

# Housing Laboratory Rodents According to the 8<sup>th</sup> Edition of the Guide

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OLAW Online Webinar

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# *Guide* Definition of Performance Standard

“Performance standard means a standard or guideline that, while describing a desired outcome, provides flexibility in achieving this outcome by granting discretion to those responsible for managing the animal care and use program, the researcher, and the IACUC.

The performance approach requires professional input, sound judgment, and a team approach to achieve specific goals...

Performance standards can be advantageous because they accommodate the consideration of many variables... so that implementation can be best tailored to meet the recommendations in the *Guide*.”

*Guide* pages 6-7

# OLAW Performance Standard Criteria

A well-established performance standard meets the following criteria:

- supports scientific objectives;
- supports the health and welfare of the animal;
- includes a justified performance index; and
- has associated outcome criteria.



# HOUSING LABORATORY RODENTS ACCORDING TO THE 8TH EDITION OF THE GUIDE: ASSESSING PERFORMANCE STANDARDS

Dr. Joseph Garner  
Stanford University

# Animal Wellbeing *IS* the ultimate Performance Standard

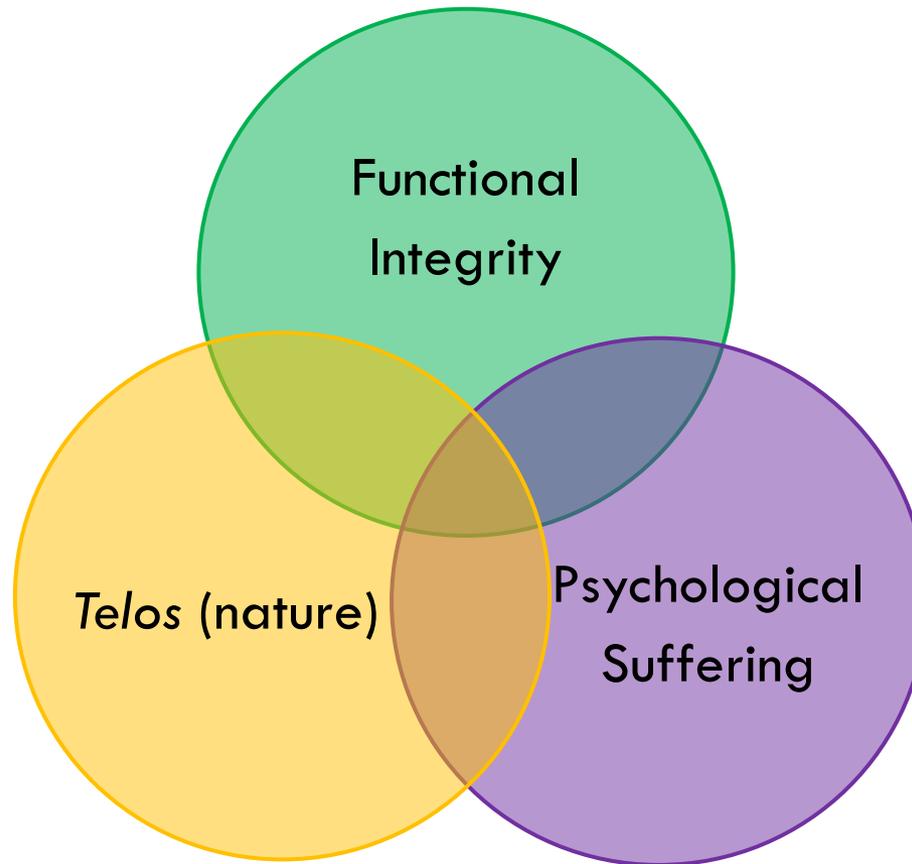
Animal wellbeing is the performance standard

Control – what does it mean to be an animal

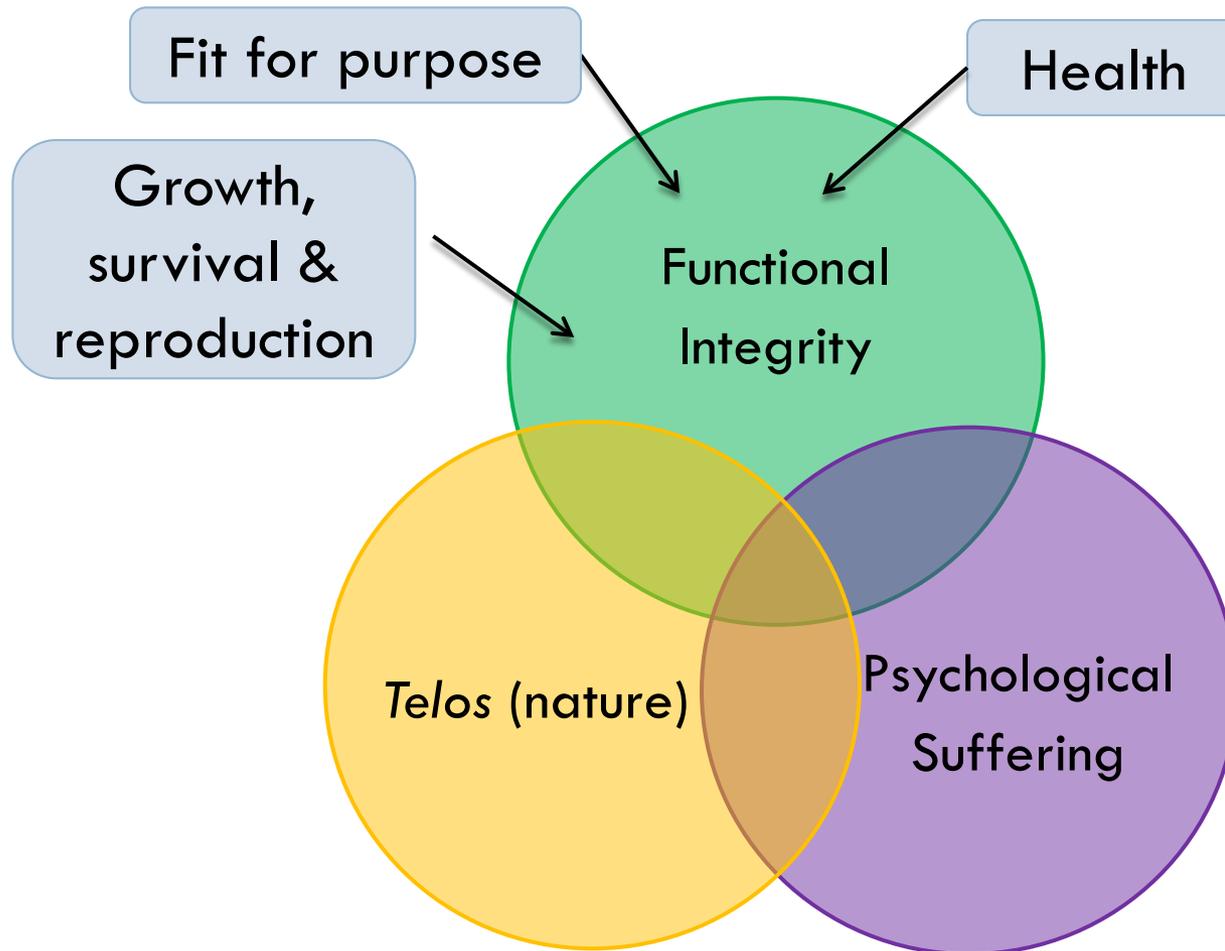
An enrichment success story

The lab from a mouse's point of view

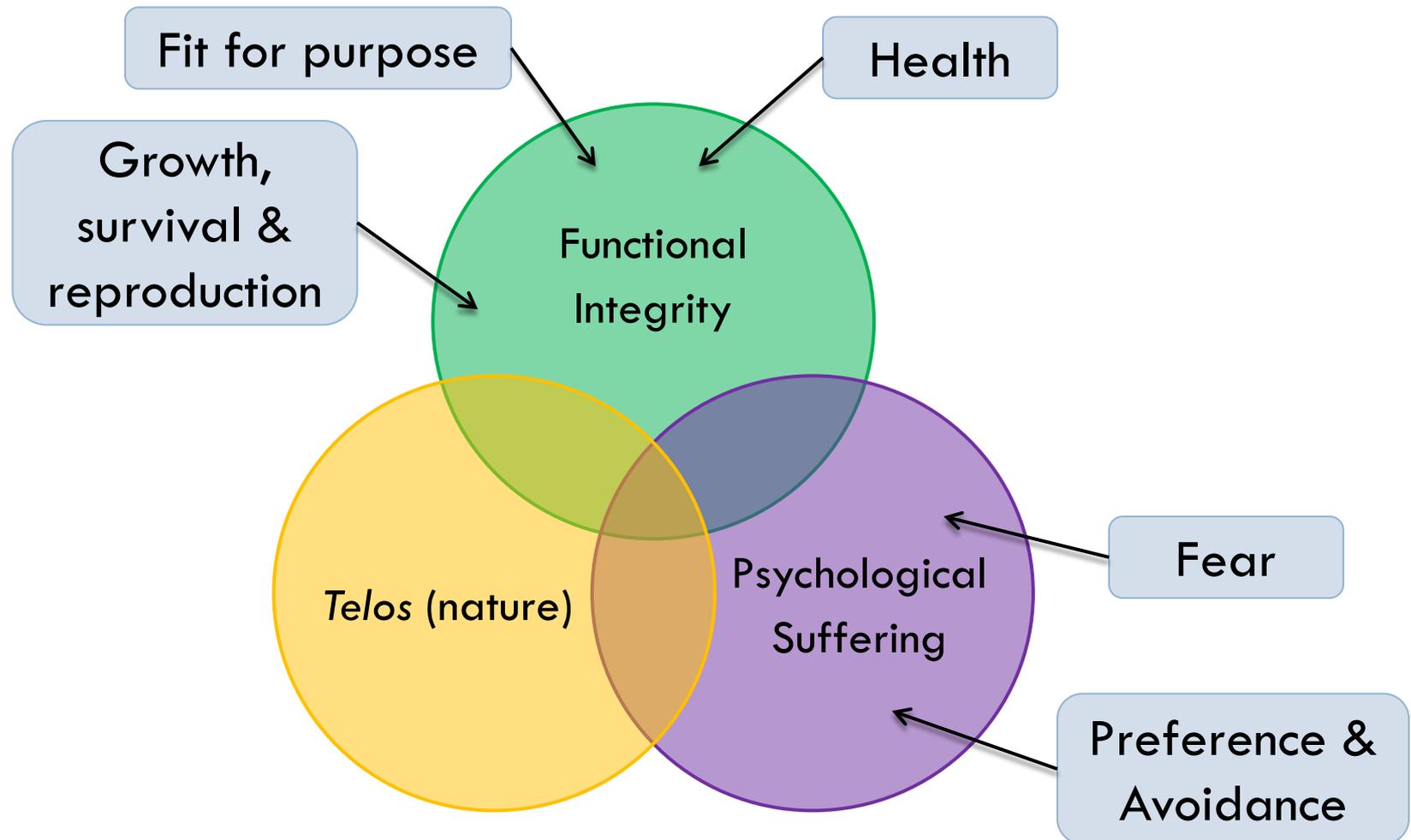
# Three different visions of wellbeing: different people have different values



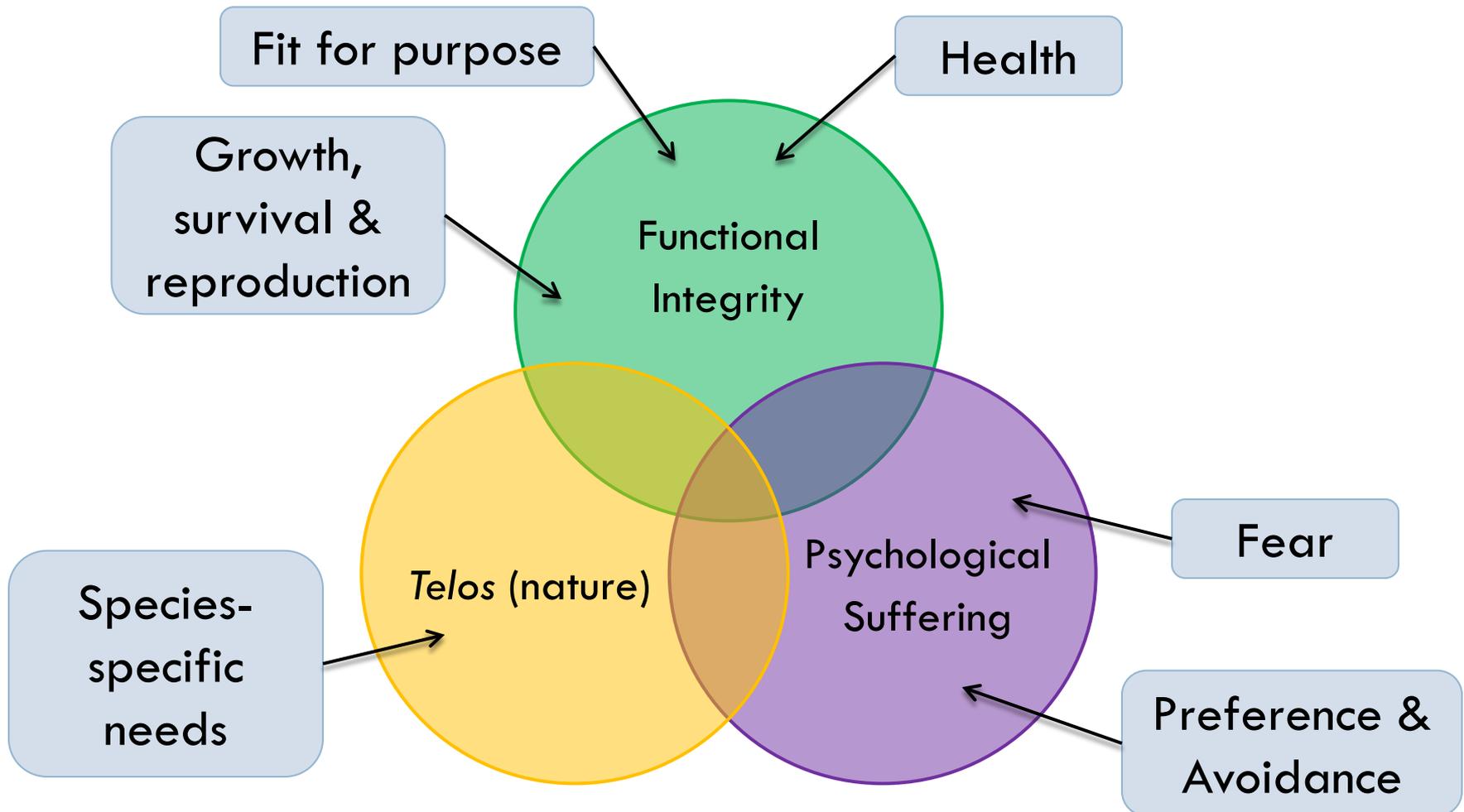
# Three different visions of wellbeing: each can be measured in different ways



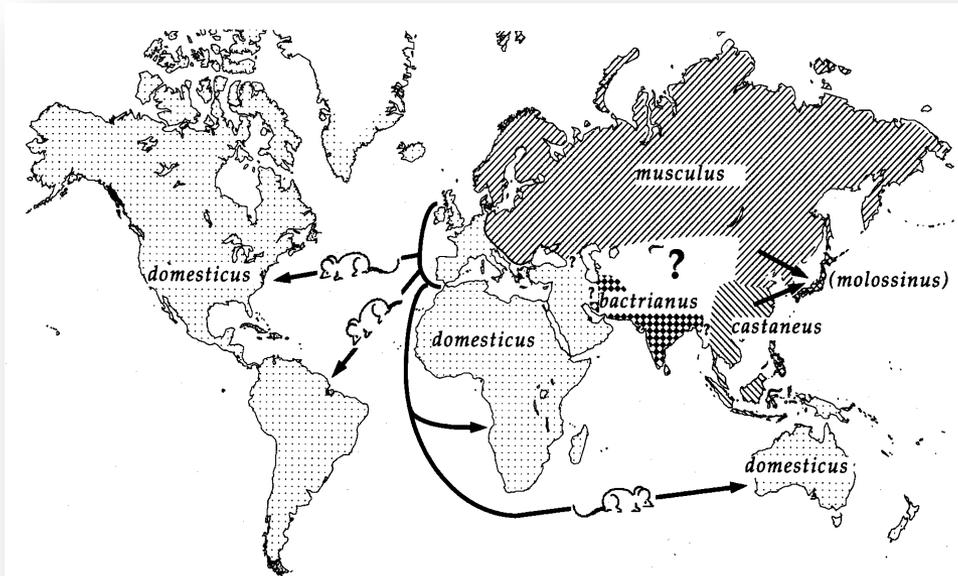
# Three different visions of wellbeing: each can be measured in different ways



# Three different visions of wellbeing: each can be measured in different ways



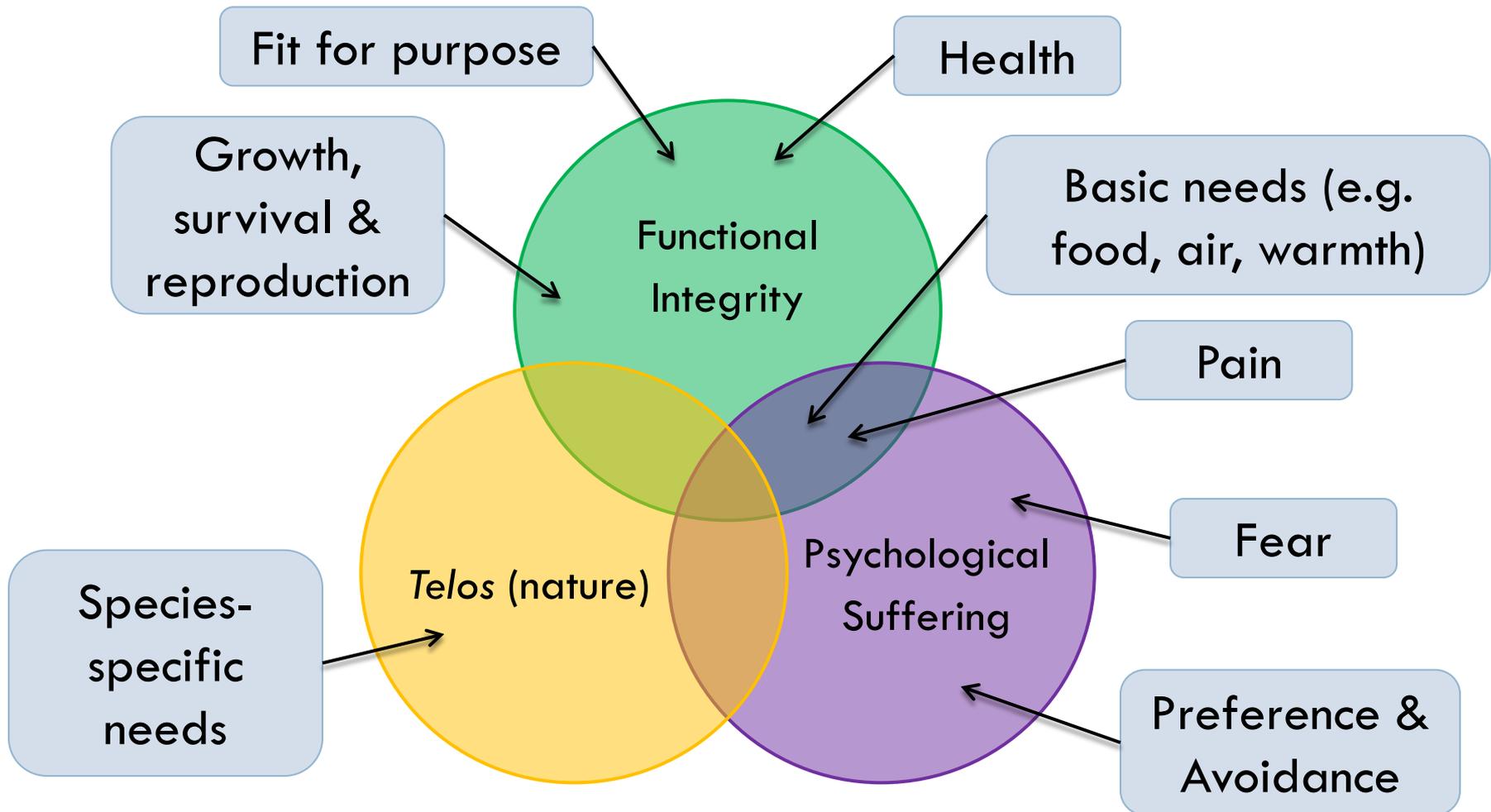
# Telos – The mouseness of mouse



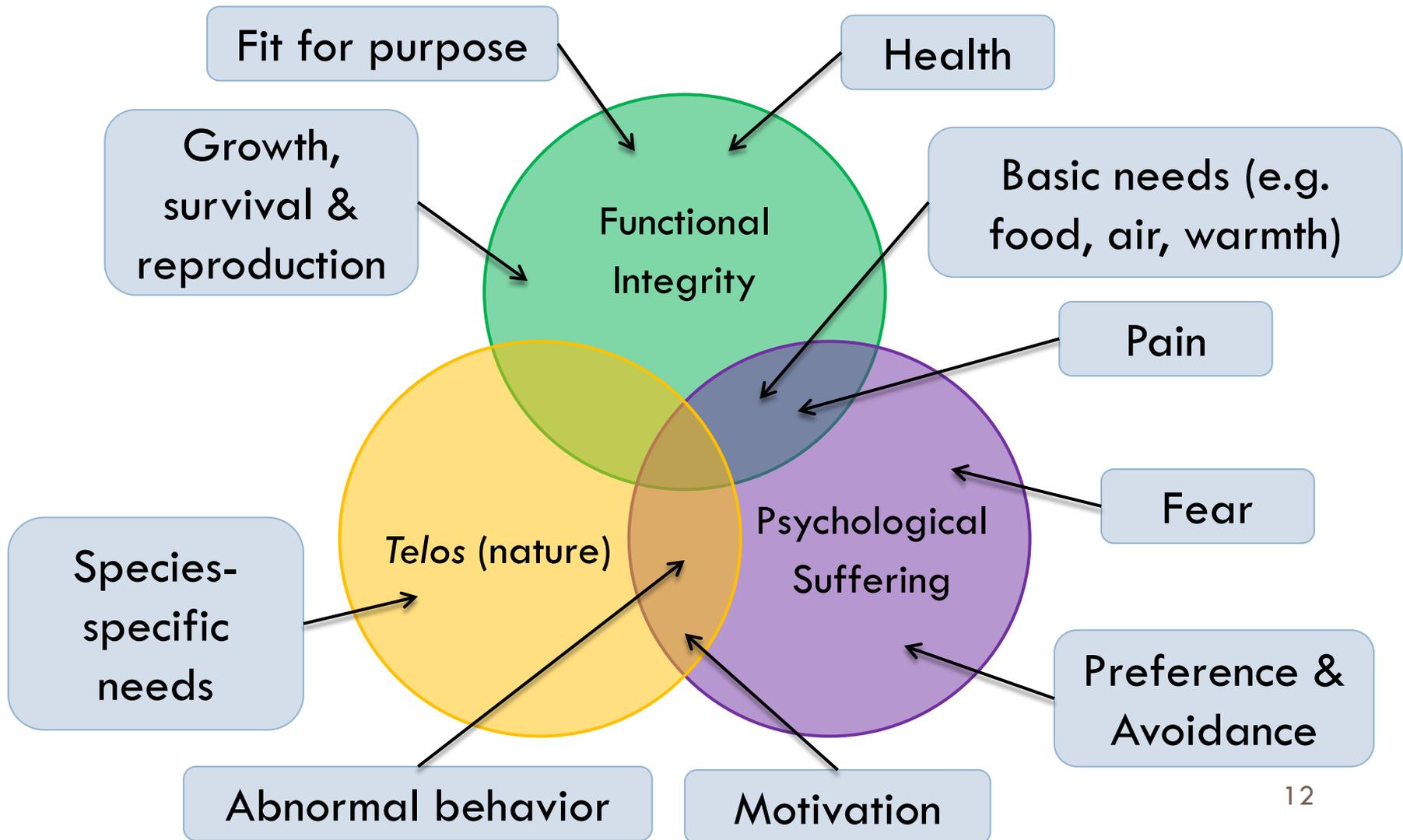
- House mice evolved in arid grasslands
- House mice are the only mice to live commensally with humans
- To follow us around the world they have had to be as adaptable to differing environments as humans
- Phenotypic plasticity

Figure: Silver, 1995

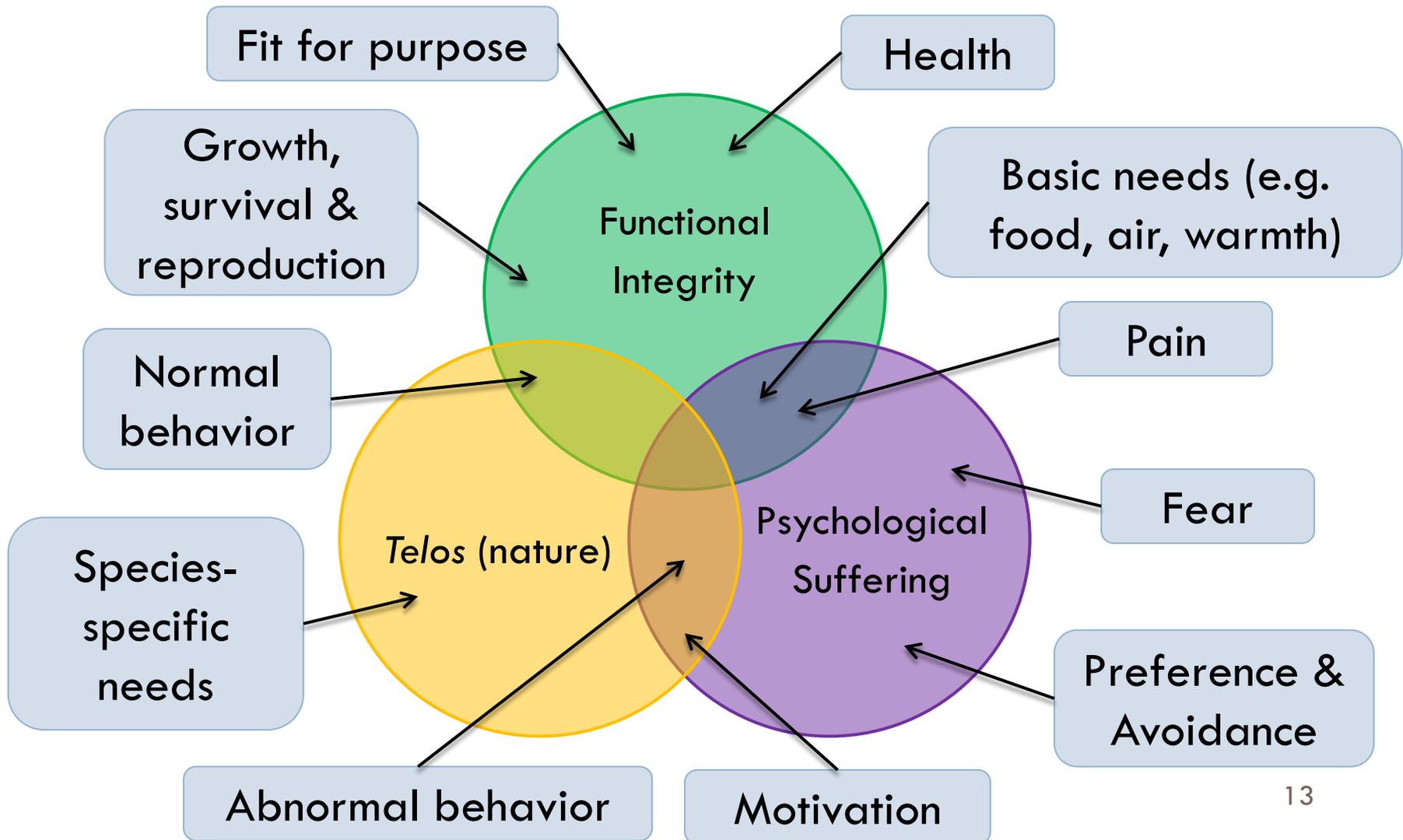
# Three different visions of wellbeing: each can be measured in different ways



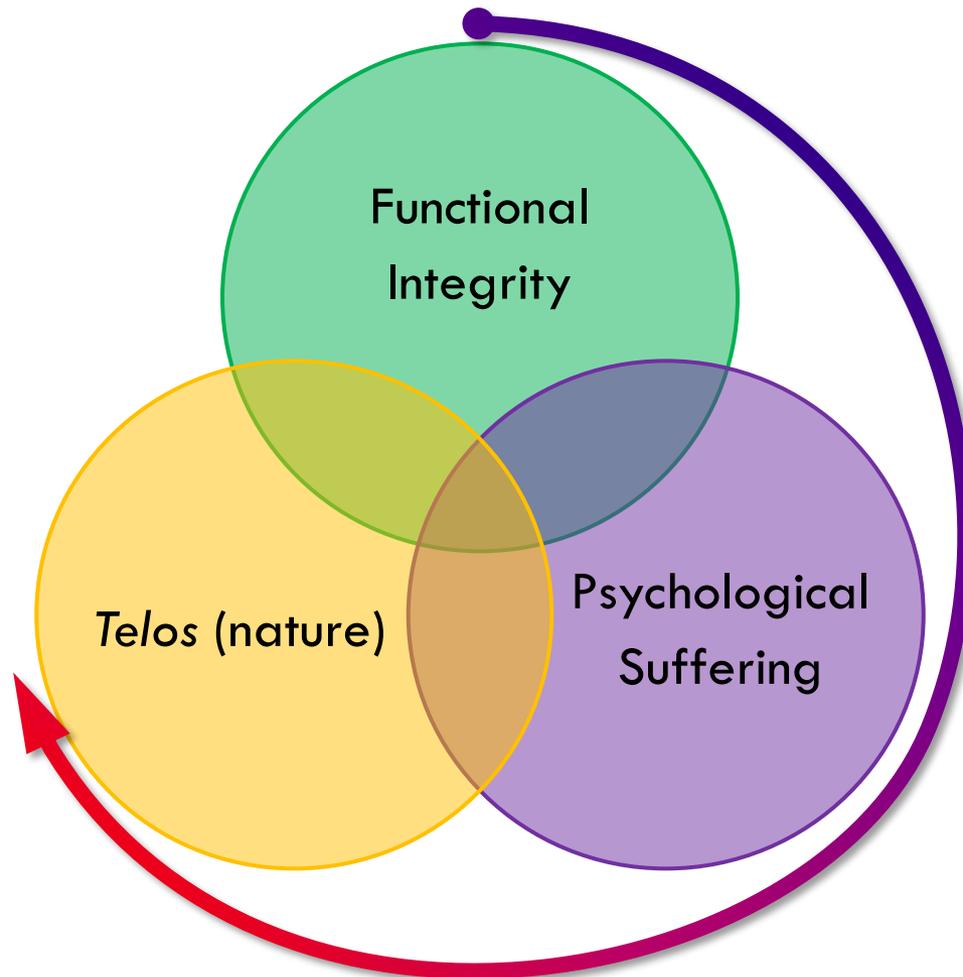
# Three different visions of wellbeing: each can be measured in different ways



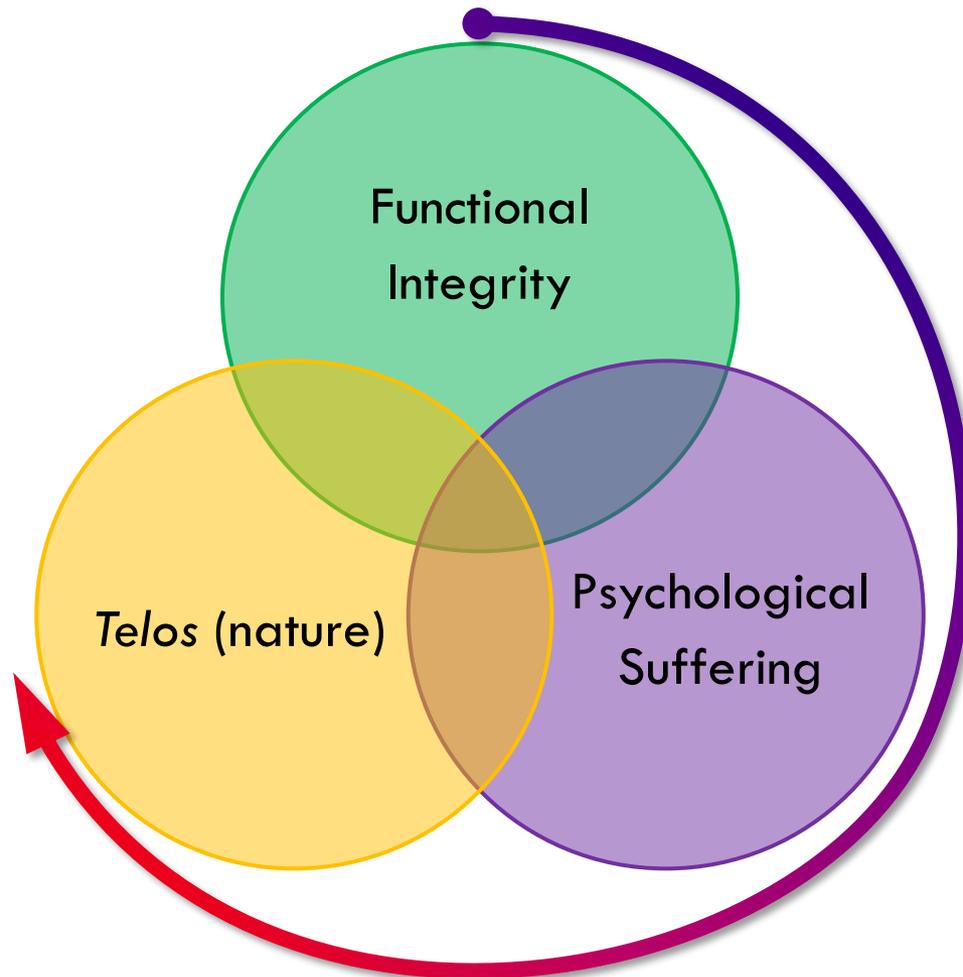
# Three different visions of wellbeing: each can be measured in different ways



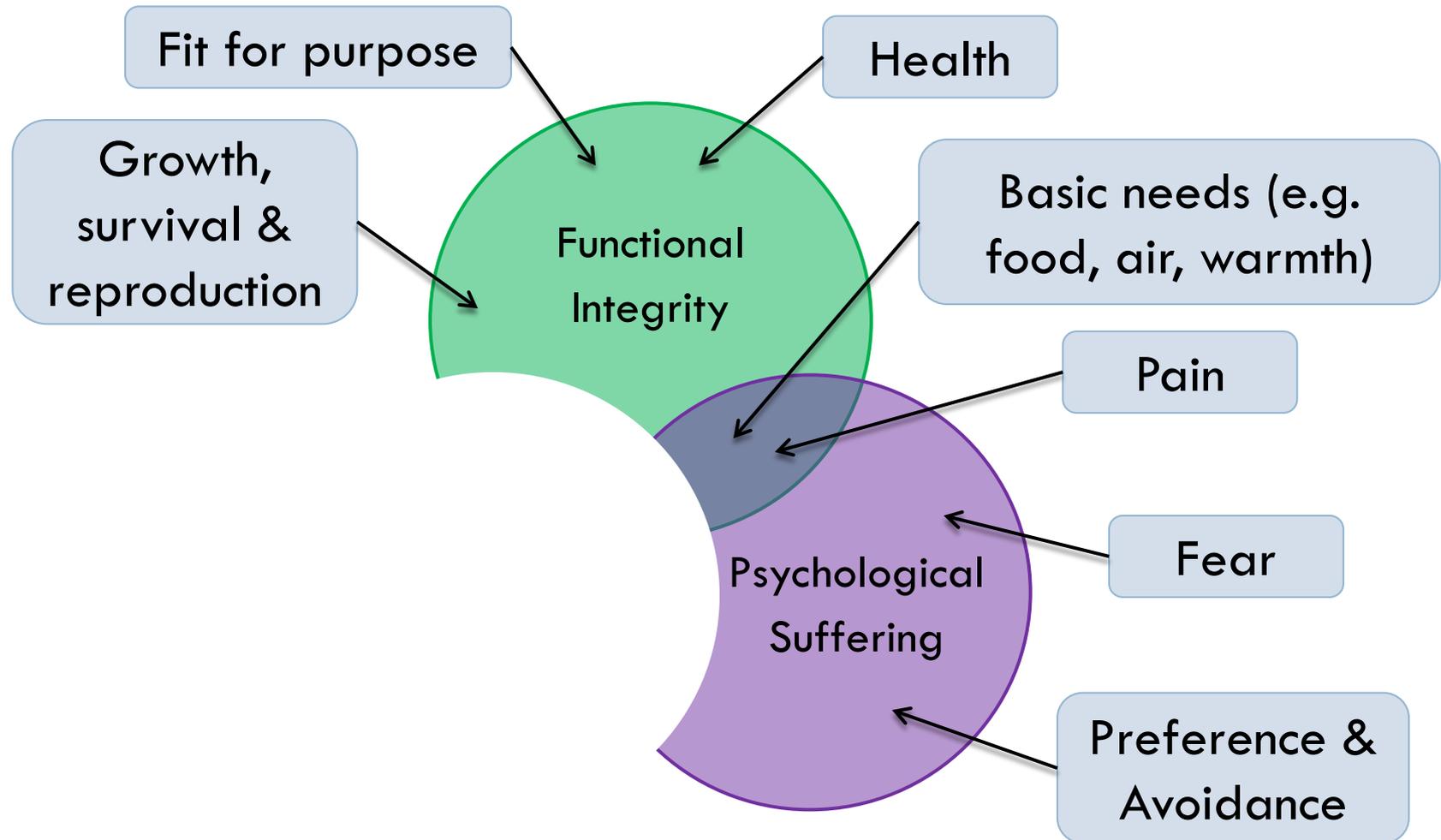
These measures are progressively more specific to the animal, but not always harder to interpret



The simple measures can be the most misleading – we have selected animals to breed despite their wellbeing



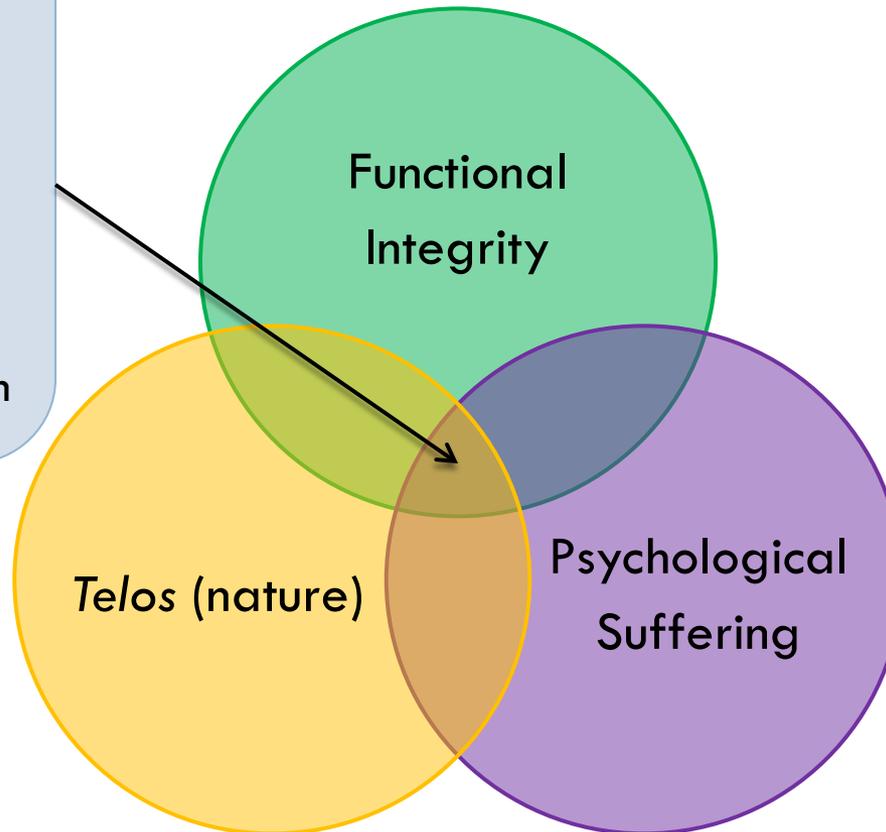
# Nevertheless, *Telos* is hard to find in the literature (and thus *The Guide*)



# The 'sweet spot' is what really matters, it's measurable, and *Telos* is the key

## Coping and distress

- **Coping behavior**
- Stress physiology
- Immune function
- Autonomic function



## Behavior and control – the *Telos* of animals

Animal wellbeing is the performance standard

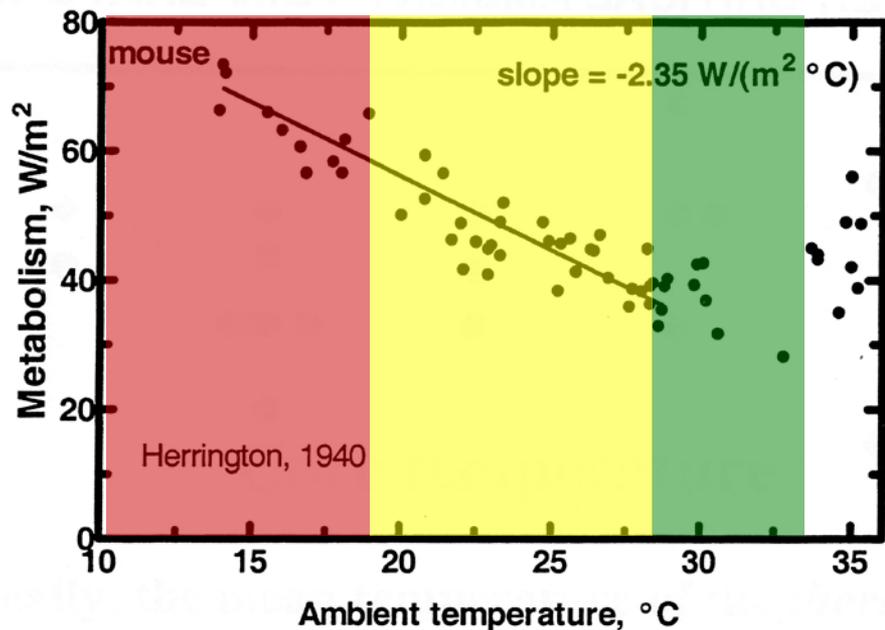
Control – what does it mean to be an animal

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# Stress and Distress (Moberg 2000)

## Example: Mouse thermoregulation



### Physiological zones of stress and distress

Thermoneutral zone: 26-34°C

Stress: 18-26°C, Metabolism increased

Distress: <18°C, Growth rate compromised

- Comfort
  - No additional resources required to maintain homeostasis
- Stress
  - Resources must be spent to maintain homeostasis
- Distress
  - No spare resources
  - **Biological functions are compromised to maintain homeostasis**
- Death

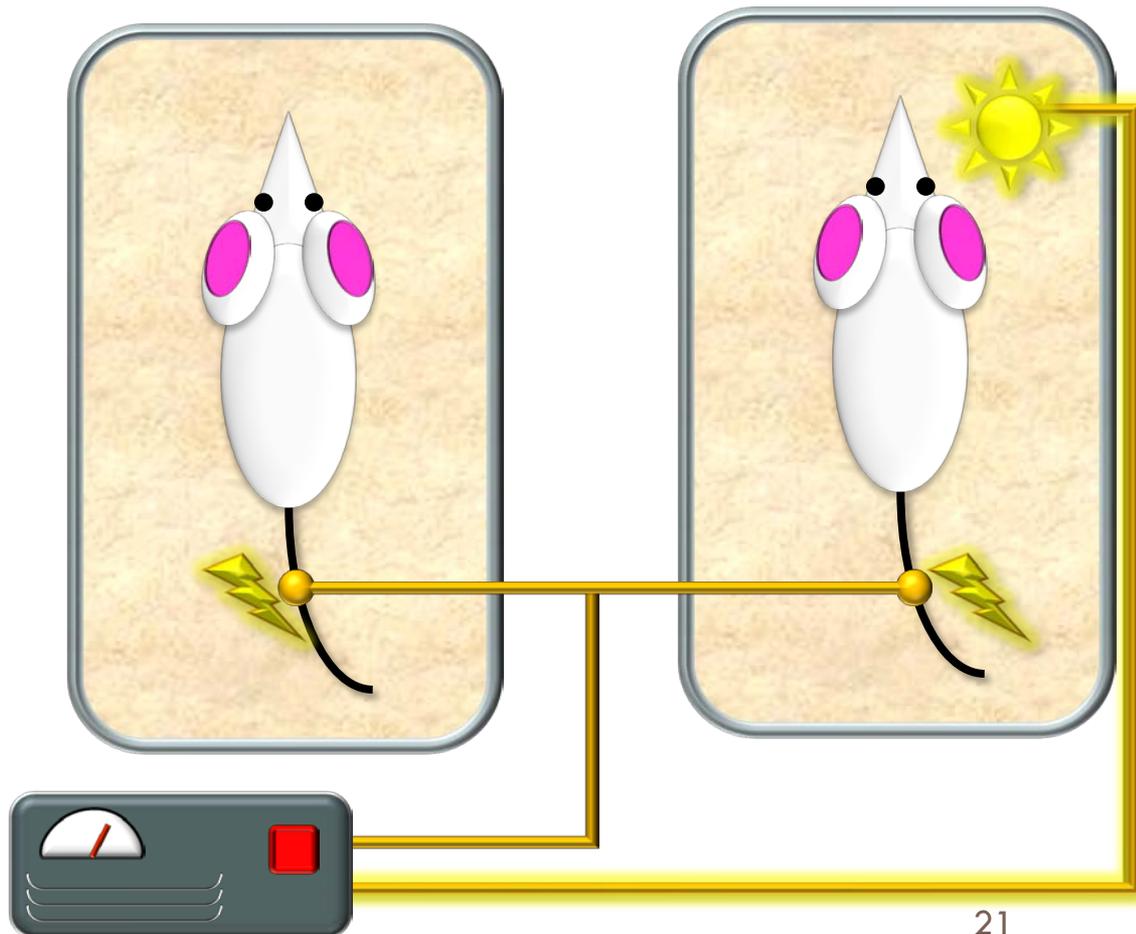
# Animals exist to behave



- Under laboratory conditions, mice become hypothermic in 12-26 minutes at  $-40^{\circ}\text{C}$
- Yet mice can live in meat freezers at  $-30^{\circ}\text{C}$  ... due to nests
- Thus the temperature of distress, depends entirely on the animals' perception of its ability to control it
- Behavior is what makes an animal an animal – plants can't build nests or walk to water

# Behavior exists to predict and control

- However it is actually the animal's PERCEPTION of its ability to predict, control, and correct a stressor that ultimately determines its physiological impact
- Weiss, 1970
- This is a key point of Moberg's argument



# What is environmental enrichment?

- Animals exist to behave
- Behavior exists to predict and control stressors
  - ▣ An animal that can't control its environment will surely die
  - ▣ So animals will do everything in their power to exert control, and when they can't, every aspect of their biology can change or even fail
  - ▣ This is the key biology predicting that enrichment should improve animal models
- **Biologically Relevant Enrichments** are ANY change in housing or husbandry that
  - ▣ Remove stressors (very hard to do)
  - ▣ Provide the animal control (much easier to do)

# An enrichment success story...

## (...the best investment you'll ever make)

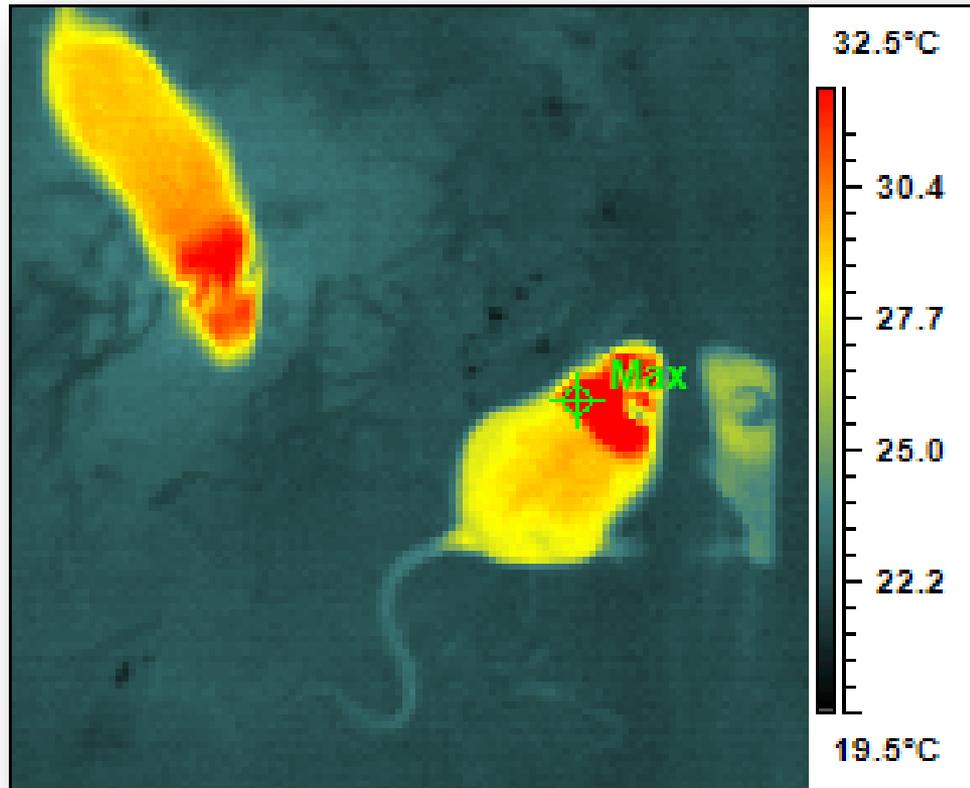
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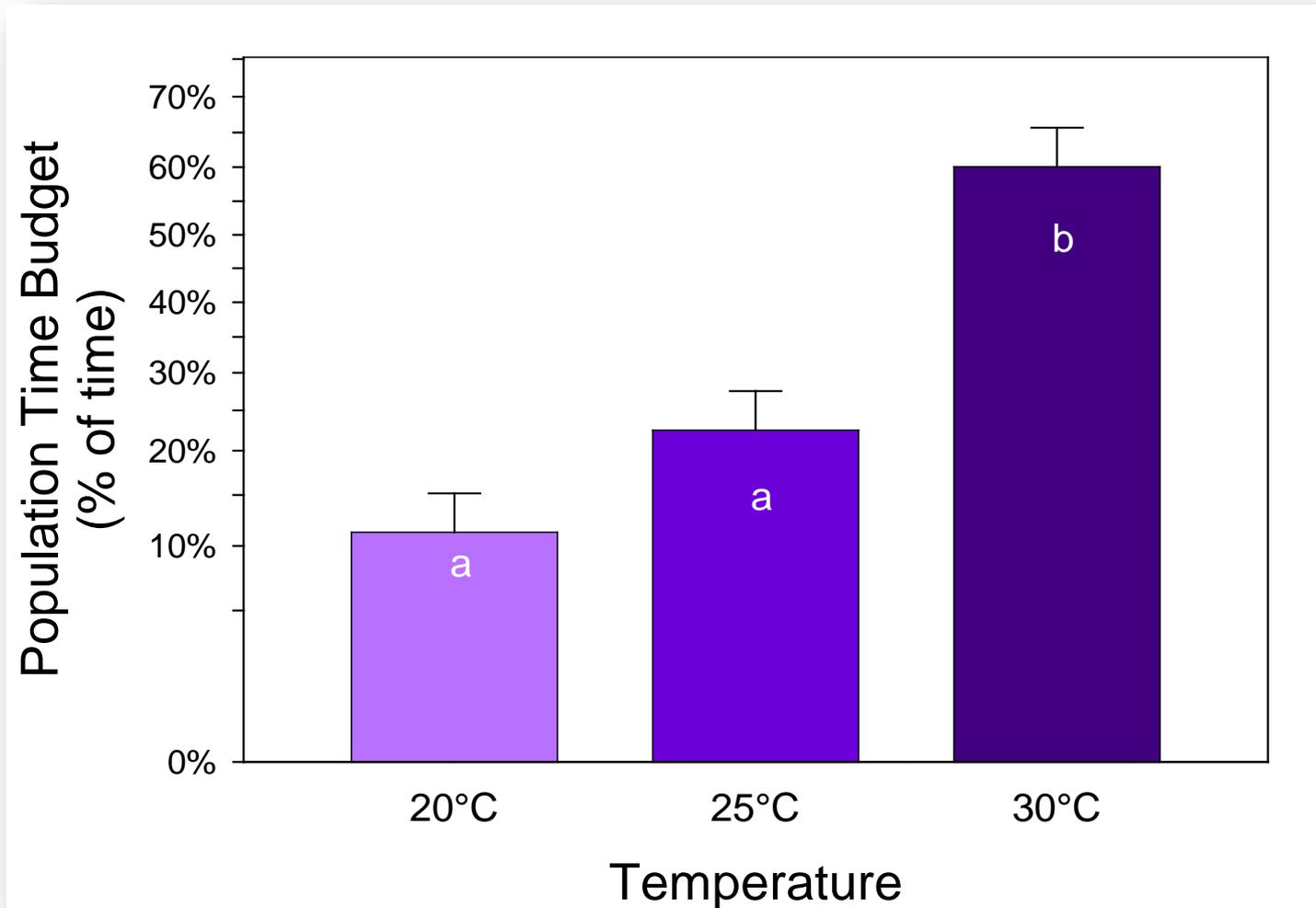
# Too cold for comfort?



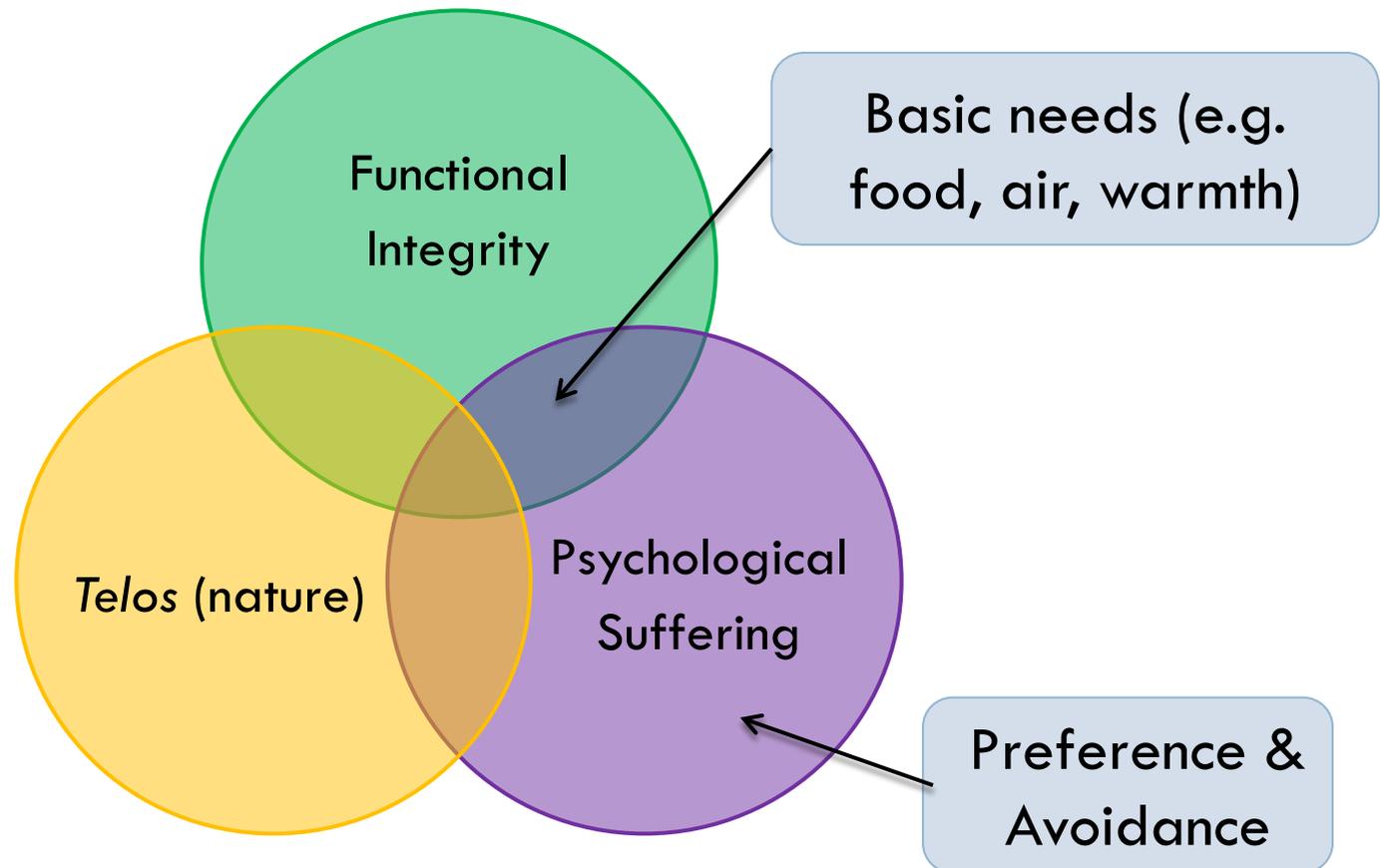
- Mice alter heat loss by altering blood flow to the tail
- A cold tail means the mouse is cold-stressed
- Core: 36-37.5°C
- Bare head: 35.4°C
- Fur: 29.5°C
- Tail: 22.6°C

# Mice prefer warmer cages...

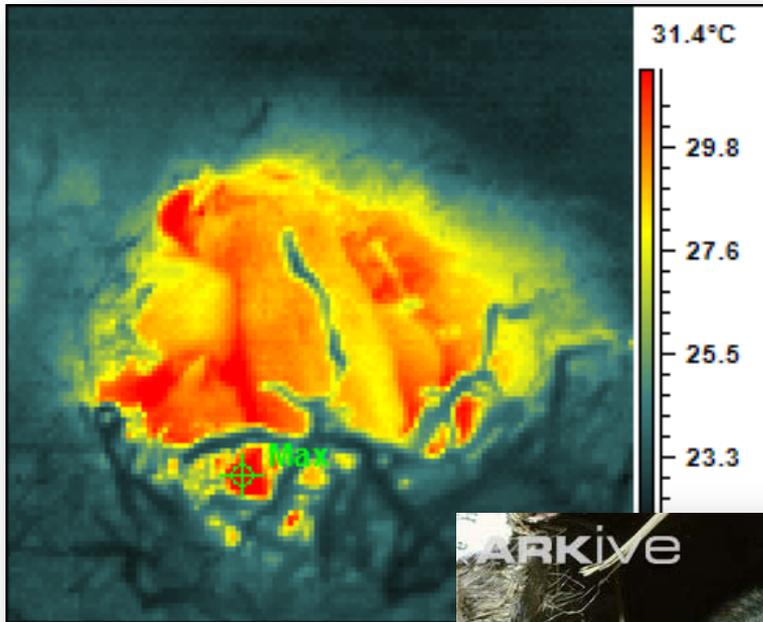
# ...but warmer cages cause aggression



# Performance standards measured

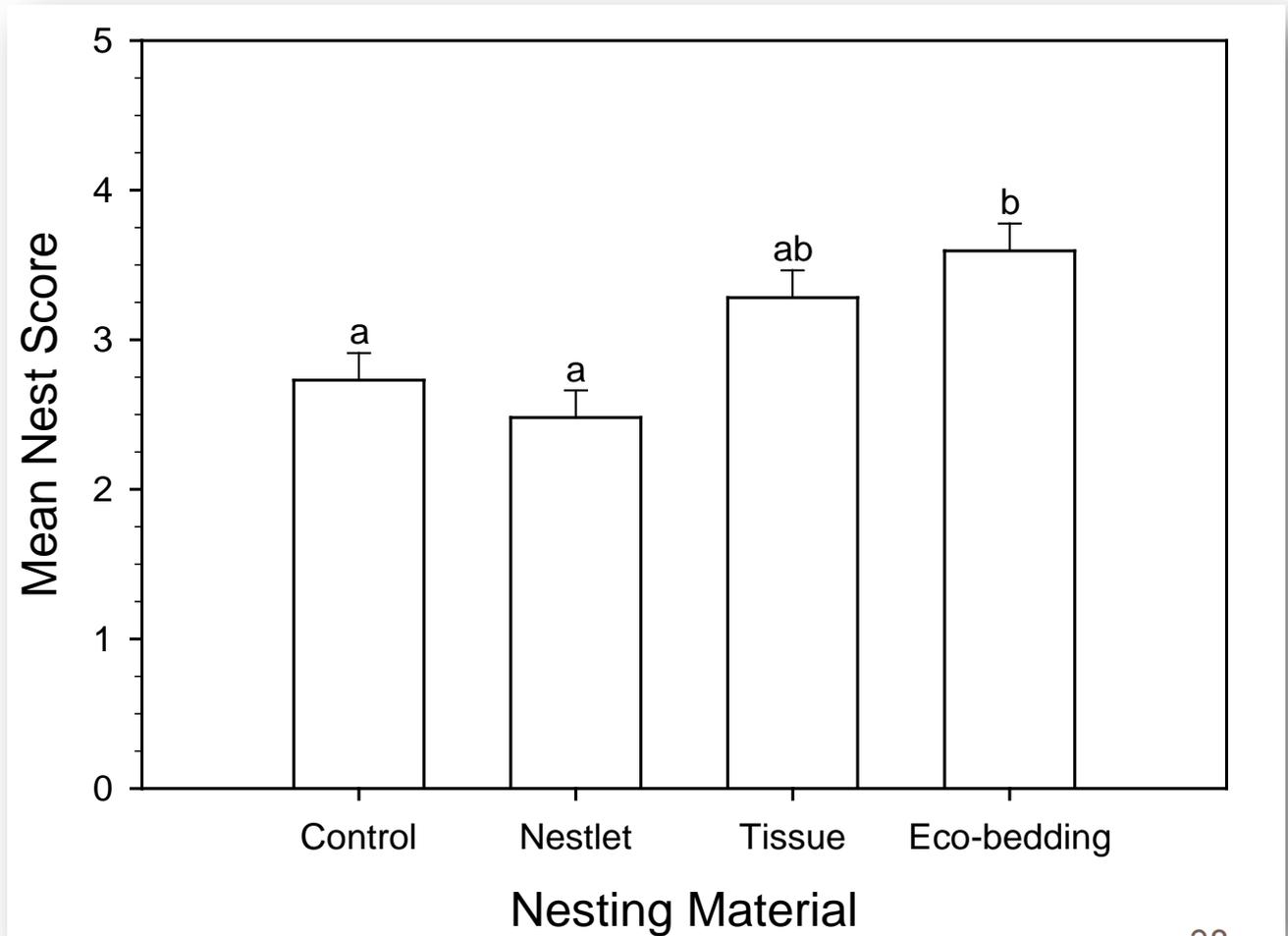


# Telos – in nature nests keep mice warm when temperatures are low and fighting is prevented



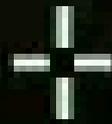
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# Biologically relevant enrichment – mice need the right kind of nesting material



Deacon, 2006;  
Hess et al, 2008

20.1



17.3

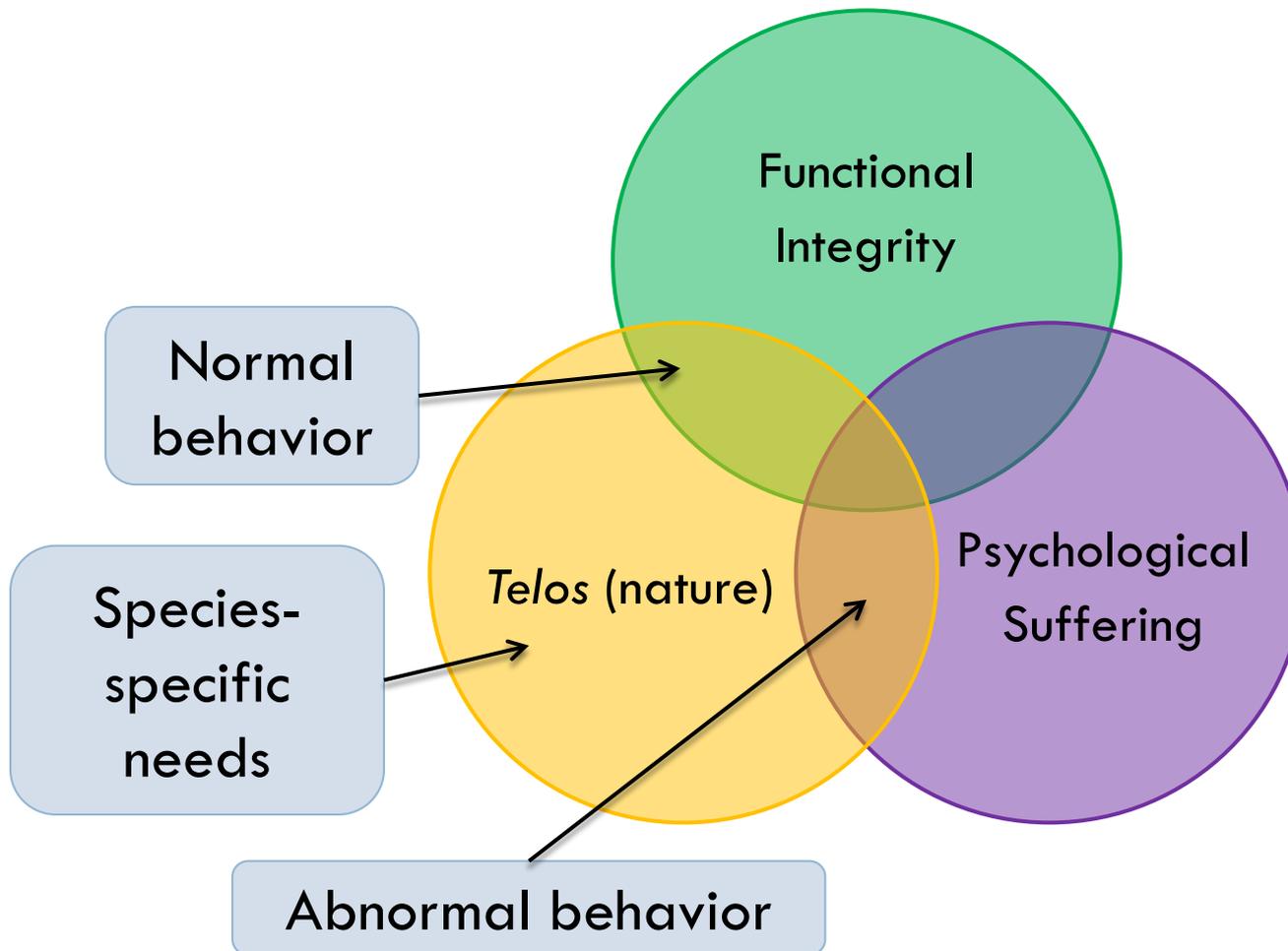
26.0

15

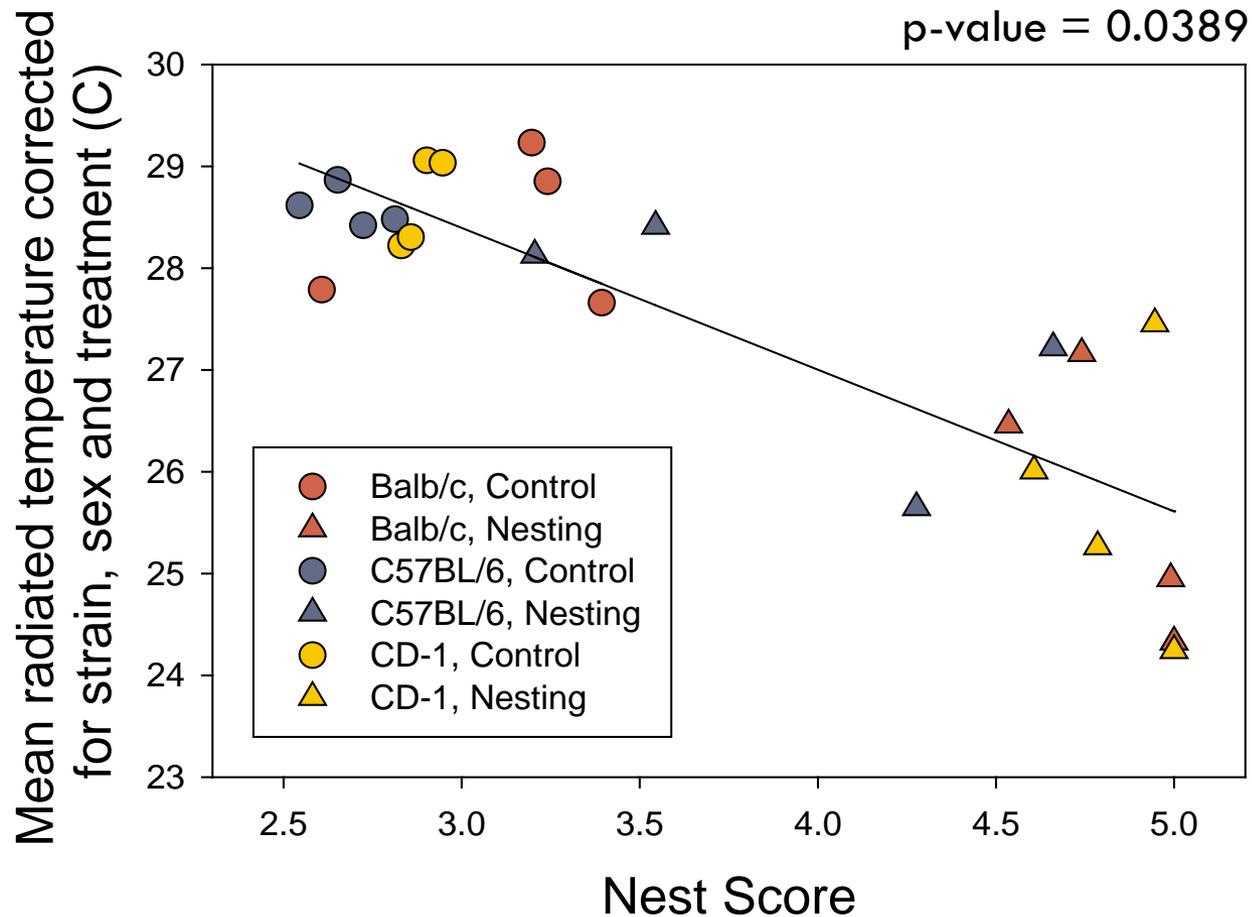


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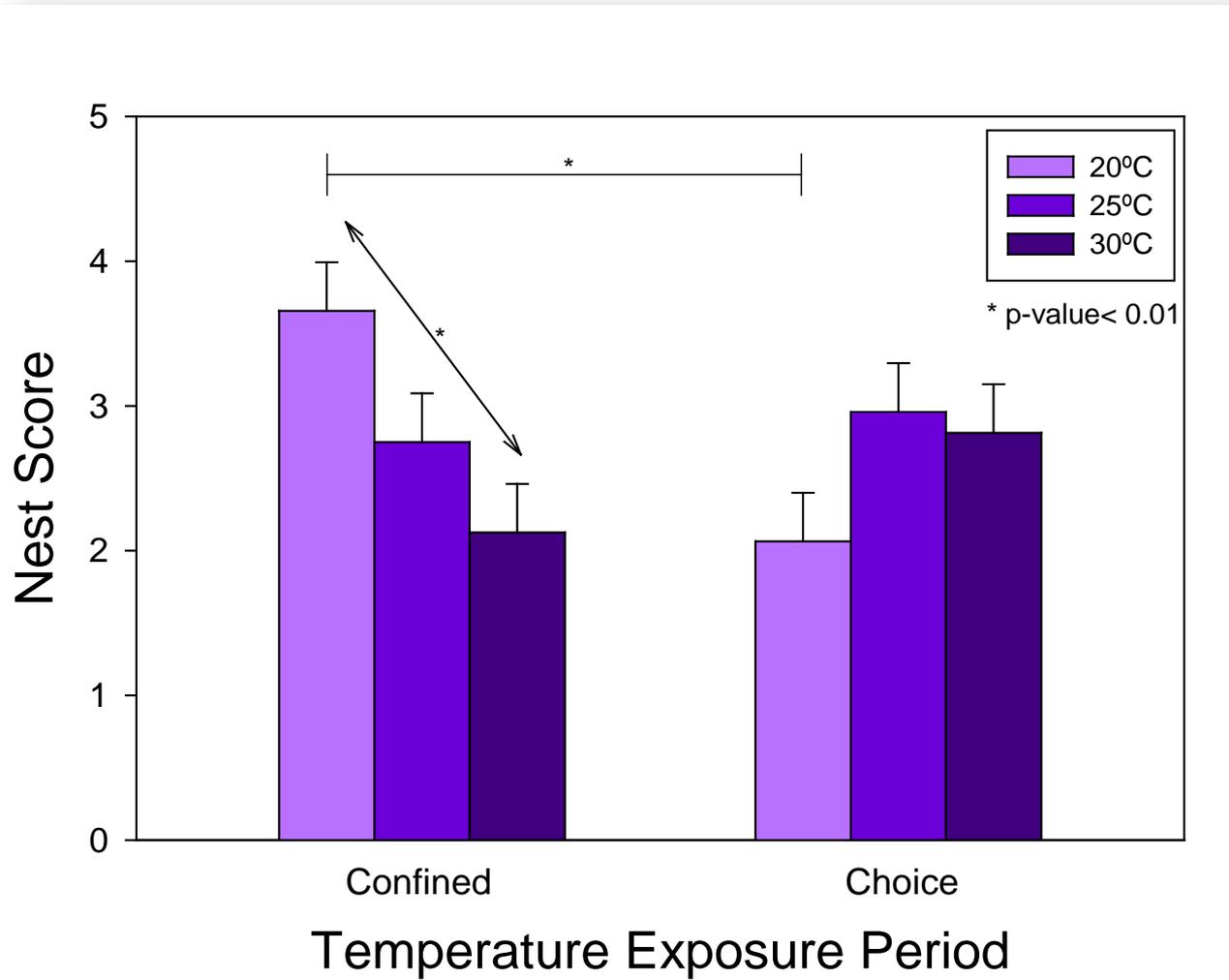
# Performance standards measured



# Heat loss decreases with better nests, regardless of treatment, strain or sex



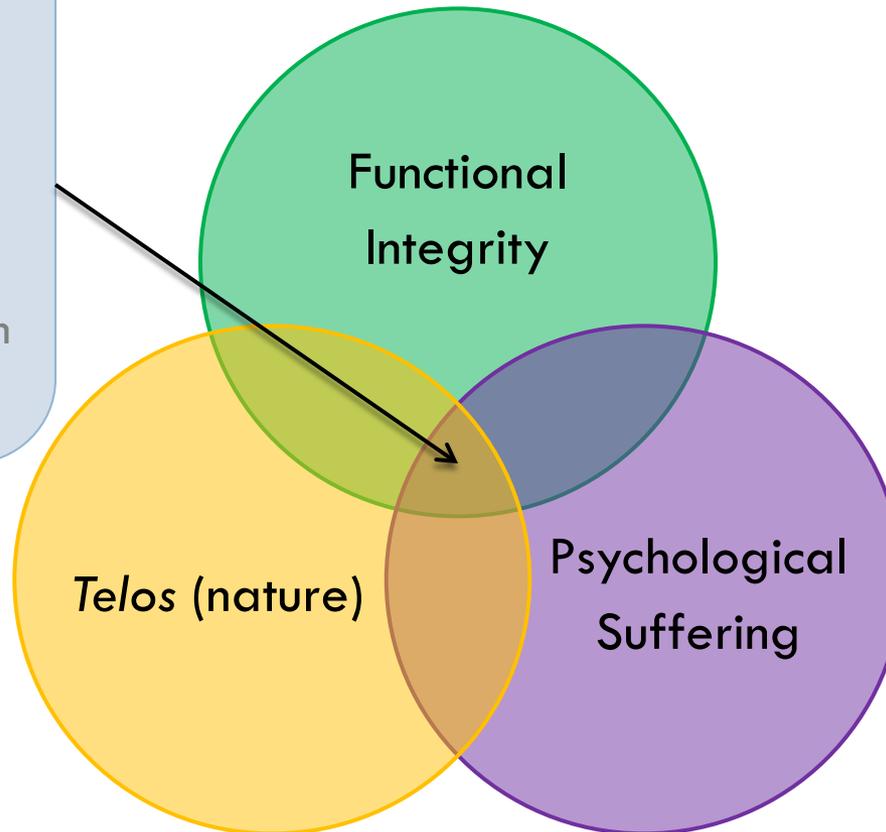
# Mice use nests to control cold they can't escape from



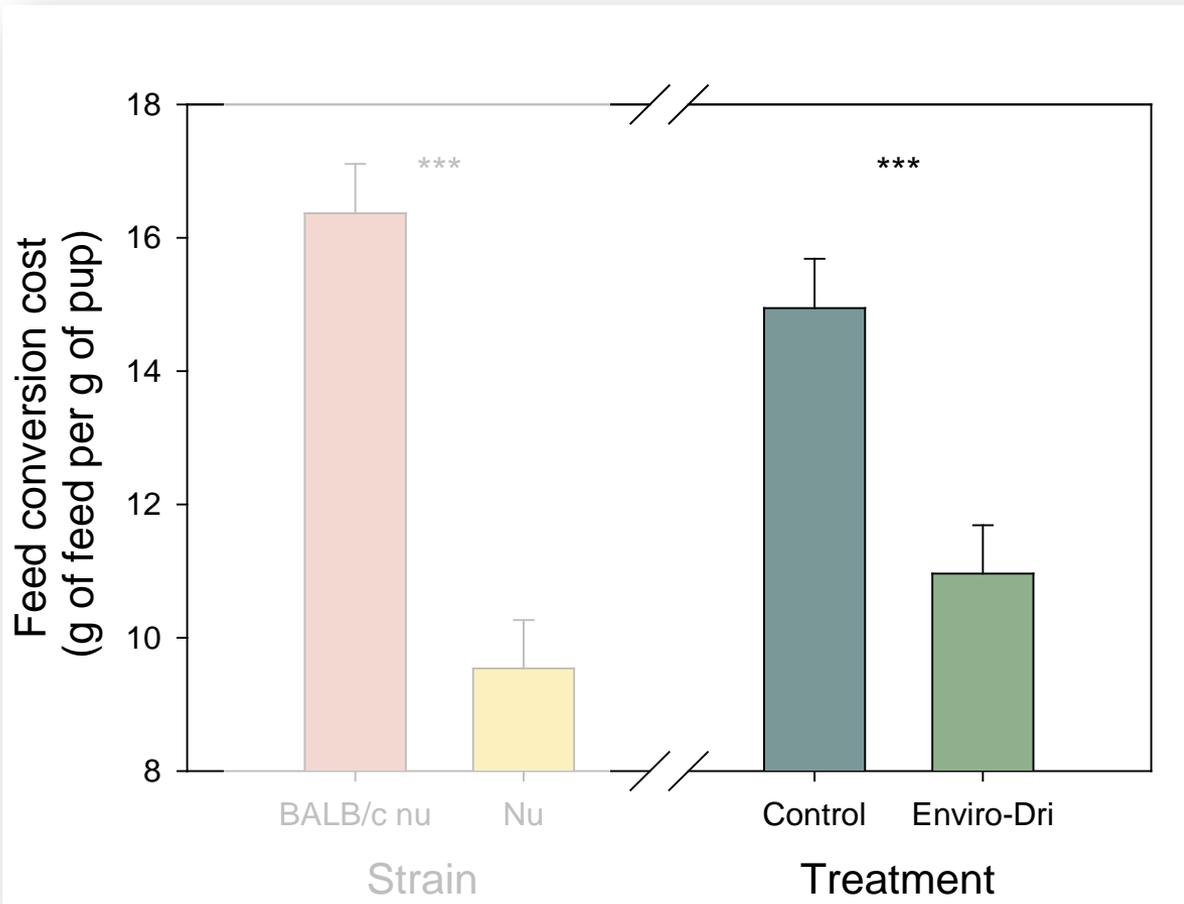
# Performance standards measured

## Coping and distress

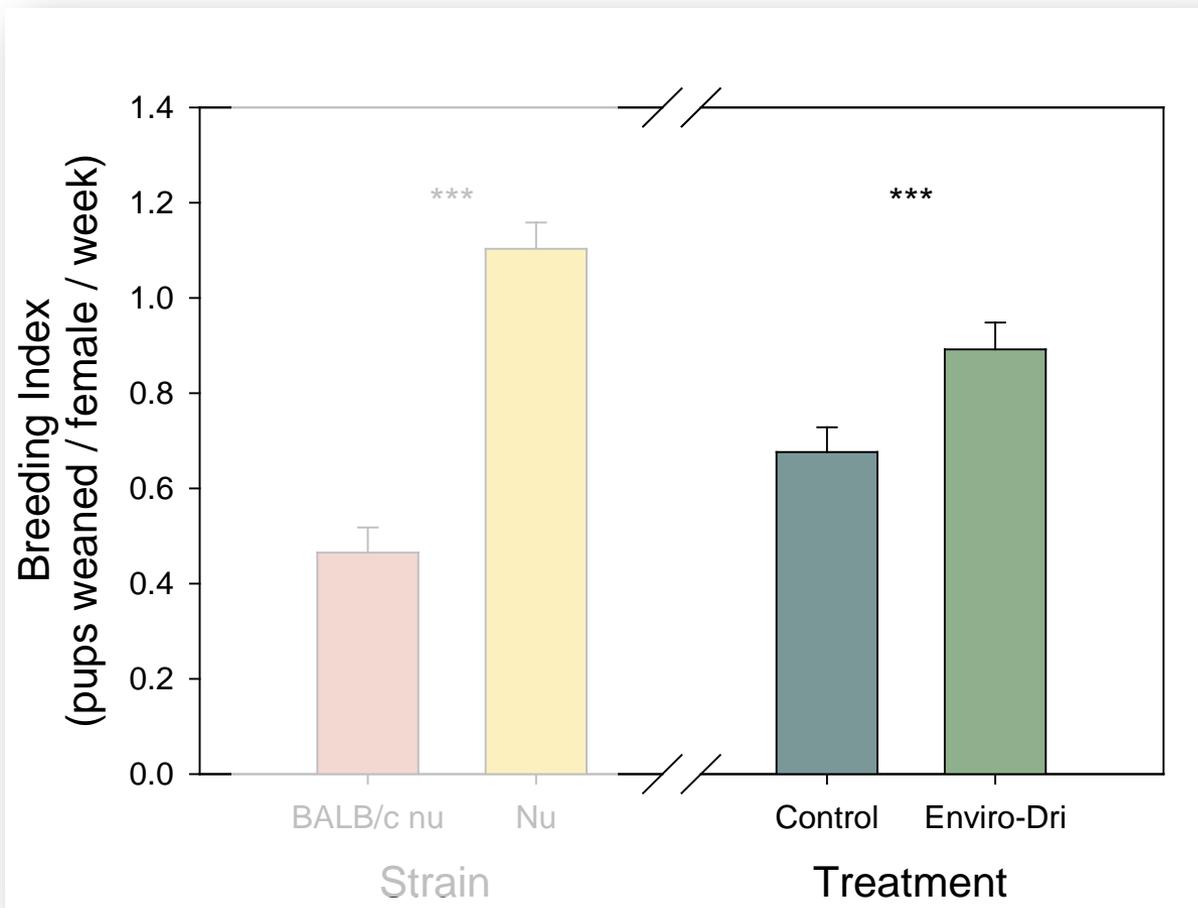
- Stress physiology
- Immune function
- Autonomic function
- Coping behavior



# Commercial scale implications – Nests conserve energy, reducing food use



# Commercial scale implications – Nests improve breeding performance



# Nesting material reduces feed cost per pup by roughly 27%

Control



Nesting

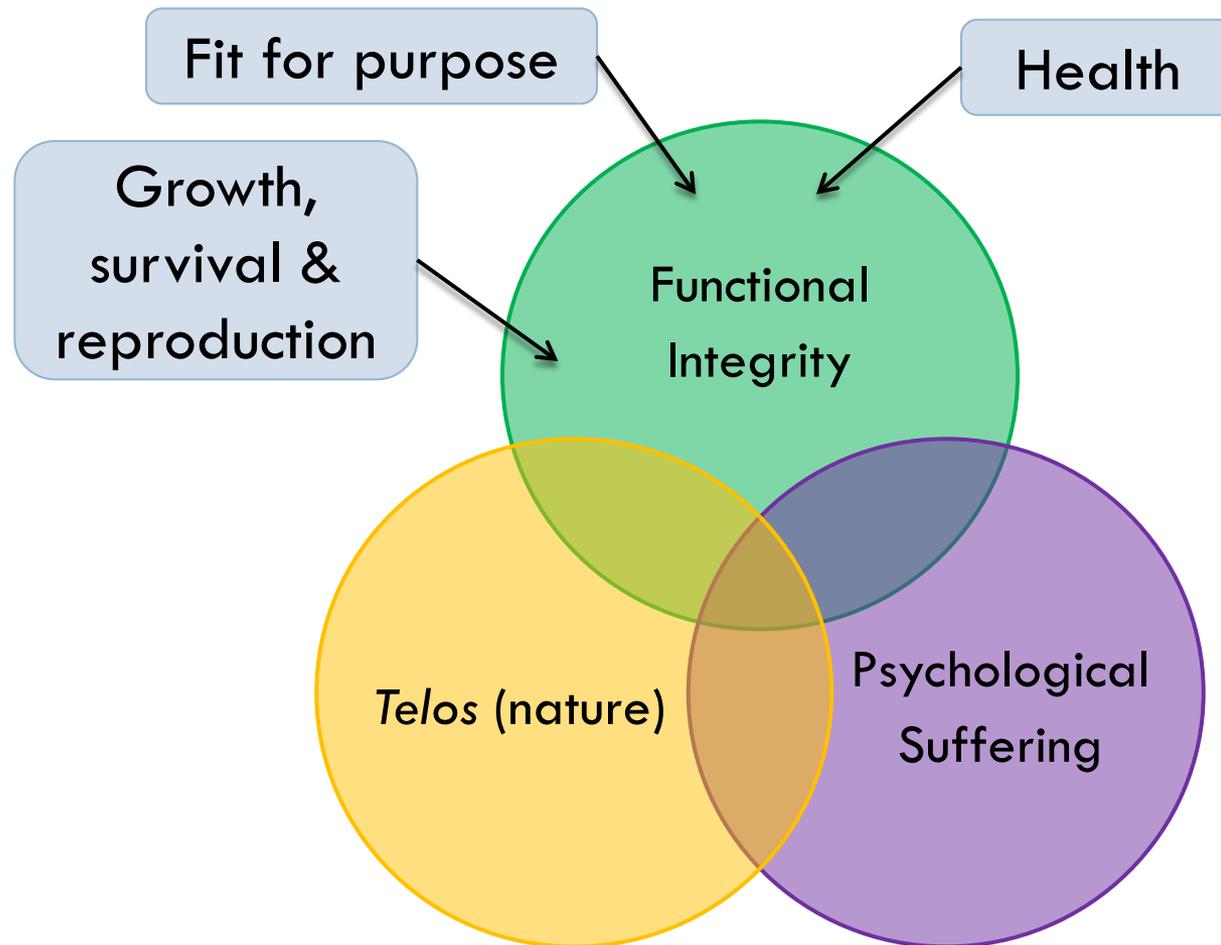


# Commercial scale implications – Return on investment

- Nesting material costs \$0.026 per cage per week
  - ▣ Total cost for 6 months = \$0.62
- Total pups gained per cage over 6 months:

	Total pups per cage with nesting material	Pups per cage without nesting material	Net pup gain with nesting material	Cost per pup	Cost of nesting material	Net gain per cage
Balb/c	34.5	16.5	18	\$47.00	(0.624)	\$845.37
NU	60.5	57.7	3	\$63.25	(0.624)	\$189.12

# Performance standards measured



# The lab from a mouse's point of view – Glass tower blocks and Tyrannosaurs

Animal wellbeing is the performance standard

Control – what does it mean to be an animal

An enrichment success story

The lab from a mouse's point of view

# Mouse environments deny *Telos*, and expose mice to stressors they cannot control

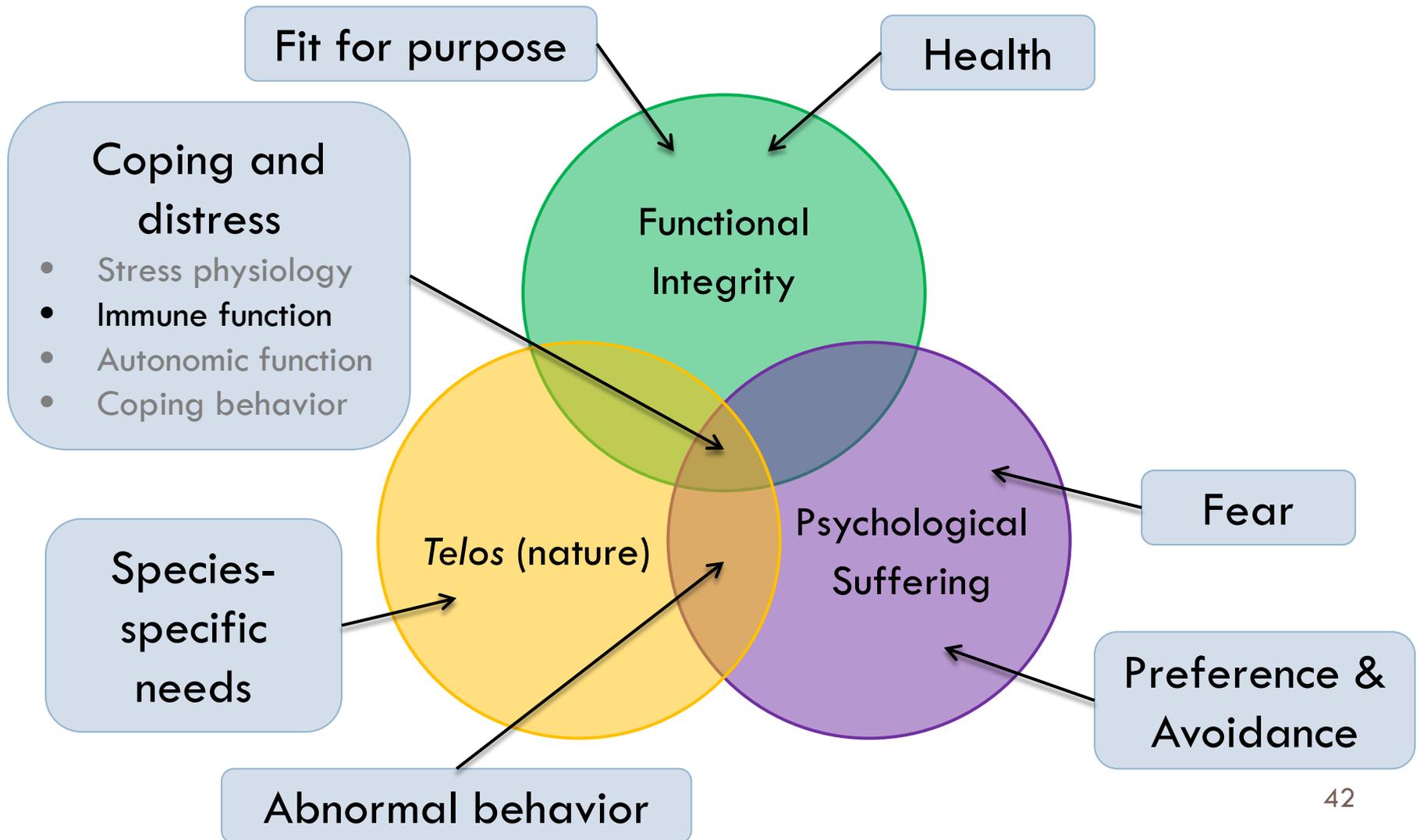
- Cold and draughts
- No shelter or nest site
- Constant presence of human predators
- Little opportunity for locomotion
- Too much light in the day, too little at night
- Disrupted social cues
  - Ultrasound
  - Cage cleaning disrupts essential odor cues
  - Shelters can provide cues that induce fighting



# The undesirable penthouse shoebox

- For ease of monitoring mouse cages are brightly lit
  - ▣ Albino strains often blinded (Brown & Wong, 2007)
- Cages are elevated, for efficiency
- Mice find brightly lit, open, elevated spaces aversive
  - ▣ The basis of anxiety / fear tests
  - ▣ Abnormal behavior and anxiety are greater on cages higher in the rack (Garner, 2004; Ader 1991)
  - ▣ Mice higher in the rack are also immune suppressed: female mice have are less likely to develop autoimmune diabetes (Ader, 1991)

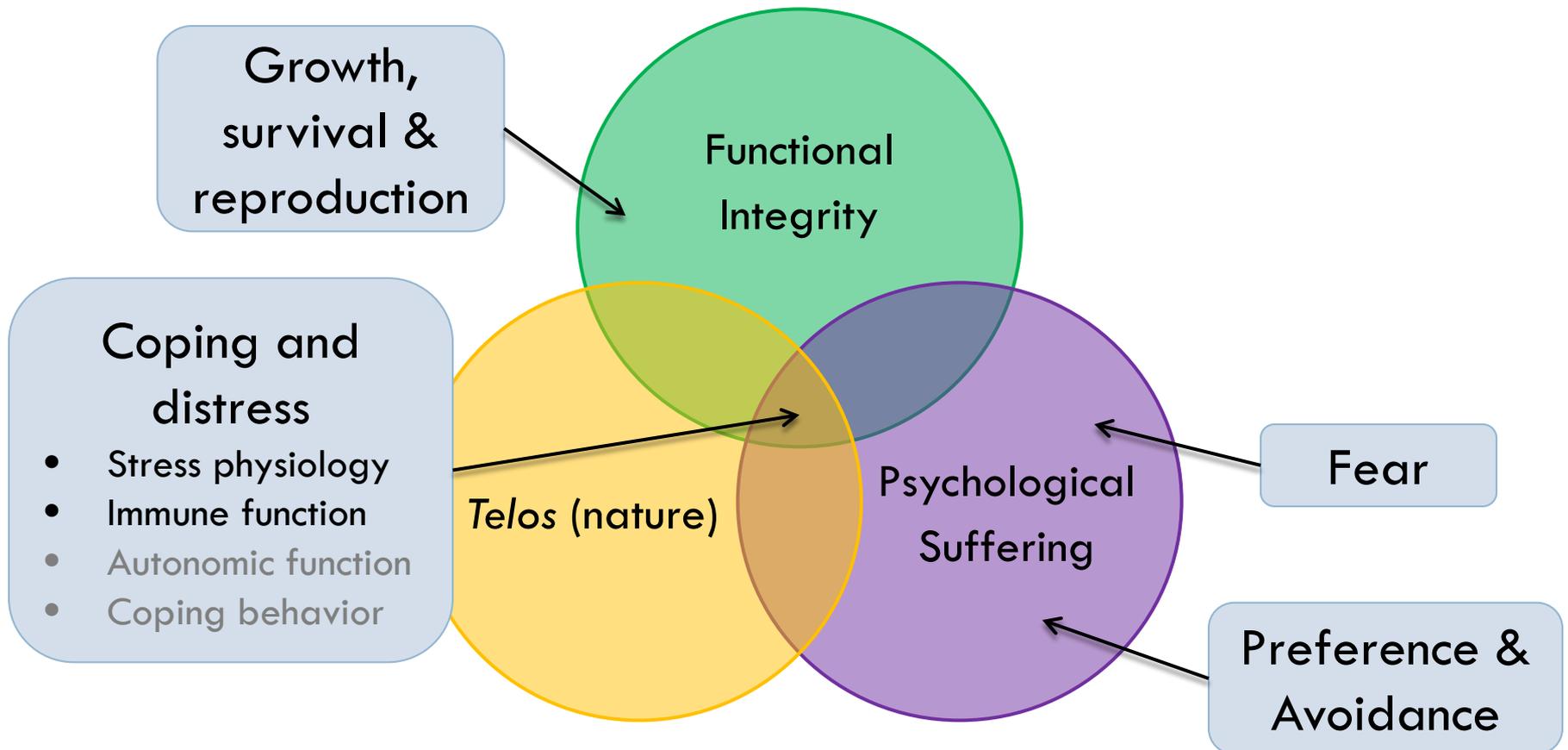
# Performance standards measured



# Too drafty for comfort?

- Ventilated cages reduce labor costs, bedding costs, and footprint
  - ▣ But is this good for mice?
  - ▣ 60 air changes an hour represents a wind speed of 3.6m/s through a 0.25" inlet for a typical shoebox
  - ▣ Bear in mind that many systems inject air around the drinker
- Mice find ventilation aversive (Baumans et al. 2002)
  - ▣ But not when provided nesting material
- Mice in ventilated cages are more fearful (Kallnick, 2007)
- Mice in ventilated cages show HPA sensitization, and are immune suppressed (Neigh, 2005)
- Mice in ventilated racks are more variable in reproductive performance (Tsai, 2003)

# Performance standards measured



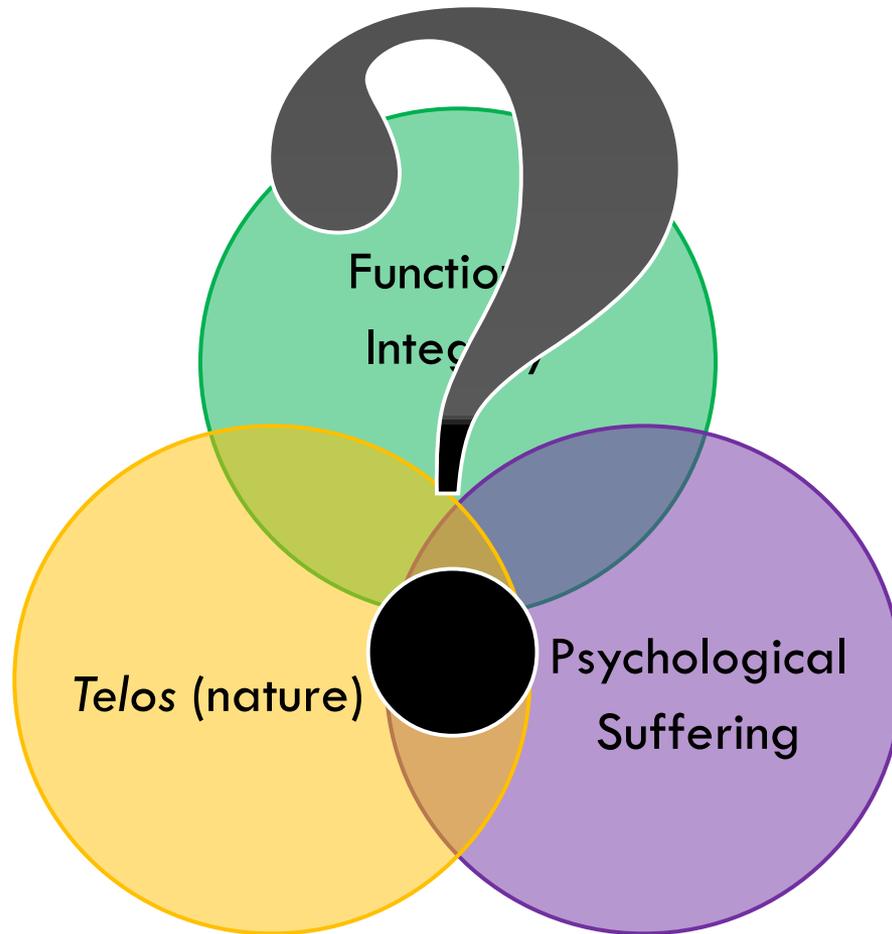
# Too crowded for comfort?

- (As a “should”) *The Guide* recommends at a minimum
  - ▣ 15in<sup>2</sup> for an adult male
  - ▣ 51in<sup>2</sup> for a female with pups
- Most people assume that these numbers are additive
  - ▣ i.e. 66in<sup>2</sup> for breeding pair, and 117in<sup>2</sup> for a trio
  - ▣ However *The Guide* is completely unclear on this point
  - ▣ *The Guide* does imply that space is additive for non-breeding cages
  - ▣ Frankly, the literature is a lot clearer than *The Guide* if one reads critically, (for instance applying some of the principles in this talk!)
- OLAW’s interpretation is that these values are not additive for breeders
  - ▣ i.e. that *The Guide* does not require an increase in space for trios
- HOWEVER, OLAW also states
  - ▣ “Blanket, program-wide departures from the Guide for reasons of convenience, cost, or other non-animal welfare considerations are not acceptable... the IACUC must ensure that cage population does not negatively impact animal welfare ...”

# This begs three questions

- Is the welfare of trios worse than pairs?
  - ▣ Perhaps
  - ▣ In wild mice trios only co-operate under very specific conditions, and pup survival is always worse if two females are breeding
  - ▣ BUT this has probably been selected against in lab mice
- Would increasing floor space solve it?
  - ▣ Probably not (i.e. essentially OLAW's position)
  - ▣ To answer this question a study needs to alter both floor space and group size (no such studies in breeders)
  - ▣ From studies in non-breeders we know that mice respond primarily to group size, not space
- Is there any benefit to trio breeding?
  - ▣ Perhaps not per female, but there is an economic benefit per cage
  - ▣ BUT OLAW expressly disallows cost as the primary basis for a decision

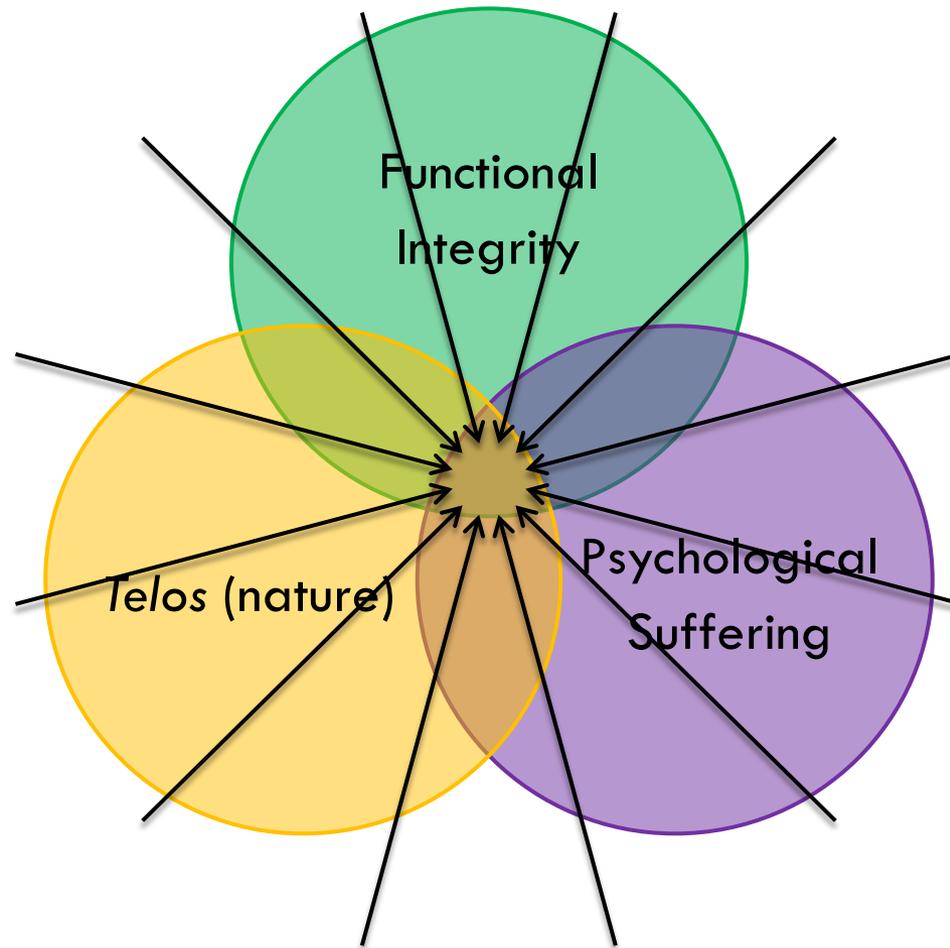
Beware! Absence of evidence is not evidence of absence.





# In summary

Animals exist to behave, behavior exists to control.  
When animals cannot control their environment,  
wellbeing, science, and performance standards suffer.



# Thanks to... and a shameless plug



[www.mousebehavior.org](http://www.mousebehavior.org)

- GLAS, UFAW
- Charles River Labs
- Students
  - ▣ Brett DuFour
  - ▣ **Brianna Gaskill**
  - ▣ Sarah Hess
  - ▣ Chris Howerton
- Collaborators
  - ▣ **Kathleen Pritchett-Corning**
  - ▣ Christopher Gordon
  - ▣ Joy Mench
  - ▣ Christina Winnicker

# Performance Measures and Cage Space Exceptions to the 8<sup>th</sup> Edition of the *Guide*

Guy B. Mulder, DVM, MS, DACLAM  
Director, Professional Services

# Overview

- Review the process Charles River is utilizing when evaluating alternatives to rodent cage space recommendations in *Guide*
  - Applies only to the commercial production of mice and rats
- Key aspects of the process
  - IACUC review and approval
  - Performance based
  - “Clinically relevant” assessment criteria
  - Performed in barrier production settings

# Background

- Charles River production facilities
  - Eight sites in North America
    - United States (7)
      - AAALAC, Int. accreditation
      - OLAW Assurance
    - Canada (1)
      - CCAC accreditation
- Commercial production of barrier and isolator-reared animals
  - Individual barrier room may contain up to ~10,000 cages
  - Limited research performed within production settings



# Cage space recommendations

- **Scientific basis** for space recommendations is **unclear**
- Studies evaluating cage space and effects of social housing, group size, density and housing conditions have reported **inconsistent results**
- "...difficult to compare these studies due to the study design and experimental variables that have been measured."
- Variables that may affect utilization of cage space
  - Species
  - Strain (and phenotype)
  - Age
  - Gender
  - Quality of space
  - Structures within the space

(p 58)

TABLE 3.2 Recommended Minimum Space for Commonly Used Laboratory Rodents Housed in Groups\*

Animals	Weight, g	Floor Area/Animal, <sup>a</sup> in. <sup>2</sup> (cm <sup>2</sup> )	Height, <sup>b</sup> in. (cm)	Comments
Mice in groups <sup>c</sup>	<10	6 (38.7)	5 (12.7)	Larger animals may require more space to meet the performance standards.
	Up to 15	8 (51.6)	5 (12.7)	
	Up to 25	12 (77.4)	5 (12.7)	
	>25	≥15 (≥96.7)	5 (12.7)	
Female + litter		51 (330) (recommended space for the housing group)	5 (12.7)	Other breeding configurations may require more space and will depend on considerations such as number of adults and litters, and size and age of litters. <sup>d</sup>
Rats in groups <sup>c</sup>	<100	17 (109.6)	7 (17.8)	Larger animals may require more space to meet the performance standards.
	Up to 200	23 (148.35)	7 (17.8)	
	Up to 300	29 (187.05)	7 (17.8)	
	Up to 400	40 (258.0)	7 (17.8)	
	Up to 500	60 (387.0)	7 (17.8)	
	>500	≥70 (≥451.5)	7 (17.8)	
Female + litter		124 (800) (recommended space for the housing group)	7 (17.8)	Other breeding configurations may require more space and will depend on considerations such as number of adults and litters, and size and age of litters. <sup>d</sup>

\*The interpretation of this table should take into consideration the **performance indices** described in the text beginning on page 55.

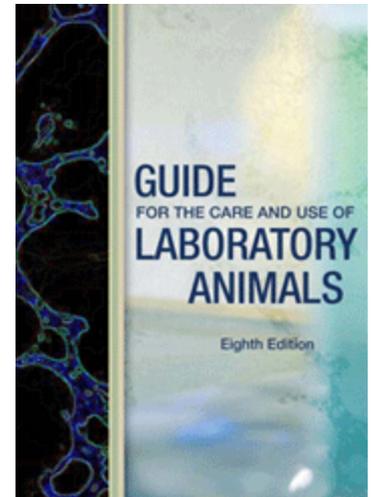
<sup>d</sup>Other considerations may include culling of litters or separation of litters from the breeding group, as well as other methods of more intensive management of available space to allow for the safety and well-being of the breeding group. Sufficient space should be allocated for mothers with litters to allow the pups to develop to weaning without detrimental effects for the mother or the litter.

(p 57)

# Performance standards

The *Guide* endorses a performance-based approach....

- Performance approach requires... (p 6)
  - Professional input
  - Sound judgment
  - Team approach
  - Clearly defined outcomes and goals
  - Monitoring on a regular basis



# Performance standards

## Space allocation

“...should be assessed, reviewed, and modified as necessary by the IACUC considering the performance indices...” (p 56)

- Health
- Reproduction
- Growth
- Behavior
- Activity
- Space utilization
- And any special needs/characteristics of the model

# IACUC review & approval

- Standardized review/proposal format
  - Specific procedure/practice for exception
  - Species affected
  - *Guide* recommendation on the subject
  - Proposed exception
  - Rationale for the exception
  - Proposed performance measures
  - Proposed methods to investigate the exception (research proposal)
    - Including statistical analysis when applicable
  - Post-approval monitoring requirements

## Request for Exception to ILAR 2011 Guide Recommendations

**Procedures or Practice:**

**Species Affected:**

**History:**

**Conditions or Circumstances Resulting in Exception Request**

**Guide Recommendations:**

**Proposed Exception:**

**Rationale for Exception:**

<input type="checkbox"/>	Equivalence of conditions
<input type="checkbox"/>	Changes required to ensure animal wellbeing
<input type="checkbox"/>	Other (explain)

**Proposed Performance Measures:**

**Study Proposal to Determine Acceptability\*:**

*(Institutional study proposal inserted here or attached to this form)*

**Other Supporting Information or Data\*\*:**

*(May be included with study proposal if attached separately)*

Request

### IACUC Actions:

Initial Study Proposal:

<input type="checkbox"/>	Approval
<input type="checkbox"/>	Disapproval
<input type="checkbox"/>	Disapprove; resubmit based upon IACUC Required Changes

IACUC Review Following Initial Study:

Summary of initial study\*\*\*:

Methods:

Results:

Analysis and interpretation (conclusions):

+

<input type="checkbox"/>	Approval
<input type="checkbox"/>	Disapproval
<input type="checkbox"/>	Disapprove; conduct additional data collection

IACUC approved post approval data collection and analysis:

Parameters:

Frequency of Data Collection and Analysis Frequency:

Frequency of IACUC Review:

### Date of IACUC Review:

Initial Study Proposal:

Review of Study Findings:

Other Reviews (including post approval monitoring):

\* A completed institutional protocol form should be submitted along with this document for initial review.

\*\* Include pertinent literature citations and other supporting data.

\*\*\* Summarizes study and results once completed.

# Performance Measures

- **Objective measures** that are **clinically relevant** and clearly **quantifiable**
- Must compare proposed “alternative/exception” to *Guide* recommendations
- Measurements should be **practical** and **collected in the animal room**
  - Not reliant on extensive instrumentation, sampling or other practices that may induce confounding variables or limit sample size
  - e.g., telemetry for heart and respiratory rate, serum and urine constituents, complex behavioral assessments, etc.
- May supplement with laboratory measurements

# Performance measures

- Should have a direct relationship (relevancy) to the alternative/variance being proposed
  - **Indirect associations**, with greater possibilities of impact by uncontrolled variables are much *less desirable*
    - e.g., breeding success (performance) should be a sensitive indicator of chronic stress/distress

# Assessing breeding colonies

- Space-related welfare issues should be detectable and quantifiable by statistically analyzing:
  - Reproductive indices
  - Behavioral observations
  - Clinical observations
- Multiple parameters from each category are utilized
- Studies collected in standard production settings
  - Barrier rooms or isolators
- If equivalency is established
  - Post-approval monitoring is performed using key performance indicators

# Reproductive performance

- Example indices
  - Production Index (PI)
    - Pups per female breeder per week
  - Litter size at birth
  - Survival to weaning
  - Sex ratio
  - Weaning weight
  - Inter-litter interval
  - Time to first plug
  - Time to female pups vaginal opening



# Behavioral assessment

- Examples
  - Aggression/Fighting
    - Maternal aggression towards pups
    - Aggression between weanlings and adults
  - Hair loss
  - Stereotypic behavior
  - Space utilization within cage
- Scoring systems
  - For each parameter
  - Most data can be collected in production rooms by husbandry technicians
    - Space utilization requires 24-7 videography, difficult to achieve in a functioning barrier room.
  - Allows quantification of data and comparison across sites

# Behavioral assessments

## Fighting/Aggression

Wound Severity Scoring:	
0 = none	No wounding observed
1 = mild	Bruising or slight scratch; barely breaks skin integrity. Smaller than 1cm total area affected. No veterinary care required.
2 = moderate	Definite wound; breaks skin integrity. 1-1.5 cm area affected. Veterinary care may be indicated.
3 = severe	Multiple wounds or severe ulceration or bruising. >1.5 cm area affected. Veterinary care definitely required; consider treatment or euthanasia.

## Barbering

0 = full pelage	No hair loss observed (to include vibrissae)
1 = mild	Up to 30% of pelt denuded
2 = moderate	Up to 50% of pelt denuded
3 = severe	Up to 75% of pelt denuded
4 = fully denuded	

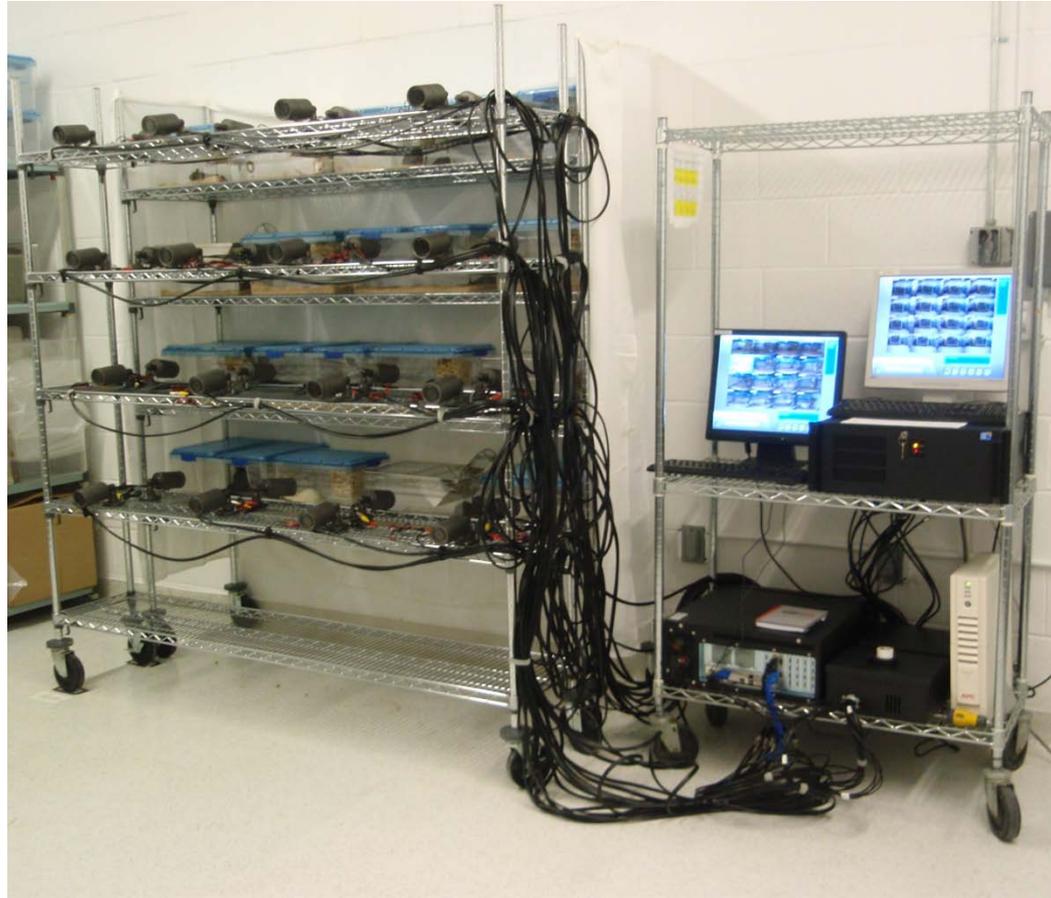
# Behavioral measures

## Stereotypic Behaviors

Behavior	Definition	Duration
Bar Chew	A bout of repeated bites into the metal bars at a particular spot on the cage lid	Count the number of episodes & the duration (frequency, duration, intensity)
Jumping	A bout of either jumping up and down or scratching with the paws along the wall in a corner of the cage	Count the number of episodes & the duration
Pace	A repeated walking pattern that appears without purpose, and is non-circular (see circle/flip)	Count the number of episodes & the duration
Circle/flip/tail carrying	A repeated walking or climbing pattern that appears without purpose. Circling is “two dimensional” and may or may not include carrying the tail in the mouth at the same time. Flipping is a “three dimensional” movement.	Count the number of episodes & the duration

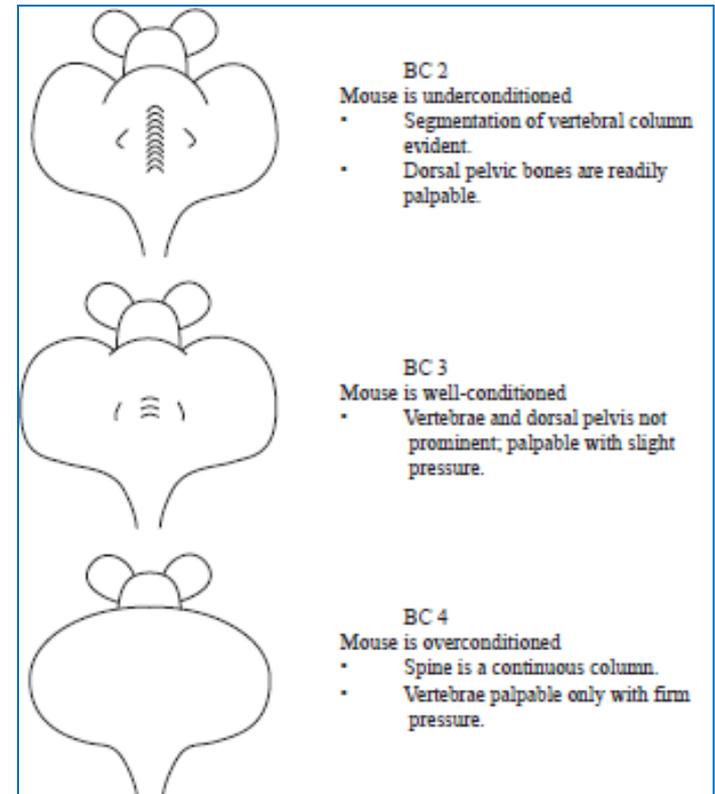
# Behavioral measures

- Space utilization
  - Performed in a behavioral lab
  - 24-7 video
  - Light and dark cycle recording



# Clinical measures

- Examples
  - Mortality
    - By sex, breeding colony segment, etc.
  - Animals euthanized for cause
  - Body conditioning scoring
  - Growth curve comparisons
    - Weaning through 10 weeks of age



LAS, 49(3), pg. 319-23, 1999

# Will all stocks, strains and genotypes be evaluated?

- **No.**
- Evaluating all possible stocks, strains and genotypes is not practical (or affordable)
- Concentrating on common lines
  - e.g., C57BL/6, CD-1, CD, etc.
  - Selected stocks and strains reflect the majority of mice and rats being produced and utilized for research

# Will animals be evaluated at every production site?

- **No.**
- There is no reason that animals studied at one site under defined environmental and husbandry conditions should react differently at another location with the same environmental and husbandry conditions.
- However, if in the IACUC's judgment there is an important difference in conditions between sites, then a comparison study may be warranted.

# Post-approval monitoring

- IACUC review of performance indicators
  - Annual review of the variance for 2 consecutive years
    - Key performance indicators
  - *De novo* review at end of year 3
    - Literature review
    - Determine if new/additional studies are warranted
- Additional ongoing review
  - Production management review production indices routinely (weekly to monthly)
  - Veterinarians involved as needed when problems arise
    - Communicate with IACUC as necessary

# What happens if differences are found?

- It depends...
  - Are the differences physiologically or behaviorally relevant?
  - Single or multiple parameters?
- It is unlikely that all findings will be in agreement between *Guide* recommendations and alternatives
- Detrimental effects to animal welfare should be able to be substantiated by additional (alternate) measures, and...
  - Include a reasonable correlation to a mechanism of action

# Post-approval monitoring

- Significant departures need to be investigated by attending veterinarian, production staff, and discussed with the IACUC
  - Just as with any health issue/adverse outcome--requires investigation with applicable diagnostic methods to establish a root cause
    - e.g., Decreased reproductive performance may be due to medical issues unrelated to cage space

# Current studies: Cage space and breeding performance

## Rats

- Breeding pairs, 2 lines
- 4 commercially available cage sizes
  - Small, CR current cage, *Guide* standard & Larger
- Performance measures
  - Breeding performance
    - Multiple indices
  - Behavioral assessments
    - Space utilization (24 hrs. video)
    - Aggression, hair loss, stereotypic behaviors, etc.
  - Clinical assessments
    - Mortality, weight curves, etc.
- Includes data collection in barrier
- Similar mouse study to start soon



# Summary

- Evaluation of equivalency based on statistically significant, clinically relevant assessments of functioning production colonies
- Multiple assessment criteria
  - Behavioral assessment
  - Reproductive performance
  - Clinical observations
- Limited to representative lines
- IACUC approval and oversight
  - Including post-approval assessment and monitoring

**Thank you**

**Questions?**

# Upcoming OLAW Online Webinars

Departures From the Guide	September 13, 2012
Topic TBA	December 13, 2012

OLAW Online Webinars are held at 1:00 PM (Eastern)



# Questions?

Type your question into the “Chat” box on your monitor and press submit. Only the OLAW staff will see your question.

