Food intake and its experimental assessment in mice

KATE ELLACOTT
VANDERBILT MMPC
& DEPARTMENT OF MOLECULAR PHYSIOLOGY AND BIOPHYSICS

Outline

● Introduction to patterns of food intake in the rodent
● Factors which affect food intake
● Food intake measures
● Diet-induced obesity
● Summary

Glossary

● Hyperphagia – Abnormally elevated food intake
● Hypophagia – Abnormally reduced food intake
● Anorectic, Anorexic, Anorexigenic – A stimulus that reduces food intake and or appetite
● Orectic, Orexic, Orexigenic - A stimulus that increases food intake and or appetite
● Cachexia – Loss of weight, particularly lean mass, and appetite due to chronic infection or disease
● Ad libitum – Latin meaning in accordance with desire
Patterns of food intake in rodents

Data obtained from animals in Mini-mitter® wheel running apparatus

Food Intake Measures

- Habituation
- Introduction to feeding assays
- Basic feeding assays
- Advanced feeding assays
  - Anorexia vs. aversion
  - Restriction paradigms
  - Others
Habituation

- Daily handling mimicking the experimental procedure
  - Individual housing
  - Handling
  - Daily sterile saline injection/restraint
  - Keep same operator
- Length of habituation?
  - 4 days or more depending on mouse strain and/or procedure
- Monitor food intake as marker of stress

Importance of habituation

Food Intake Assays

- Types of measures
  - Group vs. individual housing
  - Manual vs. automated
- Meal patterns: size of meal vs. # of meals
- Accuracy and potential errors
- Normalization?
  - Body weight
  - Feed efficiency (Kcal/△BW (g))
Nocturnal Food Intake

- **Procedure** -
  - Animals are treated immediately prior to lights out and food intake measured at various intervals post-injection

- **What it tests** -
  - The ability of a treatment to modify normal nocturnal food intake

- **Advantages** -
  - Physiological, minimal disruption to the natural behavior of the animal

- **Disadvantages** -
  - Anti-social hours for the operator
  - Difficult to weight food in the dark or under-red light illumination

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Nocturnal food intake: Example

A single dose of AgRP (i.c.v) affects food intake for 7 days post-injection.


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Fast-induced food intake

- **Procedure** -
  - Animals are fasted overnight and then given access to food immediately after treatment and intake measured at various intervals

- **What it tests** -
  - The ability of a treatment to alter food intake in the face of a strong drive to eat

- **Advantages** -
  - Easy to do and is good for treatments that give a robust response. Good for a first test but should be followed up with a nocturnal feeding assay

- **Disadvantages** -
  - Fasting creates a strong drive to eat and may mask effects that are subtle
  - The Animals are out of homeostasis
Fast-induced food intake: Example 1

- The time-course of the effect on food intake may reflect the mechanism of action of the compound e.g. short-duration of action = satiety factor
- Examine cumulative data as well as changes between time points


MC4R deficiency is associated with defects in satiety signaling

- The time-course of the effect on food intake may reflect the mechanism of action of the compound e.g. short-duration of action = satiety factor
- Examine cumulative data as well as changes between time points


Fast-induced food intake: Example 2

- Beware of treatments that completely wipe-out food intake
- Watch the animals do they look normal?
- Perform dose response curve
- Check whether animals have aversive response

3h 12h
**Day-time food intake**

- **Procedure** - Animals are treated soon after lights-on after normal nocturnal feeding and intake measured at defined intervals.
- **What it tests** - The ability of a treatment to stimulate food intake in the satiated animal.
- **Advantages** - Easy.
- **Disadvantages** - Requires a strong stimulus to see an effect. Only use secondarily to nocturnal or fast-induced feeding assays.

**Day-time food intake: Example**

- NPY: strong orexigen
- Has immediate effect but does not persist

Morley et al. 1987 Am J Physiol Regul Integr Comp Physiol 253:R316-322

**Altering normal homeostasis: Fast-induced re-feeding**

- **Procedure** - Animals are fasted during a period of high baseline consumption (e.g. overnight) and then given access to food and intake measured at various intervals and/or until body weight returns to pre-fasted values.
- **What it tests** - Is there a defect in homeostatic control of food intake, rewarding properties of food or in other mechanisms? e.g. altered satiety
- **Potential outcomes** - Normal re-feeding, Rebound hyperphagia, Hypophagia
Altering normal homeostasis: Response to high-fat diet

- **Procedure** -
  - Animals are exposed to a high-fat diet and food intake and body weight gain monitored.

- **What it tests** -
  - Is there a defect in homeostatic control of food intake in response to increased caloric density

- **Potential outcomes** -
  - Hyperphagia
  - Rapid weight gain
  - Protection from diet-induced obesity

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MC4R−/− mice show high-fat hyperphagia

[Srisai et al 2011 – Endocrinology 152(3):890-902]

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Advanced Food Intake Assays

- **Anorexia vs. Aversion**
  - Conditioned Taste Aversion (CTA)
  - Pica (kaolin/clay consumption)

- **Food restriction paradigms**
  - Pair-feeding
  - Meal-entrainment
Anorexia vs. Aversion: Signs of malaise in mice

- Immobility
- Altered gait
- Hunched posture
- Spikey coat/stops grooming
- Altered urination and/or defecation
- Porphyrin staining around eyes and nose
- Vocalization (rare)

Images from AALAS learning library – www.aalaslearninglibrary.org

Anorexia vs. Aversion: Conditioned Taste Aversion (CTA)

- Procedure –
  - Animals are conditioned to receive one novel palatable flavor after administration of a drug. A second distinct flavor is introduced separately after vehicle administration. On test day both flavors are offered to the mice and then animals can choose between them. If the drug is associated with any negative sensations such as nausea then the animal avoids the flavor that was given at the same time as the drug.

- What it tests –
  - Whether a treatment reduces food intake by inducing negative sensations in the animal such as nausea

A sample conditioned taste aversion paradigm

<table>
<thead>
<tr>
<th>Mobility phase</th>
<th>Training</th>
<th>Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day</td>
<td>1-5</td>
<td>6</td>
</tr>
<tr>
<td>6</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>9</td>
<td>10</td>
<td>11</td>
</tr>
<tr>
<td>12</td>
<td>13</td>
<td>14</td>
</tr>
<tr>
<td>Bottle 1 contents</td>
<td>Vehicle or drug</td>
<td>Vehicle or drug</td>
</tr>
<tr>
<td>Bottle 2 contents</td>
<td>Vehicle or drug</td>
<td>Vehicle or drug</td>
</tr>
<tr>
<td>Treatment</td>
<td>Vehicle</td>
<td>Vehicle</td>
</tr>
<tr>
<td>Vehicle</td>
<td>Vehicle</td>
<td>Vehicle</td>
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</tr>
<tr>
<td>Vehicle</td>
<td>Vehicle</td>
<td>Vehicle</td>
</tr>
</tbody>
</table>

Vehicle water 1

Vehicle water 2

Vehicle or drug 1

Vehicle or drug 2

None
Conditioned Taste Aversion (cont.)

- **Advantages** –
  - While time consuming it does not require any special equipment

- **Disadvantages** –
  - Interpretation can be complicated as nausea and satiety may be mediated by the same pathways.
  - The presence of aversion does not necessarily mean that the effects of a compound are non-specific.

Nausea = Extreme satiety?

- Hunger
- Satiety
- Malaise

- Food Intake

Anorexia vs. Aversion: Pica Consumption

- **Procedure** –
  - Animals are given a choice of kaolin pellets or standard chow during a 5 day habituation phase and their consumption of both noted. On the test day the animals are fasted overnight and then treated the following day and given access to both kaolin pellets and regular chow. Repeated studies may be performed to look for chronic effects.

- **What it tests** –
  - Kaolin consumption is believed to be a proxy for nausea in rodents. Often used to test chemotherapeutics.
Pica Consumption (cont.)

- **Advantages** –
  - Simple to perform

- **Disadvantages** –
  - Need to purchase or make Kaolin pellets
  - Like CTA interpretation can be complicated

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An example of a pica test paradigm

<table>
<thead>
<tr>
<th>Day</th>
<th>Habituation</th>
<th>Acute Test</th>
<th>Chronic Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-5</td>
<td>Chow</td>
<td>Kaolin</td>
<td>Chow</td>
</tr>
<tr>
<td>6</td>
<td>Chow</td>
<td>Kaolin</td>
<td>Chow</td>
</tr>
<tr>
<td>7</td>
<td>Kaolin</td>
<td>Chow</td>
<td>Kaolin</td>
</tr>
<tr>
<td>8</td>
<td>Kaolin</td>
<td>Chow</td>
<td>Kaolin</td>
</tr>
<tr>
<td>9</td>
<td>Vehicle</td>
<td>Vehicle</td>
<td>Vehicle</td>
</tr>
</tbody>
</table>

- **Hopper 1 contents**
  - Chow
  - Vehicle or drug

- **Hopper 2 contents**
  - Kaolin
  - Vehicle or drug

- **Treatment**
  - Vehicle

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Food restriction paradigms: Pair-feeding

- **Procedure** –
  - Treated animals have their food intake reduced to the same level as control animals resulting in three experimental groups – 1) Control *ad lib*; 2) Treated *ad lib*; 3) Treated *pair fed*
  - If the treatment reduces food intake then a second control group is pair-fed to the treated group resulting in the following experimental groups - 1) Control *ad lib*; 2) Treated *ad lib*; 3) Control *pair fed*

- **What it tests** –
  - The effects of treatment independent of changes in food intake
Sample pair-feeding paradigms

A. Pair-feeding to examine the effect of treatment on body composition independent of increased food intake induced by treatment

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control a (g)</td>
<td>C (g)</td>
<td>E (g)</td>
<td></td>
</tr>
<tr>
<td>Treated b (g)</td>
<td>D (g)</td>
<td>F (g)</td>
<td></td>
</tr>
<tr>
<td>Treated pair-fed to control a (g)</td>
<td>N.D.</td>
<td>A (g)</td>
<td>C (g)</td>
</tr>
</tbody>
</table>

N.D. = Not determined

B. Pair-feeding to examine the effect of treatment on body composition independent of decreased food intake induced by treatment

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control a (g)</td>
<td>C (g)</td>
<td>E (g)</td>
<td></td>
</tr>
<tr>
<td>Treated b (g)</td>
<td>D (g)</td>
<td>F (g)</td>
<td></td>
</tr>
<tr>
<td>Control pair-fed to treated a (g)</td>
<td>N.D.</td>
<td>B (g)</td>
<td></td>
</tr>
</tbody>
</table>

N.D. = Not determined

Pair-feeding example 1

Ste Marie et al 2000 – PNAS 97(22):12339-12344
Pair-feeding example 2

Smart et al 2006 – JCI 116 (2): 495-505

Pair-feeding (cont.)

- **Advantages** –
  - Simple and does not require special equipment. Can be used as a proxy for alterations in metabolic rate, however, will not replace calorimetry studies

- **Disadvantages** –
  - Time consuming
  - Stressful for food restricted animals
  - Pair-fed groups need to be staggered

Food restriction paradigms: Meal-entrainment

- **Procedure** –
  - Animals are trained to receive their meals at a set time of the day usually during the light-cycle. Animals are usually allowed to eat freely during that time point and will often consume a day's worth of food in a short time period. Requires habituation.

- **What it tests** –
  - Enables the investigator to look at meal-anticipation and satiation as the meal-time is more regimented.
  - Also used for circadian studies
Meal-entrainment example 1


Meal-entrainment example 2

Johnstone et al 2006 – Cell Metabolism 4: 313-321

Meal-entrainment

- **Advantages**
  - Animals’ meal-times are set
  - Can switch the animals to eating during the light cycle

- **Disadvantages**
  - Stressful for the animals
  - Changes circadian rhythm
  - Alters normal physiology
Other Advanced Food Intake Assays


Hyperphagic obese mouse models

- Ay mouse - Picture from www.jax.org
- Ob/Ob mouse - Picture from www.jax.org
- MC4R-null mouse

Diet-induced obesity

- Importance of diet choice
  - Correct control diet
  - Cafeteria diets
  - Calorific liquids

- Frequency and time of measurement

- Limited numbers of DIO mice available from commercial vendors
### Commercially available diet comparison

<table>
<thead>
<tr>
<th></th>
<th>Std Chow</th>
<th>Purina 5001</th>
<th>Control</th>
<th>D12450B</th>
<th>45% Fat</th>
<th>D12451</th>
<th>60% Fat</th>
<th>D12494</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein (% kcal)</td>
<td>29</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carbohydrate (% kcal)</td>
<td>53</td>
<td>70</td>
<td>35</td>
<td>20</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fat (% kcal)</td>
<td>13</td>
<td>10</td>
<td>45</td>
<td>60</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>kcal/g</td>
<td>3.36</td>
<td>3.85</td>
<td>4.73</td>
<td>5.24</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type CHO</td>
<td>Fiber</td>
<td>Sucrose&gt; corn starch&gt; maltodextrin</td>
<td>Sucrose&gt; maltodextrin&gt; corn starch</td>
<td>Maltodextrin&gt; sucrose</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type fat</td>
<td>?</td>
<td>Soybean oil&gt;lard</td>
<td>Lard&gt;soybean oil</td>
<td>Lard&gt;soybean oil</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Diet-induced obesity

- Importance of diet choice
  - Correct control diet
  - Cafeteria diets
  - Calorific liquids

- Frequency and time of measurement

- Limited numbers of DIO mice available from commercial vendors
Useful Resources

- AALAS learning library
  - www.aalaslearninglibrary.org

- Research Diets and Test diet
  - www.researchdiets.com
  - www.testdiet.com

- Mouse phenome database - For baseline information
  - http://phenome.jax.org/pub-cgi/phenome/mpdcgi?rtn=docs/home

Sample decision chart for assessment of the effect of a drug/compound on energy intake
A sample decision chart for mouse phenotyping