

# Monitoring For Humane Endpoints: Developing An Appropriate Strategy



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# **MONITORING FOR HUMANE ENDPOINTS: DEVELOPING AN APPROPRIATE STRATEGY**

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# OBJECTIVES

- Definition of endpoints
- Assessment of pain and chronic distress
- Development of humane endpoints
- Case studies



# DEFINITION OF ENDPPOINTS

# STUDY ENDPOINTS

Established at the beginning of the study.

Desired experimental outcomes and expected times of data collection.

# STUDY ENDPOINTS: TOXICITY TESTING

Day 0

Give test compound

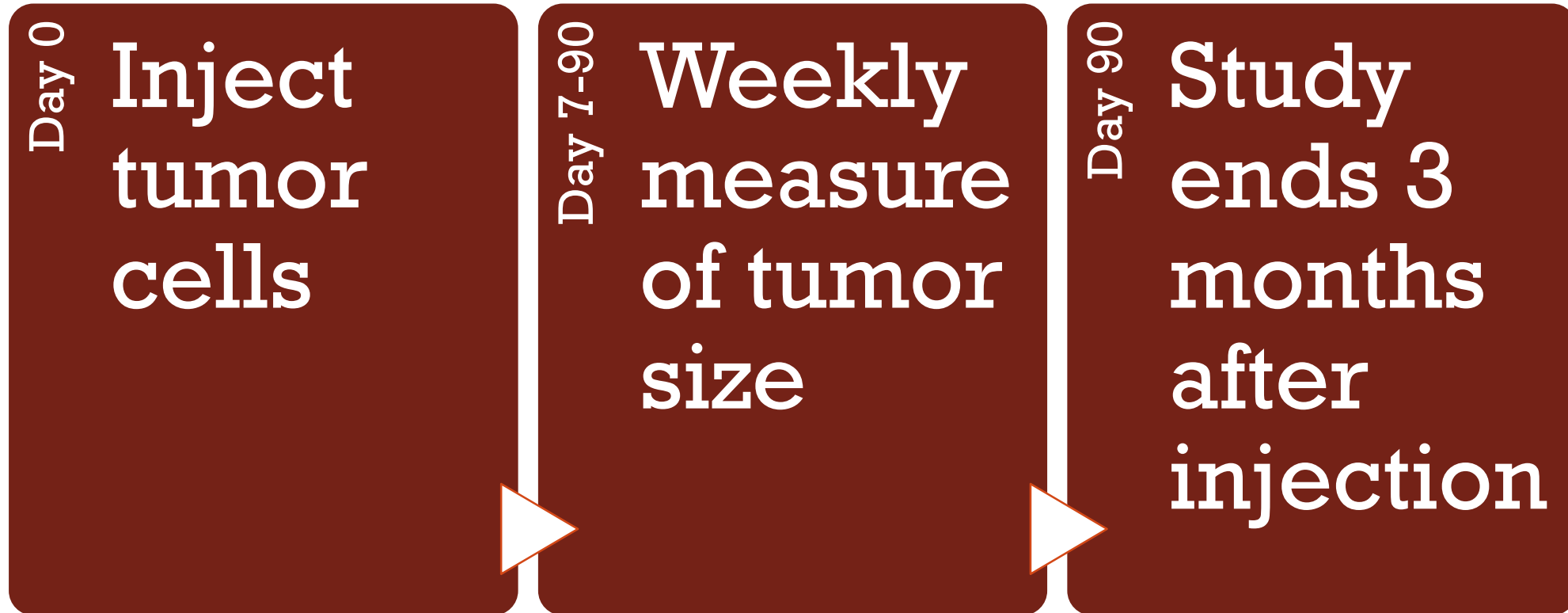
Day 7-90

Weekly measure of BUN & Creatinine

Day 90

Euthanize for tissue collection

# STUDY ENDPOINTS: TUMOR STUDY



# STUDY ENDPOINTS: BEHAVIORAL TESTING

Day 0

Start training\*

\*includes fasting

Day 1-15

Continue daily behavioral assessment

Day 16

Study ends



# STUDY ENDPOINTS: MULTIPLE SCLEROSIS

Day 0

Induce MS  
(experimental  
autoimmune  
encephalopathy,  
aka EAE)

Day 1-30

Daily treatment  
with proposed  
therapeutic  
agent

Day 30

Animals  
euthanized for  
tissue collection  
and histology

# HUMANE ENDPOINTS

The criteria that are used to determine when to terminate the study for an individual animal (or cohort of animals) **before** the defined experimental endpoint for humane reasons.

# **HUMANE ENDPOINTS, CONTINUED**

**Does not always mean euthanasia – can mean terminating a painful procedure and/or giving treatment to alleviate pain and/or distress.**

# HUMANE ENDPOINTS: 3RS



R.L. Burch and W.M.S. Russell

## Refinement

- Minimize pain and/or distress

## Replacement

- Non-animal models
- “Less sentient” animal models

## Reduction

- Appropriate animal number use

# HUMANE ENDPOINTS: FIVE FREEDOMS

- Freedom from hunger or thirst
- Freedom from discomfort
- Freedom from pain, injury or disease
- Freedom to express (most) normal behavior
- Freedom from fear and distress

# GENERIC HUMANE ENDPOINTS

- Weight loss
- Inability to ambulate
- Labored respiration
- Dehydration
- Hunched posture
- Poor coat (piloerection)
- Wounds or hair loss
- Ocular or respiratory discharge
- Inability to access food or water





# WHAT TOOLS DO WE HAVE TO BE MORE OBJECTIVE?



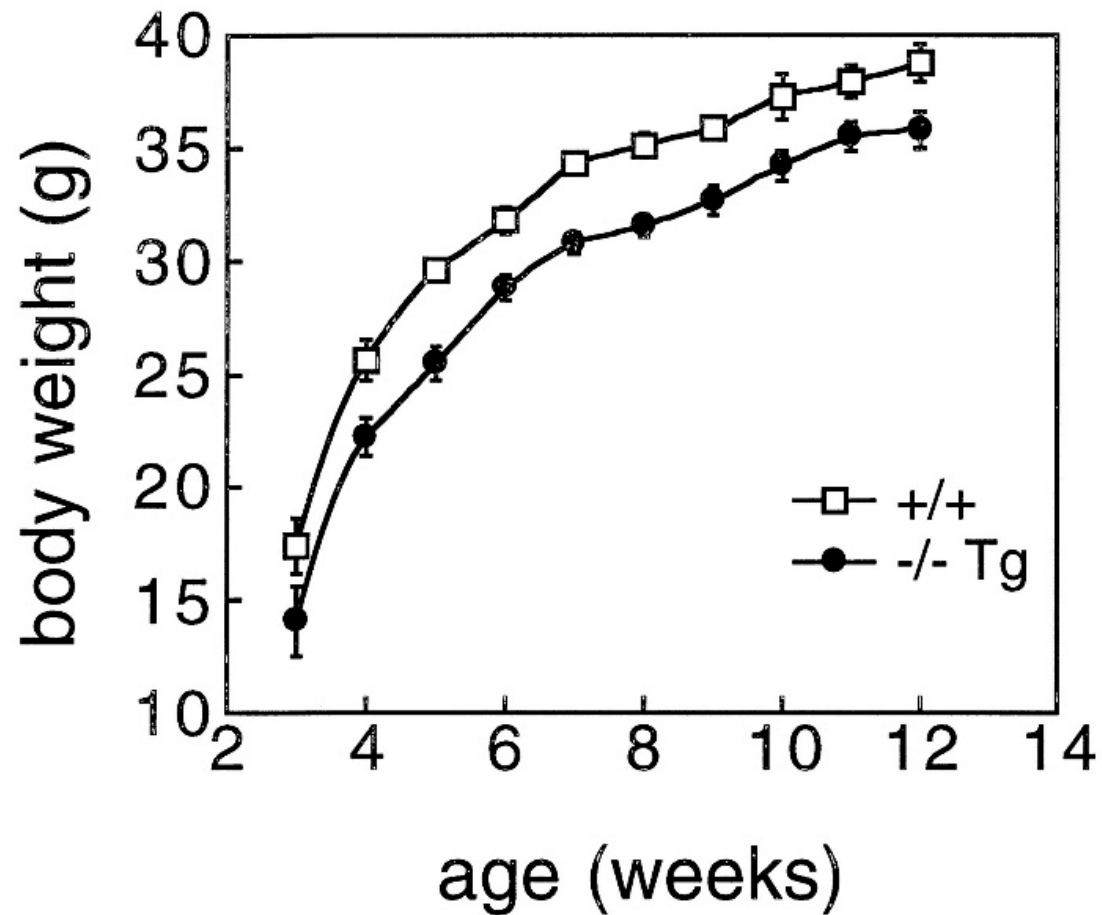
# HOW DO WE MEASURE WELL-BEING?





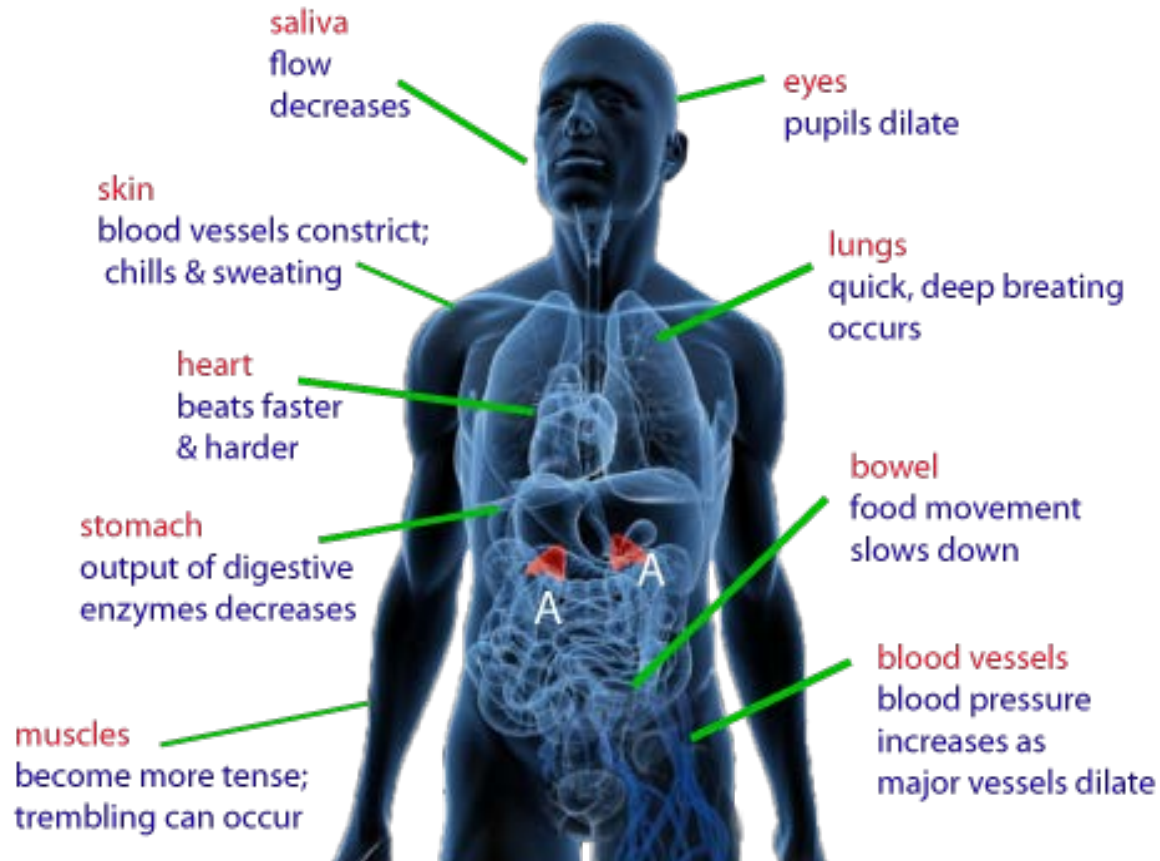
**DAVID FRASER**

# BASIC HEALTH & FUNCTIONING: GROWTH



# BASIC HEALTH & FUNCTIONING: PHYSIOLOGY

## Fight or Flight Response



# CLINICAL EXAM

- Temperature, pulse, respiratory rate (TPR)
  - Increase or decrease
  - Expected changes dependent upon model
- Body weight
- Bloodwork

# BODY TEMPERATURE



Infrared thermometer

Telemetry transmitter



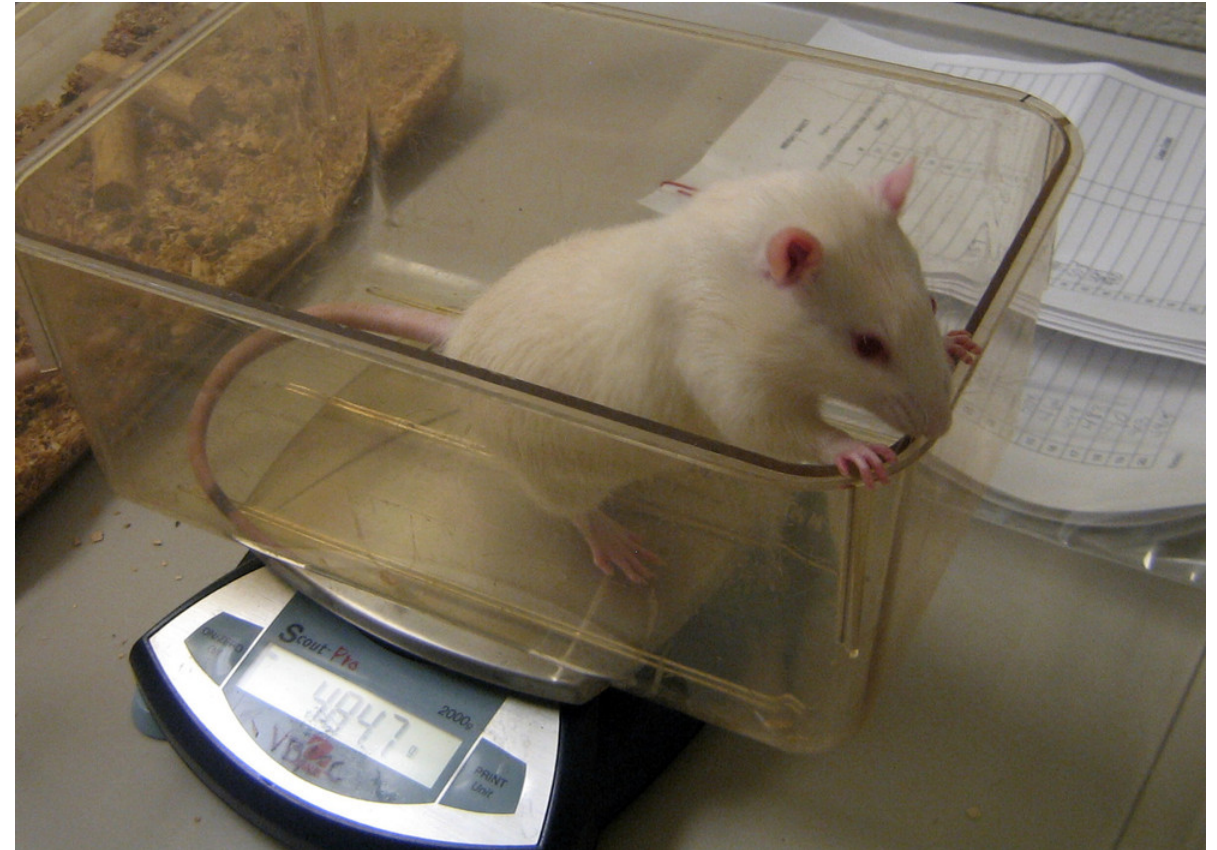
Infrared thermometer



Rectal thermometer

# BODY WEIGHT

- Labor intensive
- Requires specialized equipment
- Assessment of change
  - Age dependent
  - Tumor growth can mask cachexia



# BODY CONDITION SCORE: MICE

- Does not require baseline
- Does not require specialized equipment
- Age independent
- Appropriate for many tumor studies
- Available for multiple species



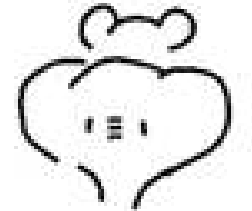
**BC 1**

- Mouse is emaciated.
- *Skeletal structure extremely prominent; little or no flesh cover.*
- *Vertebrae distinctly segmented.*



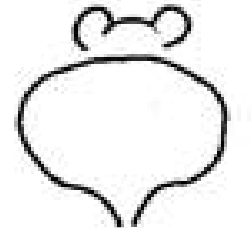
**BC 2**

- Mouse is underconditioned.
- *Segmentation of vertebral column evident.*
- *Dorsal pelvic bones are readily palpable.*



**BC 3**

- Mouse is well-conditioned.
- *Vertebrae and dorsal pelvis not prominent, palpable with slight pressure.*



**BC 4**

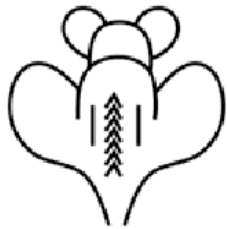
- Mouse is overconditioned.
- *Spine is a continuous column.*
- *Vertebrae palpable only with firm pressure.*



**BC 5**

- Mouse is obese.
- *Mouse is smooth and bulky.*
- *Bone structure disappears under flesh and subcutaneous fat.*

# BODY CONDITION SCORE: RAT



## BC 1 Rat is emaciated

- Segmentation of vertebral column prominent if not visible.
- Little or no flesh cover over dorsal pelvis. Pins prominent if not visible.
- Segmentation of caudal vertebrae prominent.



## BC 2 Rat is under conditioned

- Segmentation of vertebral column prominent.
- Thin flesh cover over dorsal pelvis, little subcutaneous fat. Pins easily palpable.
- Thin flesh cover over caudal vertebrae, segmentation palpable with slight pressure.



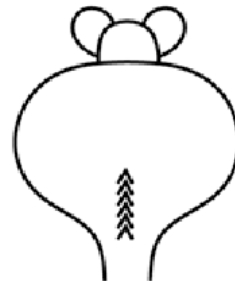
## BC 3 Rat is well-conditioned

- Segmentation of vertebral column easily palpable.
- Moderate subcutaneous fat store over pelvis. Pins easily palpable with slight pressure.
- Moderate fat store around tail base, caudal vertebrae may be palpable but not segmented.



## BC 4 Rat is overconditioned

- Segmentation of vertebral column palpable with slight pressure.
- Thick subcutaneous fat store over dorsal pelvis. Pins of pelvis palpable with firm pressure.
- Thick fat store over tail base, caudal vertebrae not palpable.



## BC 5 Rat is obese











- Segmentation of vertebral column palpable with firm pressure; may be a continuous column.
- Thick subcutaneous fat store over dorsal pelvis. Pins of pelvis not palpable with firm pressure.
- Thick fat store over tail base, caudal vertebrae not palpable.



# BODY CONDITION SCORE: RABBIT

Size-O-Meter Score:

Characteristics:

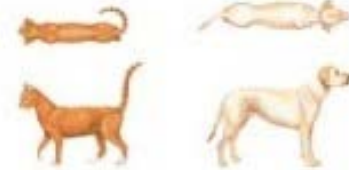
1	Very Thin			<ul style="list-style-type: none"><li>• Hip bones, ribs and spine are very sharp to the touch</li><li>• Loss of muscle and no Fat cover</li><li>• The rump area curves in</li></ul>
2	Thin			<ul style="list-style-type: none"><li>• Hip bones, ribs and spine are easily Felt</li><li>• Loss of muscle and very little Fat cover</li><li>• Rump area is Flat</li></ul>
3	Ideal			<ul style="list-style-type: none"><li>• Hip bones, ribs and spine easily Felt but are rounded, not sharp - Ribs Feel like a pocket Full of pens!</li><li>• No abdominal bulge</li><li>• Rump area is Flat</li></ul>
4	Overweight			<ul style="list-style-type: none"><li>• Pressure is needed to Feel the ribs, spine and hip bones</li><li>• Some Fat layers</li><li>• The rump is rounded</li></ul>
5	Obese			<ul style="list-style-type: none"><li>• Very hard to Feel the spine and hip bones - Ribs can't be Felt!</li><li>• Tummy sags with obvious Fat padding</li><li>• Rump bulges out</li></ul>

# BODY CONDITION SCORE: COMPANION ANIMALS

1

20% below  
ideal body weight

Ribs easily felt with no fat cover



2

10% below  
ideal body weight

Bones raised with minimal tissue  
between the skin and bone



3

**Ideal  
body weight**

Ribs can be felt through slight fat cover



4

10% above  
ideal body weight

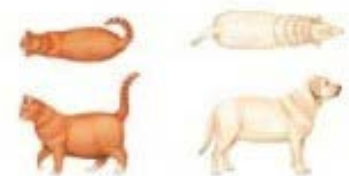
Difficult to feel ribs through moderate fat cover. A slightly  
sagging abdominal fat pad may be seen in cats















5

20% above  
ideal body weight

Ribs are difficult to feel under thick fat. Cats have a  
prominent sagging abdominal fat pad

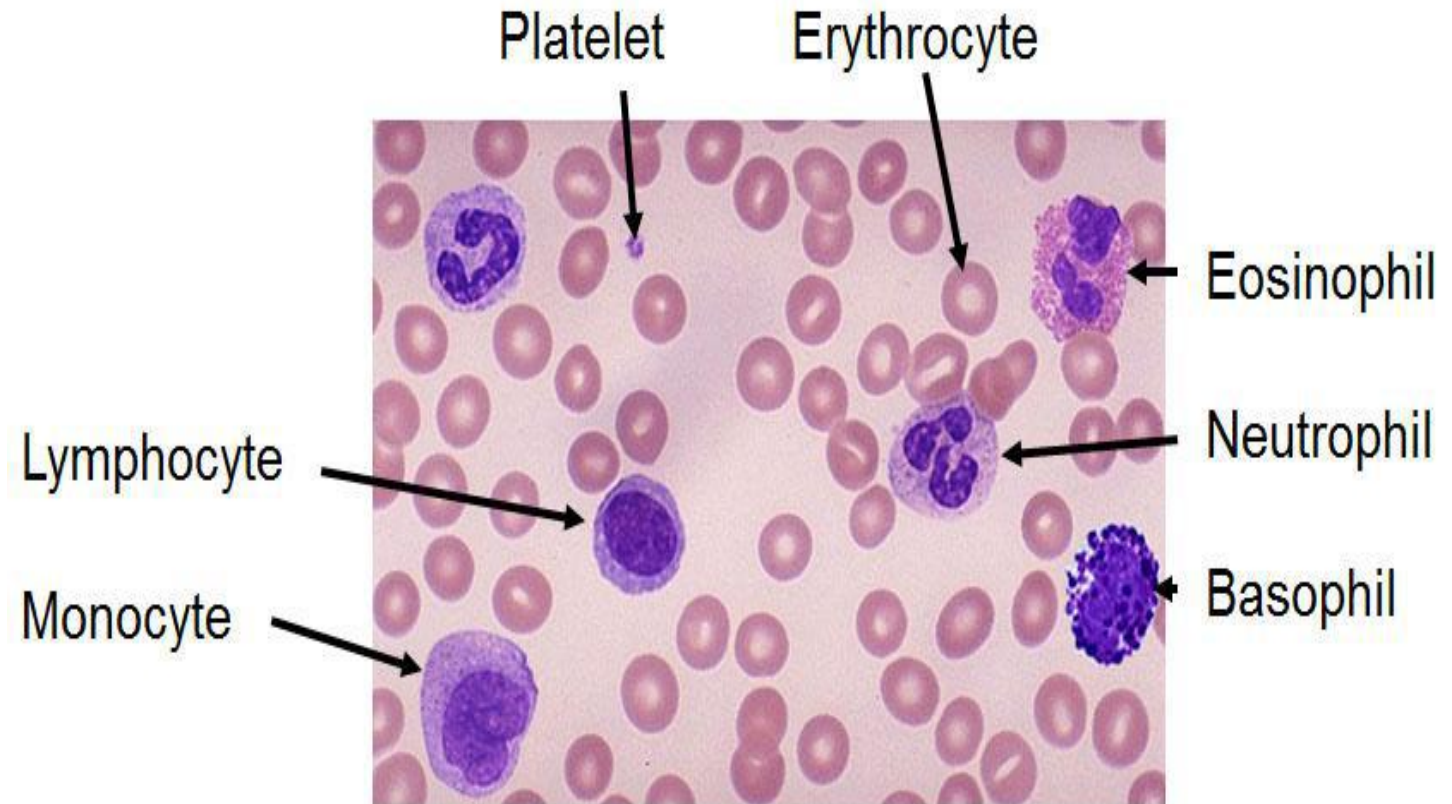


# BODY CONDITION SCORE: MACAQUE

		Ambulating	Right Lateral Viewed from Back
1	<b>EMACIATED</b> – Very prominent hip bones (easily palpable and likely visible), prominent facial bones, spinous processes and ribs. Minimal to no muscle mass is palpable over ileum or ischium. Anus may be recessed between ischial callosities. Body is very angular, no subcutaneous fat layer to smooth out prominences.		
1.5	<b>VERY THIN</b> – Hips, spinous processes, and ribs are prominent. Facial bones may be prominent. There is very little muscle present over the hips and back. Anus may be recessed between ischial callosities. Body is angular, no subcutaneous fat to smooth out prominences		
2	<b>THIN</b> – Very minimal fat reserves, prominent hip bones and spinous processes. Hips, spinous processes and ribs are easily palpable with only a small amount of muscle mass over hips and lumbar region.		
2.5	<b>LEAN</b> – Overlying muscle gives hips and spine a more firm feel. Hip bones and spinous processes are readily palpable, but not prominent. Body is less angular because there is a thin layer of subcutaneous fat.		
3	<b>OPTIMUM</b> – Hip bones, ribs and spinous processes are palpable with gentle pressure but generally not visible. Well developed muscle mass and subcutaneous fat layer gives spine and hips smooth but firm feel. No abdominal, axillary or inguinal fat pads.		
3.5	<b>SLIGHTLY OVERWEIGHT</b> – Hip bones and spinous processes palpable with firm pressure but are not visible. Bony prominences smooth. Rib contours are smooth and only palpable with firm pressure. Small abdominal fat pad may be present.		
4	<b>HEAVY</b> – Bony contours are smooth and less well defined. Hip bones, spinous processes and ribs may be difficult to palpate due to more abundant subcutaneous fat layer. May have fat deposits starting to accumulate in the axillary, inguinal or abdominal areas.		
4.5	<b>OBESE</b> – This animal will often have prominent fat pads in the inguinal, axillary or abdominal region. Abdomen will be pendulous when animal sitting or ambulating. Hip bones and spinous processes difficult to palpate. Bony contours smooth and poorly defined.		
5	<b>GROSSLY OBESE</b> – Obvious, large fat deposits in the abdominal, inguinal and axillary regions. Abdominal palpation is very difficult due to large amount of mesenteric fat. Pronounced fat deposits may alter posture/ambulation. Hip bones, rib contours and spinous processes only palpable with deep palpation.		

# BLOODWORK

- Total number of white blood cells
- Ratio of neutrophils to lymphocytes
  - Evidence of infection
  - Evidence of chronic stress
- Hematocrit (HCT/PCV)
- Hemoglobin





**DAVID FRASER**

# EXPLORATION OF NATURAL LIVING: STRATEGIES

## Natural History

- Burrow
- Build nests
- Forage
- Gnaw
- Social groups

## Enrichment Strategies

- Deep bedding
- Nesting materials
- Supplementary diets
- Chewing toys
- Social housing

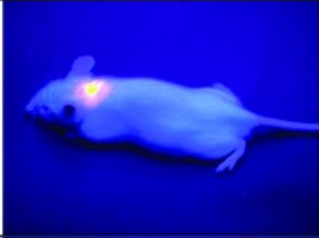
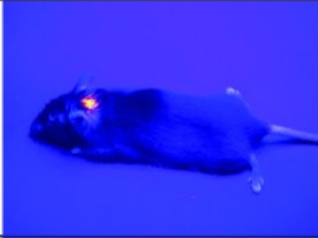








# NESTING MATERIAL INTEGRATION

- Can provide information about mouse behavior
- References
  - Rock et al 2014
  - Yuan et al 2018
  - Corder et al 2018
  - Oliver et al 2018
  - <https://www.jove.com/video/51012/nest-building-as-an-indicator-of-health-and-welfare-in-laboratory-mice>



# QUANTIFIABLE GROOMING

- Use of non-toxic fluorescent powder in mineral oil
- Measure and score time to groom

Score	Description	Example Image	
		CD1	C57BL6
1	A strong fluorescent signal is present at the application site on the forehead between the ears		
2	Fluorescence present at the application site as well as the front and/or rear nails		
3	Fluorescence present at the application site and the ears. Front and/or rear nails may also fluoresce		
4	Fluorescence is absent from the nails and ears but remains present in trace amounts at the application site		
5	Fluorescence is no longer detected		



# ZEBRAFISH BEHAVIOR

(C) **Acute predator (Leaf fish) exposure**

Low-stress control fish (divider present)      Predator (Leaf fish) home tank      Stressed 'exposed' fish (divider removed)

(D) **Social behaviors**

Shoaling      Automated behavior recognition

3-point tracking

Tail (T)      Center (C)      Nose (N)

**Social interaction:** 'approach' (N1 and N2 are very close, heading head-to-head towards each other)

Normal children

**Social investigation:** 'follow/chase' (N1 and T2 are very close, heading nose-to-tail in the same direction)

**Lack of social interest:** (N1 and N2 points are very far, two fish heading in different directions)

Autistic children

TRENDS in Neurosciences

# GRIMACE SCALE: MICE

- Assessment of pain
- Cageside “analgesia”
- Retrospective and requires specialized equipment



# GRIMACE SCALE: RAT, RABBIT

## Rabbit Pain Face

- Keating et al, 2012.

Not present "0"	Moderate "1"	Obvious "2"
	<b>Orbital Tightening</b>	
	<b>Nose/Cheek Flattening</b>	
	<b>Ear Changes</b>	
	<b>Whisker Change</b>	

Nose Shape		
Not Present (0)	Moderately Present (1)	Obviously Present (2)
The nares (nostril slits) are drawn vertically creating a more pointed nose that resembles a 'V' more than a 'U'. The tip of the nose may also be tucked under towards the chin exaggerating this appearance.		
Whisker Position		
Not Present (0)	Moderately Present (1)	Obviously Present (2)
Whiskers are straightened and extended horizontally or pulled back toward the cheeks instead of the normal position where whiskers tend to have a gentle downward curve.		
Ear Position		
Not Present (0)	Moderately Present (1)	Obviously Present (2)
Normally the ears are roughly perpendicular to the head, facing forward or to the side, held in an upright position away from the back and sides of the body with a more open and loosely curled shape. In pain the ears rotate away from normal position to face towards the hindquarters, tend to move backward and be held closer to the back or sides of the body and have a more tightly folded or curled shape (i.e. more like a tube).		

Orbital Tightening		
Not Present (0)	Moderately Present (1)	Obviously Present (2)
The eyelid is partially or completely closed. The globes themselves may also be drawn in toward the head so that they protrude less. If the eye closure reduces the visibility of the eye by more than half, it would be scored as '2' or 'obviously present'.		

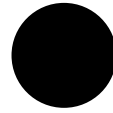
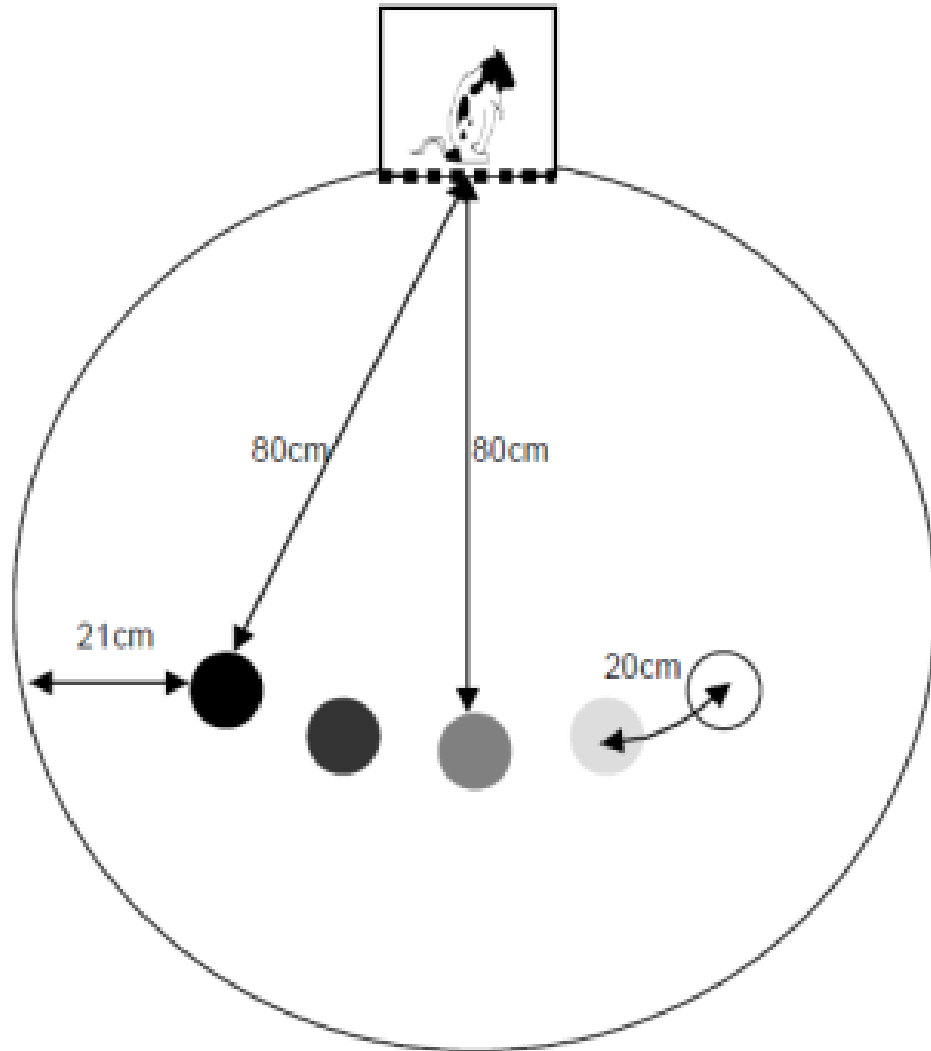
Cheek Flattening		
Not Present (0)	Moderately Present (1)	Obviously Present (2)
Contraction around the muzzle so that the whisker pads are pressed against the side of the face. The side contour of the face and nose is angular and the rounded appearance of the cheeks to either side of the nose is lost.		

<https://www.nc3rs.org.uk/grimacescales>

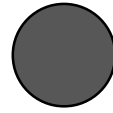


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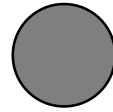
# AFFECTIVE STATE



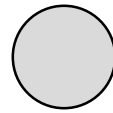
Unrewarded location



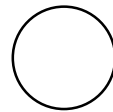
Probe nearest unrewarded location



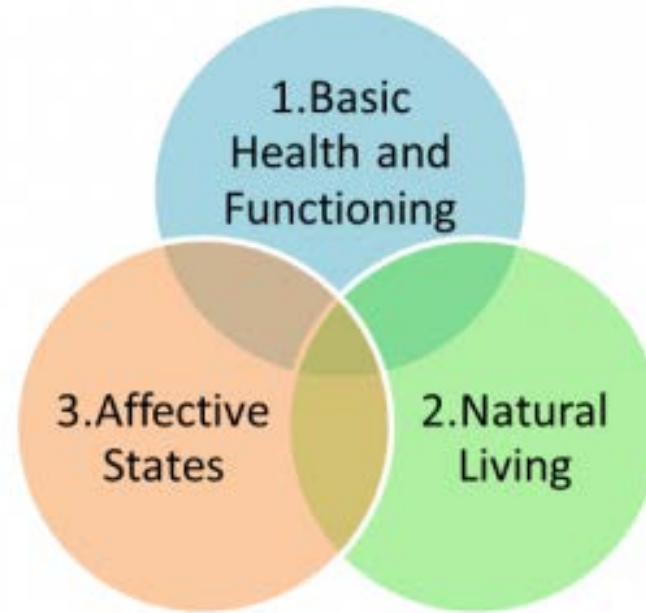
Probe halfway



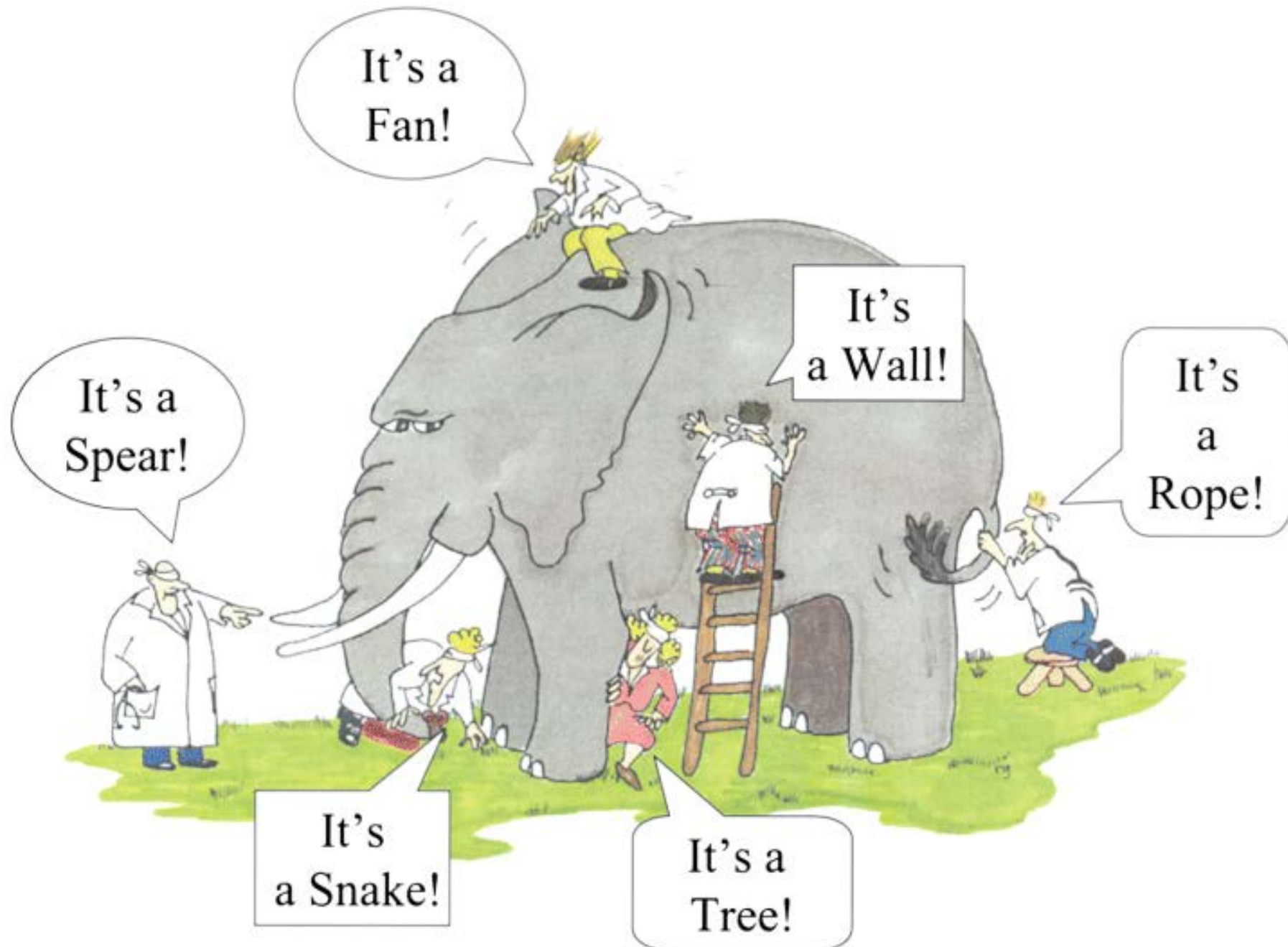
Probe nearest rewarded location



Rewarded location



**THOU SHALT NEVER PERFORM A  
WELL-BEING STUDY THAT EVALUATES  
ONLY ONE COMPONENT**



It's a Fan!

It's a Wall!

It's a Rope!

It's a Snake!

It's a Snake!

It's a Tree!

They can't see red, so they feel nice and secure

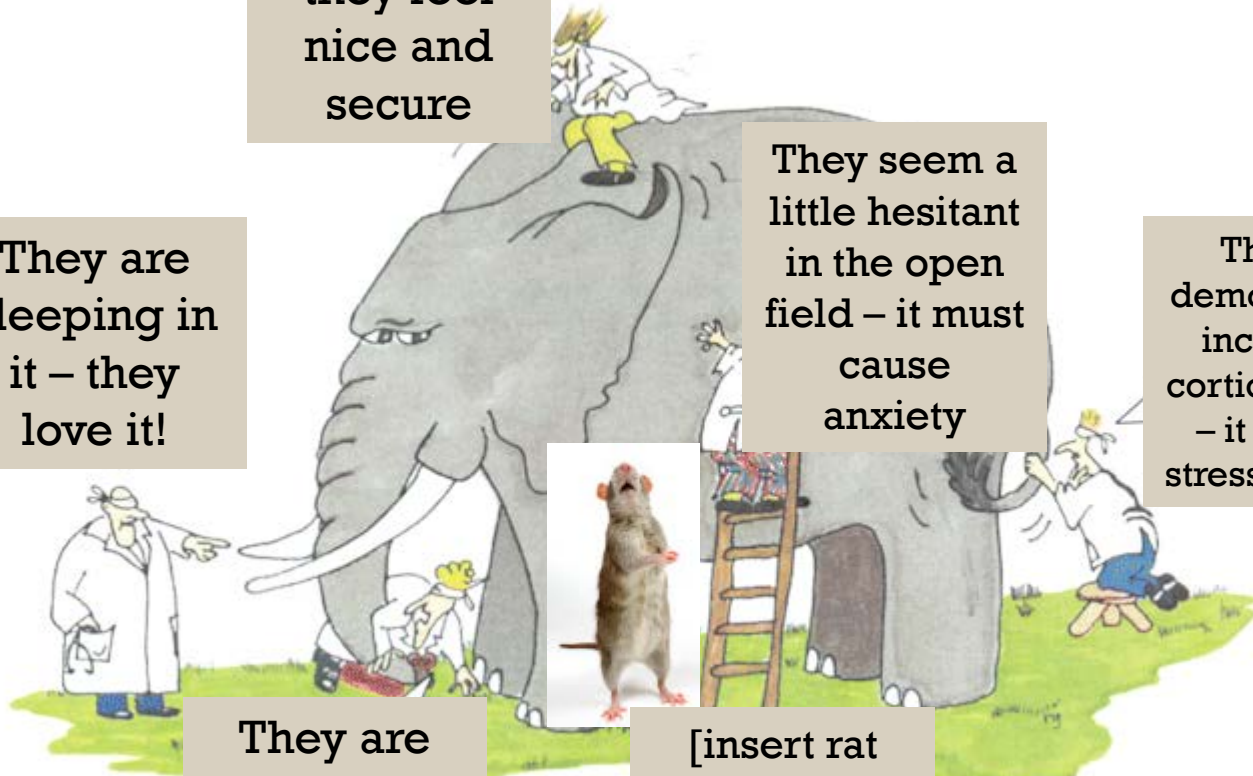
They are sleeping in it – they love it!

They seem a little hesitant in the open field – it must cause anxiety

They are demonstrating increase in corticosteroids – it must be stressing them.

They are fighting over it – bad idea!

[insert rat language to describe their opinion]





He is sleeping in his nest, he must be just fine.

I don't see anything that looks like the animal is in pain.

Are those eyes squinty or is the mouse just sleeping?

There is a shift in the NE:LY ratio – animal must be stressed out.

He is ignoring the nesting material provided – must not be good

[insert mouse language to describe their opinion]





# DEVELOPMENT OF HUMANE ENDPOINTS



# THEORY OF DEVELOPING HUMANE ENDPOINTS

- What is happening to the animal?
- What is the expected response?
- What kinds of complications can be predicted?
- What specific criteria will be used to determine that it is time to treat?
- What specific criteria will be used to determine that it is time to remove from study (including euthanasia)?

Very study dependent



# STUDY ENDPOINTS: TOXICITY TESTING

Day 0

Give test compound

Day 7-90

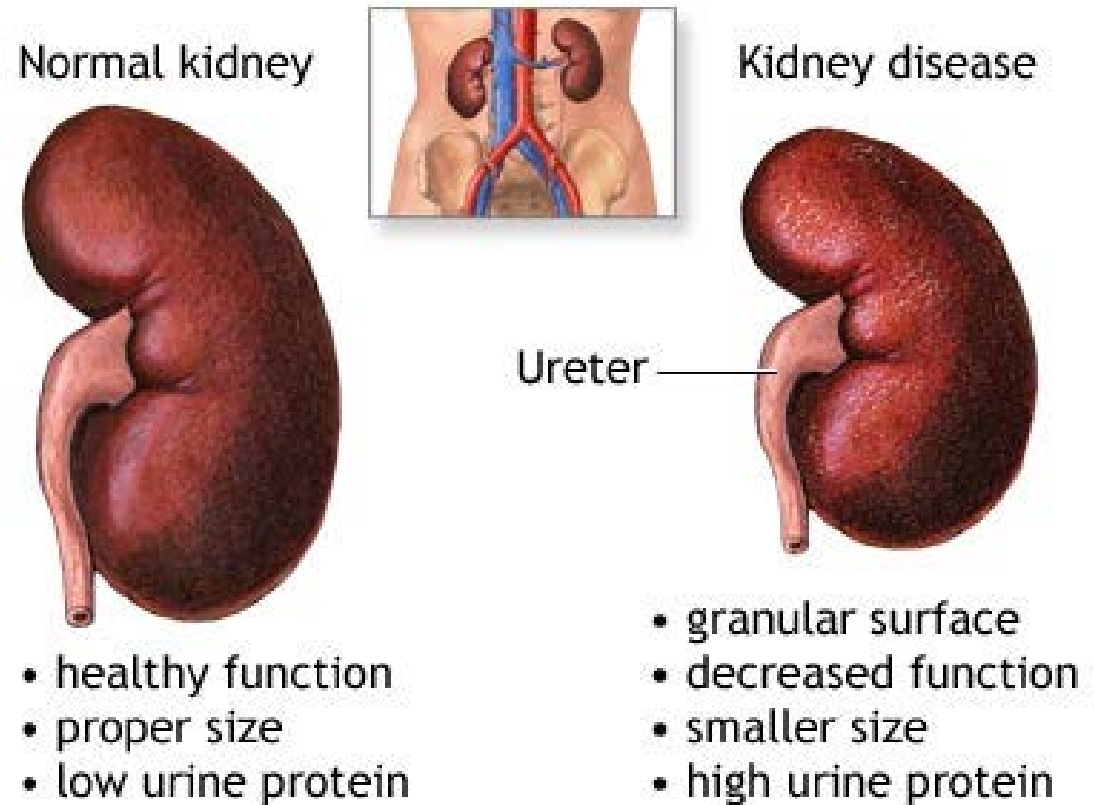
Weekly measure of BUN & Creatinine

Day 90

Euthanize for tissue collection

# TOXICITY TESTING: HUMANE ENDPOINTS

- Body weight/body condition score
- Hydration status
  - Skin tent
  - Blood work
- Renal function
  - Blood work
- Imaging
  - Ultrasound
- Behavior
  - Nest building
  - Grooming



# TOXICITY TESTING: OBJECTIVE ENDPOINTS

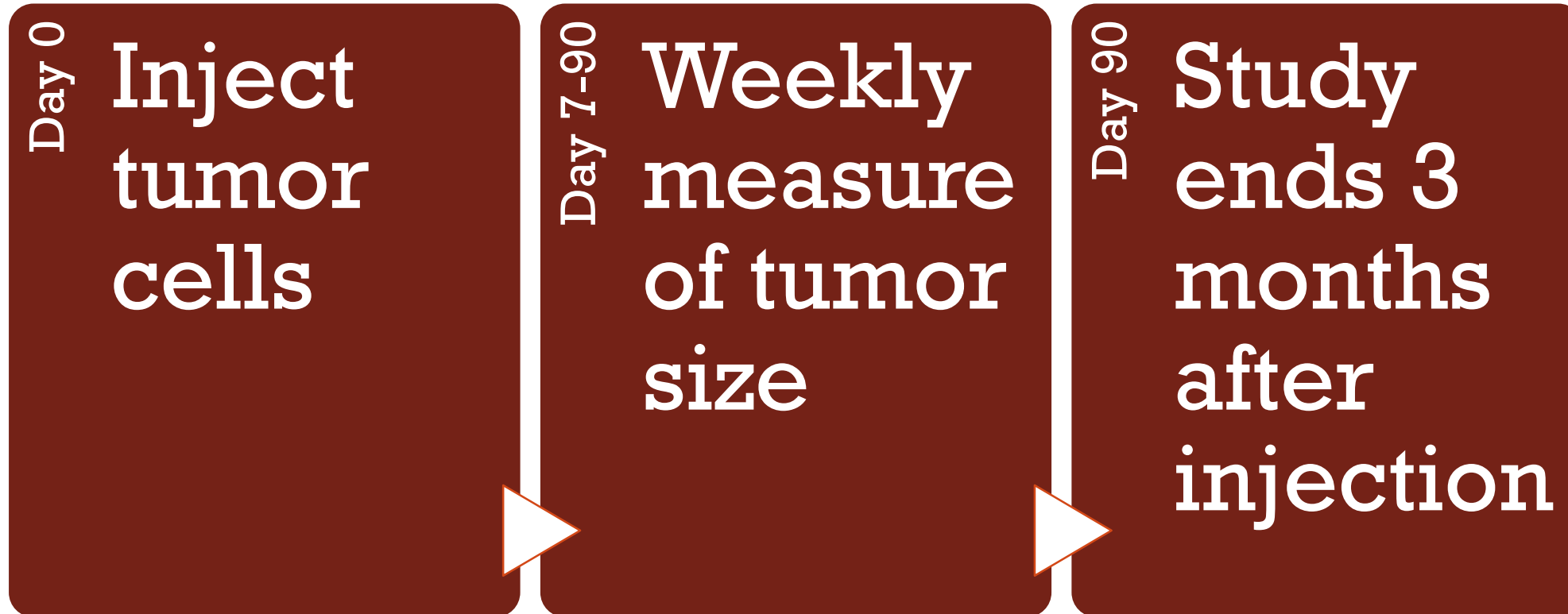
- Mouse model criteria for euthanasia
  - BCS of 1
  - BUN >45 mg/dL
  - Creatinine >1.2 mg/dL
  - Time to integrate nesting material >15 minutes

# TOXICITY TESTING: ZEBRAFISH

General Health Swimming	<ol style="list-style-type: none"><li>0. normal</li><li>1. intermittent loss of equilibrium</li><li>2. frequent loss of equilibrium</li><li>3. complete loss of equilibrium</li></ol>
Body Score (Estimated)	<ol style="list-style-type: none"><li>0. normal</li><li>1. loss of 10-15% BW</li><li>2. loss of 15-20% BW</li><li>3. loss of &gt;20% BW</li></ol>
Abnormal abdominal muscle tone	<ol style="list-style-type: none"><li>0. normal</li><li>1. mild</li><li>2. moderate</li><li>3. severe</li></ol>
Abdominal Distension	<ol style="list-style-type: none"><li>0. normal</li><li>1. mild</li><li>2. moderate</li><li>3. severe</li></ol>
Behaviour	<ol style="list-style-type: none"><li>0. normal</li><li>1-3. all fish at surface gasping for air</li></ol>

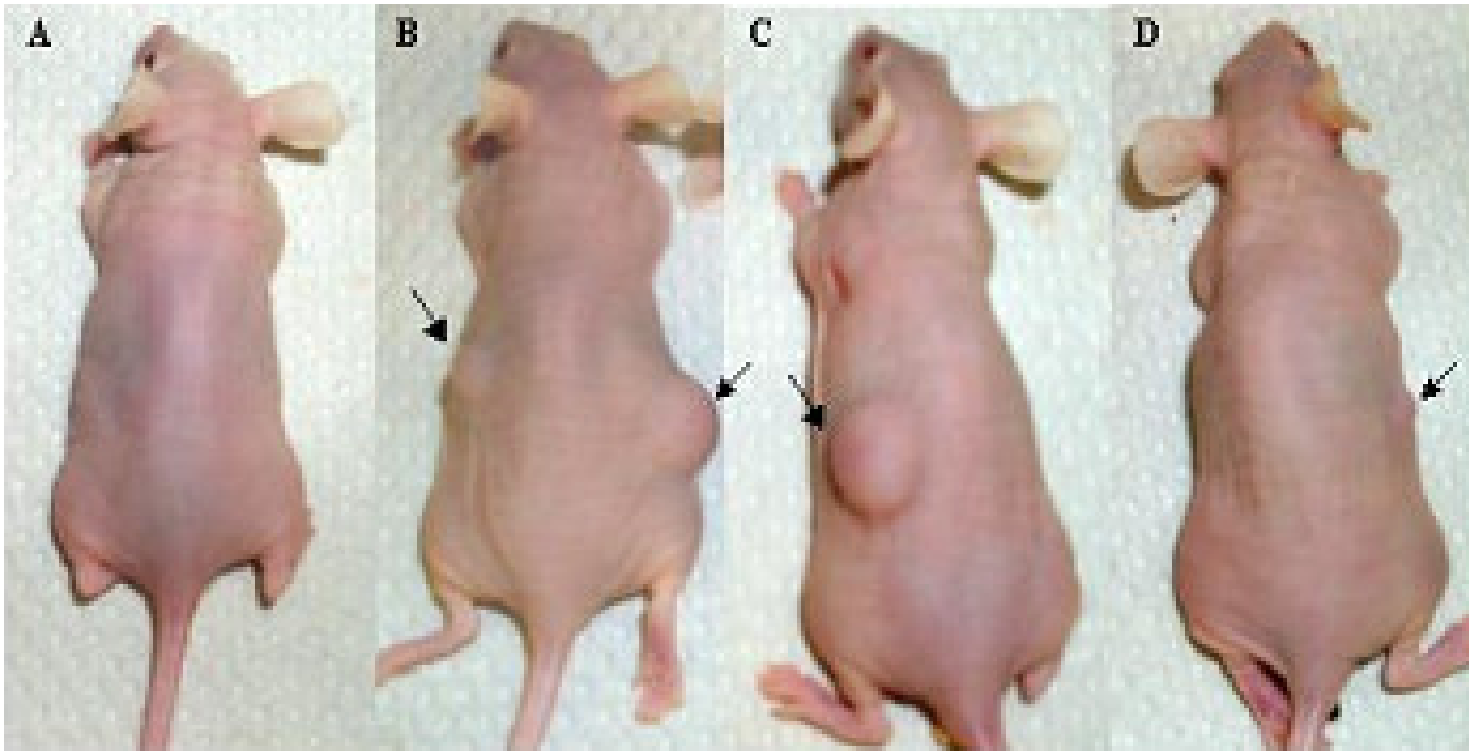
**0 = normal: no action**  
**1-4 = moderate changes:  
should be monitored  
daily**  
**5-8 = significant  
changes: monitor  
twice daily**  
**>8 = euthanize**

# STUDY ENDPOINTS: TUMOR STUDY





# TUMOR STUDY: HUMANE ENDPOINTS



- Body condition score
  - *Not body weight!*
- Tumor size/ulceration
- Mouse behavior
  - Nesting score

# STUDY ENDPOINTS: BEHAVIORAL TESTING

Day 0

Start training\*

\*includes fasting

Day 1-15

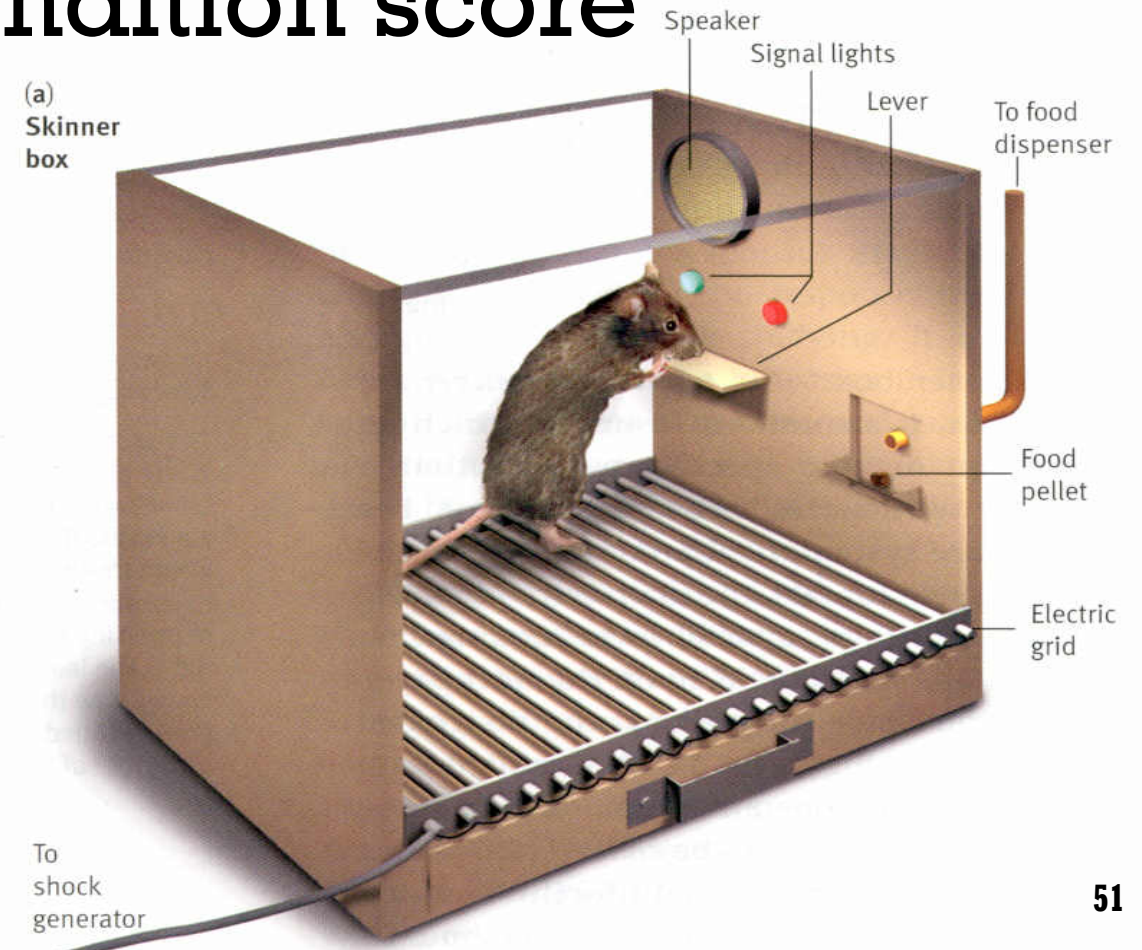
Continue daily behavioral assessment

Day 16

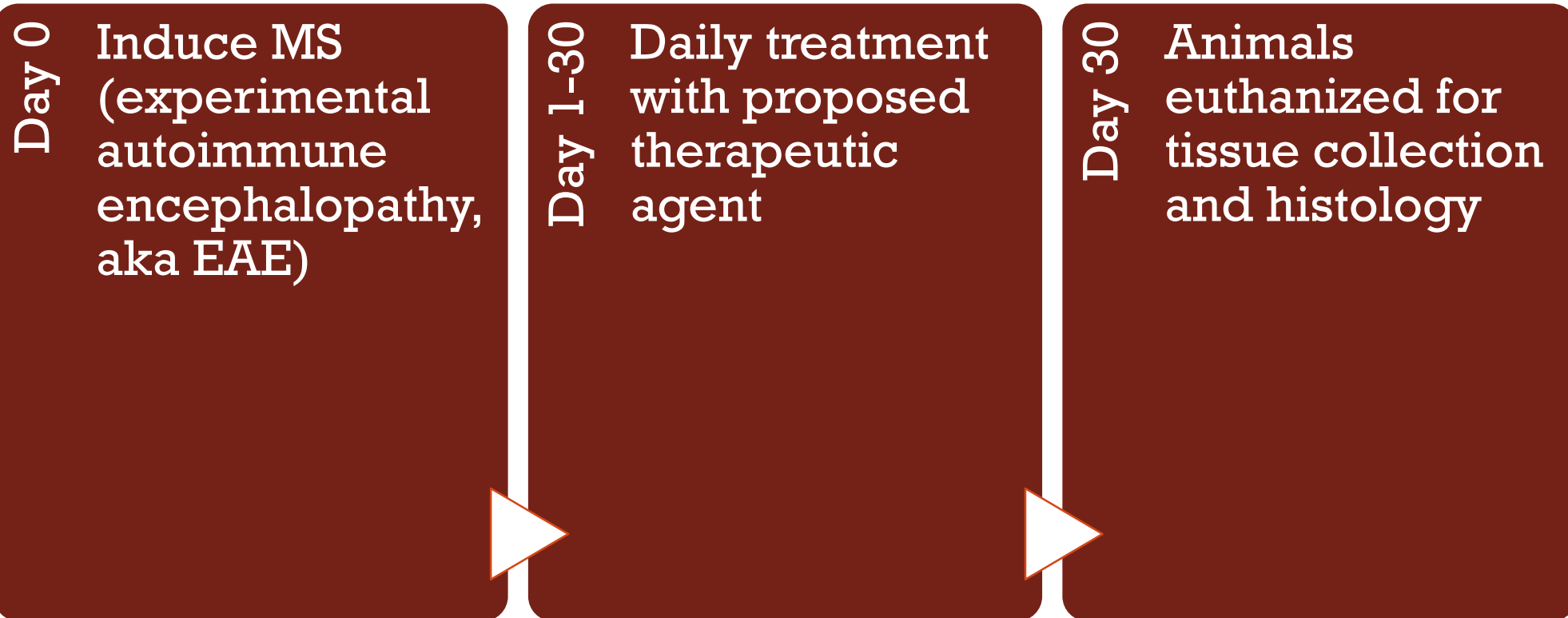
Study ends

# BEHAVIORAL TESTING: HUMANE ENDPOINTS

- Body weight/body condition score
- Passive Behavior
  - Nesting
  - Grooming
- Active Behavior



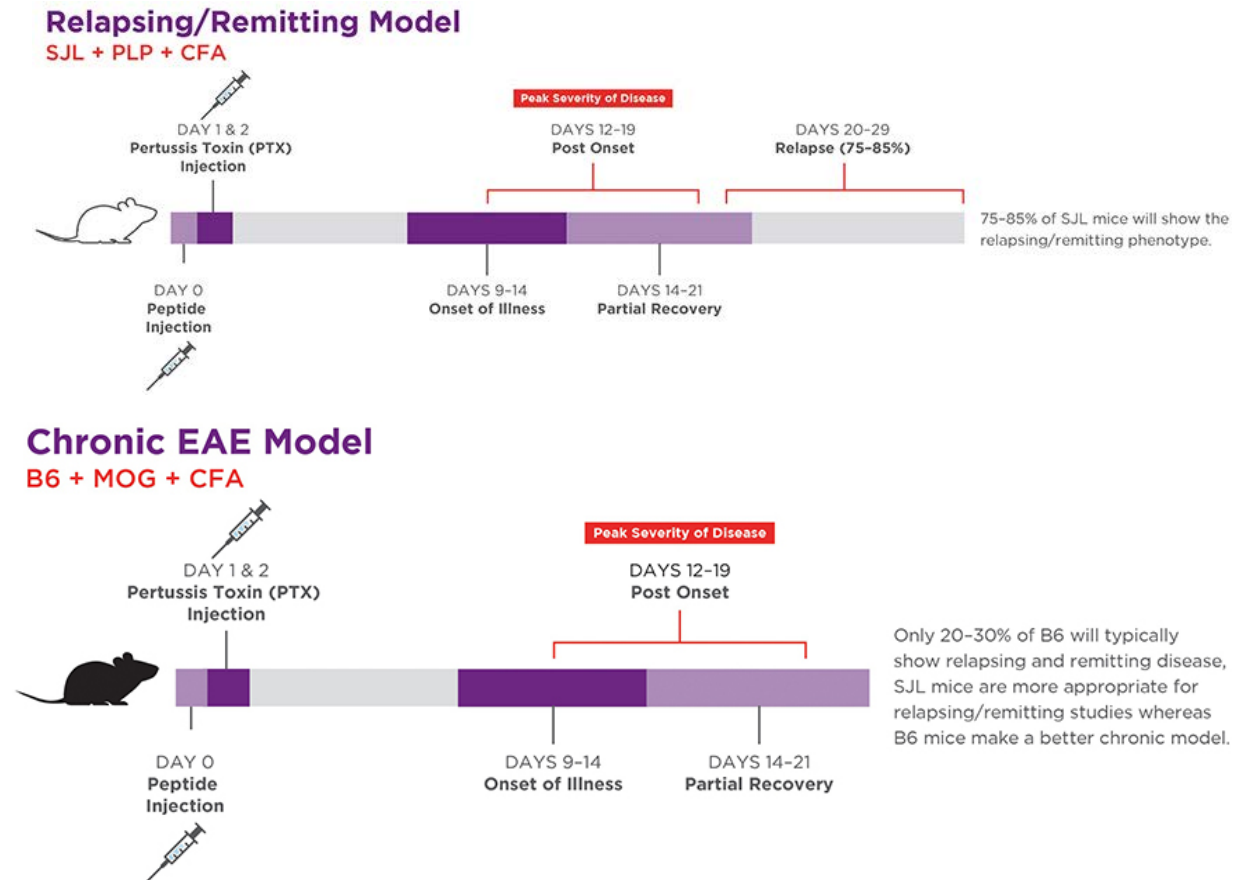
# STUDY ENDPOINTS: MULTIPLE SCLEROSIS



# MULTIPLE SCLEROSIS (EAE): EXPECTED OUTCOMES

## Know your model!

- Relapsing/Remitting Model
  - SJL mice
  - Will get very sick, then will improve
- Chronic Model
  - B6 mice
  - Progressively worse over time

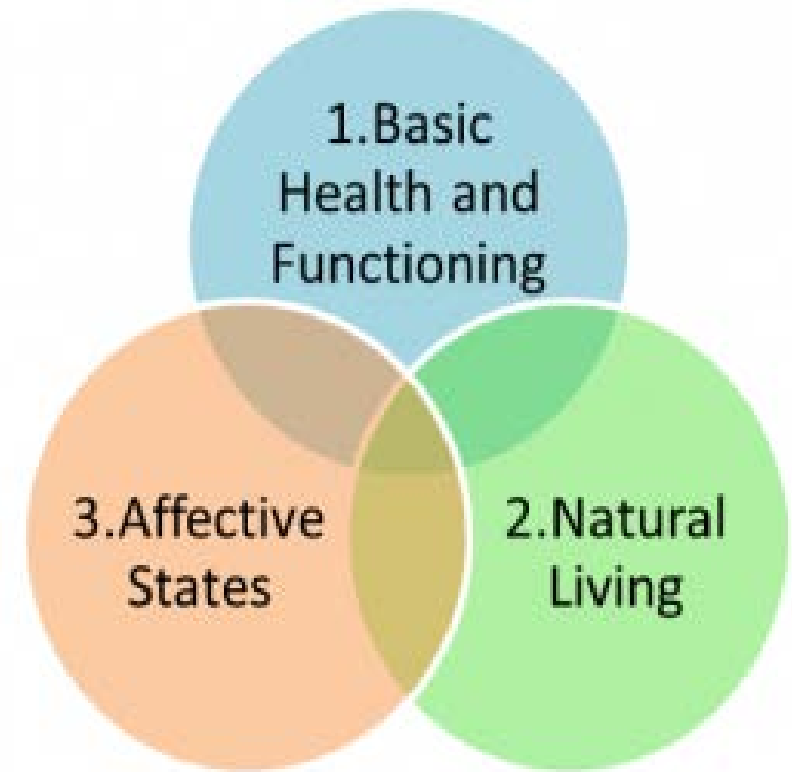


# MULTIPLE SCLEROSIS (EAE): HUMANE ENDPOINTS

- Body weight/body condition score
- Hydration status
  - Skin tent
  - Blood work
- Passive Behavior
  - Nesting
  - Grooming

# CONCLUSION

- What is happening to the animal?
- What is the expected response?
- What kinds of complications can be predicted?
- Look at available assessments of well-being to construct appropriate humane endpoints



Very study dependent

# QUESTIONS



# QUESTION 1

Where can you find guidelines and regulations on humane endpoints?

# ANSWER 1

<https://www.humane-endpoints.info/en#>

# QUESTION 2

Are there set humane endpoints like those you described in your talk or can humane endpoints be “customized” depending on the research and animal model?

# **ANSWER 2**

**Customization is necessary and encouraged!**

# QUESTION 3

I am particularly interested in hearing thoughts on assessing endpoints for monkeys engaged in neuroscience (electrophysiological and behavioral) experiments.

There is a delicate balance between maximizing the information gleaned from any one animal given the extensive behavioral training and preparation that goes into preparing each animal and specific experiments. I am interested in hearing about guidelines for these determinations.

# QUESTION 4

What are the principal considerations in developing humane endpoints in any study?

# ANSWER 4

- What is happening to the animal?
- What is the expected response?
- What kinds of complications can be predicted?
- What specific criteria will be used to determine that it is time to treat?
- What specific criteria will be used to determine that it is time to remove from study (including euthanasia)?

# QUESTION 5

Who should be involved in the establishment of species-specific and study-appropriate humane endpoints?



# ANSWER 5

- **Scientist**
- **Veterinarian**
- **IACUC**
- **Outside subject matter experts**

# QUESTION 6

At what phase of the study should humane endpoints be clearly defined?

# **ANSWER 6**

**Prior to the start of the study.**

# QUESTION 7

What are your thoughts about death as an endpoint?

# QUESTIONS

**Now:** Type your questions into the chat box on GoToMeeting dashboard.

**Later:** email your questions to [OLAWDPE@mail.nih.gov](mailto:OLAWDPE@mail.nih.gov)



# Semiannual Program Review



OLAW Online Seminar

December 13, 2018

Dawn O'Conner and Bill Greer

University of Michigan