>Lean control</th>
<th>db/db</th>
<th>Lean control</th>
<th>db/db</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (weeks)</td>
<td>5</td>
<td>5</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Group size (n)</td>
<td>18–20</td>
<td>12–13</td>
<td>18–20</td>
<td>12–13</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>19.63 ± 0.28</td>
<td>24.14 ± 0.6*</td>
<td>25.18 ± 0.56</td>
<td>35.97 ± 1.2*</td>
</tr>
<tr>
<td>Blood glucose (mg/dL)</td>
<td>145.25 ± 4.7</td>
<td>230.38 ± 13.25*</td>
<td>140.32 ± 8.81</td>
<td>583.20 ± 35.86*</td>
</tr>
<tr>
<td>Plasma insulin (ng/ml)</td>
<td>ND</td>
<td>ND</td>
<td>1.27 ± 0.21</td>
<td>4.61 ± 1.52*</td>
</tr>
<tr>
<td>Food intake (g/day/mouse)</td>
<td>4.17 ± 0.13</td>
<td>7.43 ± 0.43*</td>
<td>3.85 ± 0.11</td>
<td>6.74 ± 0.37*</td>
</tr>
<tr>
<td>Water intake (g/day/mouse)</td>
<td>7.05 ± 0.13</td>
<td>15.66 ± 1.69*</td>
<td>9.76 ± 0.89</td>
<td>20.15 ± 2.39*</td>
</tr>
<tr>
<td>% Fat mass</td>
<td>ND</td>
<td>ND</td>
<td>17.94 ± 1.01</td>
<td>58.92 ± 0.75*</td>
</tr>
<tr>
<td>% Lean mass</td>
<td>ND</td>
<td>ND</td>
<td>70.43 ± 1.01</td>
<td>42.78 ± 1.40*</td>
</tr>
<tr>
<td>% Body water</td>
<td>ND</td>
<td>ND</td>
<td>59.62 ± 1.00</td>
<td>35.85 ± 1.16*</td>
</tr>
</tbody>
</table>

ND, not determined. *p<0.05 versus age-matched lean controls, unpaired t-test.
Abnormal behavior in db/db mouse

a) Elevated Plus Maze
b) Y-maze
c) Porsolt forced swim
Motor skills

- Rotorod
- Motor ability and also motor/procedural learning across trials (cerebellum)
• **Gait**
  Measured by specialized equipment or with (non-toxic) ink and paper

• **Grip strength**
  Measured by specialized equipment or by a rating scale
Motor skills

- Inverted screen
Motor skills

- Horizontal beam
- Wire hang

Score according to performance
- grip, posture correction
Learning and memory

• There are a lot of different tasks
• There are a lot of versions of each task
• There are a lot of bad behavioral experiments in the literature
• Explicit/declarative v Implicit/procedural
Y-maze - alternation

Working memory
Mice prefer novelty

To explore a new area, you must remember where you have already been
T-maze

- Hippocampal-dependent, spatial learning
- Rule: Non-match to sample
- Forced-choice trial followed by rewarded free-choice trial
- Advantage inclusion of time delays
Morris water maze

• First establish that your animal is physically capable of performing the task
  Rats are naturally better swimmers than mice

• **Visible/Cued platform**
  Above surface
  Flag/marker

• **Then Hidden Platform**
  Use extra-maze cues to locate the platform
Data interpretation

A

B

C

Pct. time in quadrant

Target Left Right Opp

Target Left Right Opp

Platform crossings

A B C

Search Error cm

A B C
• Water maze is more stressful to mice - elicits greater levels of serum corticosterone.

• High corticosterone correlates with poorer performance in MWM but not BM.

Fig. 1. Cognitive testing elicits corticosterone. Corticosterone levels were significantly greater in the Barnes-maze group than in Naïve mice, and significantly greater in the Water maze group than in either Barnes-maze or Naïve groups. Group means are represented by horizontal bars. *** p < .001 significantly different from Naïve mice; +++ p < .001 significantly different from both Naïve and Barnes-maze groups.

Fear conditioning

• Learn and remember an association between an aversive experience and an environmental cue
• Memory of shock inferred from level of freezing on subsequent trials when exposed to the same arena

8 weeks treatment with leptin improved performance in APP/PSEN1 mice
Fear conditioning
Dietary interventions

• Caloric restriction
  – Improves cognition
  – Increases longevity
  – Increases arousal and activity
  – Physiological changes

• High fat diet
  – Impairs cognition

• Dietary change
  – Change in consumption
Pharmacological interventions

- Must be able to cross blood brain barrier
- Routes of administration
  - subcutaneous (s.c.) and intraperitoneal (i.p.)
    - most common
- Dose required
- Therapeutic response window
- Side effects
Pharmacological interventions

- Leptin
- Ghrelin
- Neuropeptide Y

- Effects on activity
- Effects on consumption
Other considerations

- Noise
- Smells
- Experimenter
- Room size
- Room cleanliness
- Single housing
- Time of day
- Time of year

“Hey Ted, I would rather work with Tiffany”
Labs I & II - Aims

- Gain confidence with handling of animals and restraint techniques
- Learn injection techniques
- See examples of behavioral tests
- See responses to pharmacological treatments
Lab I: Working with mice

- Location: Murine neurobehavioral lab
- 1:30-4:30 pm

- All groups: Mouse handling
- Group 1: Neuromuscular testing
- Group 2: Elevated plus maze
- Group 3: Locomotor activity
- If time allows - a tour of the rest of the behavior facility
Lab II: Working with rats

- Location: Murine neurobehavioral lab
- 1:30-5 pm
- Analisa Thompson-Gray & team
- Rat handling & injections (s.c., i.p.)
- Irwin screen with oxotremorin
- Rat rotorod
Recommended reading