

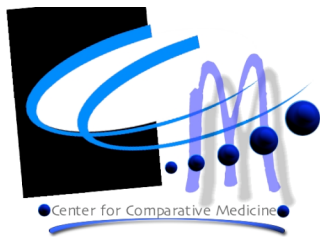
# Opportunities for Detecting Pain and Distress Associated with Acute Systemic Toxicity

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Steven M. Niemi, DVM  
Center for Comparative Medicine  
Massachusetts General Hospital

NICEATM Workshop on Acute Chemical Safety Testing  
Bethesda, MD  
February 7, 2008



# The Four R's

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Replacement

Reduction

Refinement

Relevance

# Acute Toxicity Objectives

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- Identify full spectrum of possible effects
- Detect earliest symptoms and signs
- Apply to “normal” victims
  - Covers majority of likely cases (*i.e.*, in reasonable health)
  - Provides regulatory simplicity

# Common Symptoms of Poisoning

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abominal pain	depression	headache	nausea
agitation	dimmed vision	incoherence	nervousness
angina	dizziness	irritability	palpitations
blindness	drowsiness	irritation of eyes	paranoia
burning feet	emotional lability	leg cramps	paresthesia
burning GI pain	euphoria	lethargy	psychotic-like states
chills	excitation	lumbar pain	respiratory tract irritation
choking	excitement	malaise	thirst
colic	fatigue	mania	tinnitus
confusion	hallucinations	mucosal irritation	vertigo
delirium			

# Common Clinical Signs of Poisoning

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abnormal breath odor	cough	hyperpyrexia	ocular, facial palsy	skin vesicles
abnormal urine odor	cyanosis	hypertension	oliguria	smoky urine
abortion	deafness	hyperthermia	oxaluria	sneezing
alopecia	<b>death</b>	hyperventilation	pallor	spasticity
angioedema	decreased respiration	hypotension	paralysis	stained lips
anorexia	dehydration	hypothermia	parkinsonism	stimulation
anuria	diaphoresis	incoordination	perspiration	stomatitis
areflexia	diarrhea	increased activity	pinpoint pupils	stupor
asphxia	dilated pupils	insomnia	pneumonia	sweating
ataxia	dry mouth	iridocyclitis	prostration	tachyarrhythmia
bloody diarrhea	dry skin	jaundice	pulmonary congestion	tachycardia
bradycardia	dysphagia	lacrimation	pulmonary edema	tachypnea
bright red venous blood	dyspnea	laryngeal edema	QT prolongation	tetany
brown urine	emphysema	loss of corneal reflex	rales	throat constriction
brown mucous membranes	exaggerated reflexes	menorrhagia	rashes	torticollis
burns	fever	miosis	restlessness	tremors
cardiac arrest	flushing	muscle fasciculations	retching	unconsciousness
cardiac arrhythmias	frothing at the mouth	muscle spasms	retinal injury	urinary retention
carpedal spasm	gangrene of feet	muscular rigidity	rhinorrhea	urticaria
cataracts	glottal edema	mydriasis	salivation	violent behavior
CNS depression	glottal spasm	myodystonia	sedation	vomiting
CNS excitation	hematemesis	narcosis	seizures	wakefulness
<b>coma</b>	hematuria	nystagmus	shallow respirations	weakness
convulsive movements	hepatomegaly	obtundation	skin irritation	

(<http://www.merck.com/mmpe/index.html>)

# Usual Signalment of Poisoning Victims

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- Infants, adolescents, young adults, elderly
- Exposure accidental, habitual, intentional
- Varied racial, ethnic, economic status
- Varied diet
- “Housed” with families, neighborhoods
- “Enriched” environment

# A Flawed Premise

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Animal dosed in a spartan setting



Animal's health declines to  
moribundity or death



*In vitro* surrogate

# A Clinically Oriented Approach

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Animal dosed in more natural situation



Detect **early and common** Sx



Determine sequence & severity of  
organs/tissues affected



*In vitro* surrogate**s**



# Early Symptom of Poisoning ≈ Malaise

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“A feeling of general discomfort or uneasiness, an ‘out-of-sorts’ feeling, often the first indication of an infection or other disease.” (<http://www.stedmans.com>)

*Can one detect malaise or discomfort in animals?*

# Abnormal Behavior Cues

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- ↓ General activity
- ↑ General activity (restlessness, fighting)
- ↓ Appetite (eating)
- ↓ Libido

# Abnormal Activity Metrics (1)

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***Clever Sys Inc.***  
***The Behavior Recognition Company***

<http://www.cleversysinc.com/>

## **TopScan Software Suite**

*LocoScan*

Locomotor and open field behavior.

*MazeScan*

Advanced maze tasks.

*ObjectScan*

Object recognition and novelty seeking behavior.

*TopScan*

Top-view video tracking system.

## **DepressionScan Software Suite**

*DSRScan*

Dominant and submissive behaviors.

## **HomeCageScan Software Suite**

*HomeCageScan*

Home-cage monitoring and circadian rhythm studies.

## **NeurodegenScan Software Suite**

*CylinderScan*

Spontaneous forelimb tasks.

*RunwayScan*

Voluntary locomotion gait analysis.

## **Independent Software Programs**

*DrugEffectScan*

Detection of common behavioral side-effects.

*FreezeScan*

Fear conditioning and freezing tasks.

*PrimateScan*

Home-cage monitoring for primates.

*SeizureScan*

Seizure detection for small lab animals.

*SonoScan*

Capture and analysis of ultrasonic vocalizations.

*StereoScan*

Home-cage monitoring from top and side views.

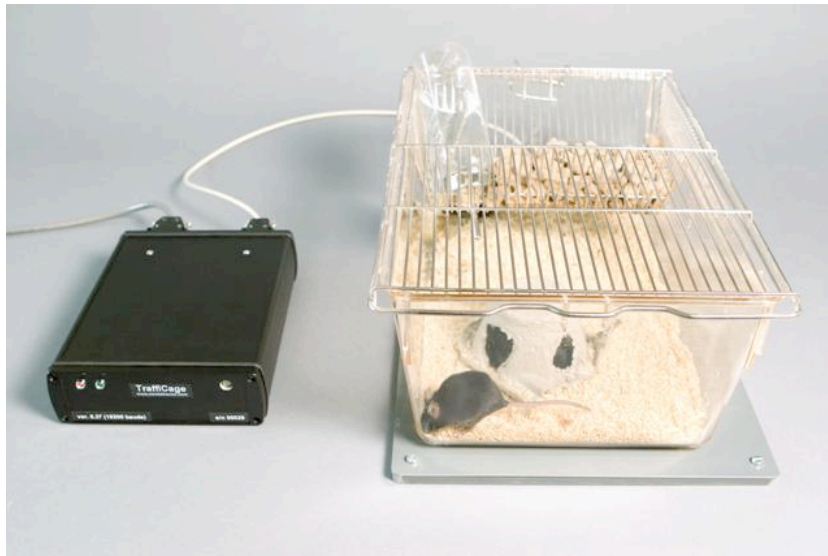


# Abnormal Activity Metrics (2)

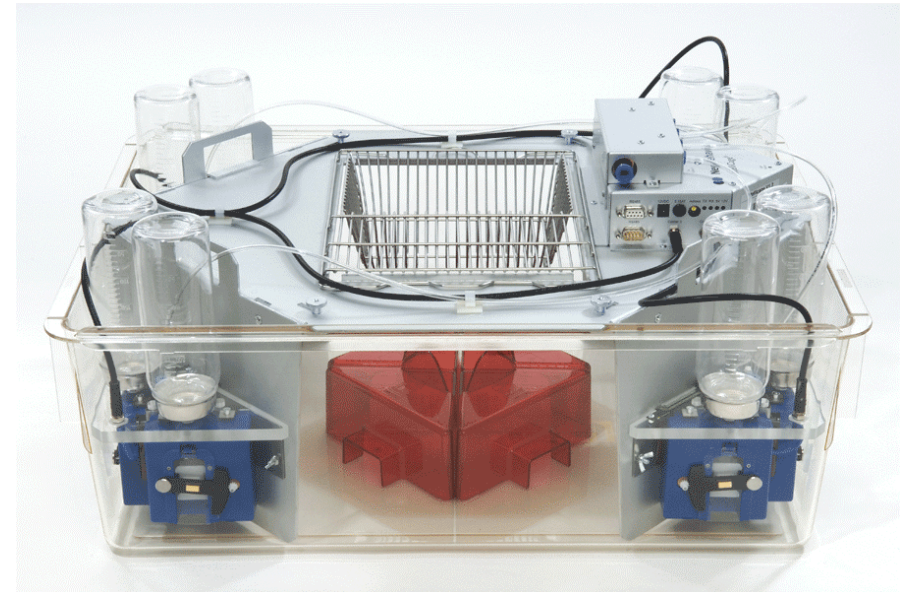


<http://www.newbehavior.com/>

**TraffiCage<sup>®</sup>**



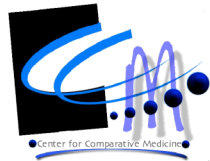
**IntelliCage<sup>®</sup>**



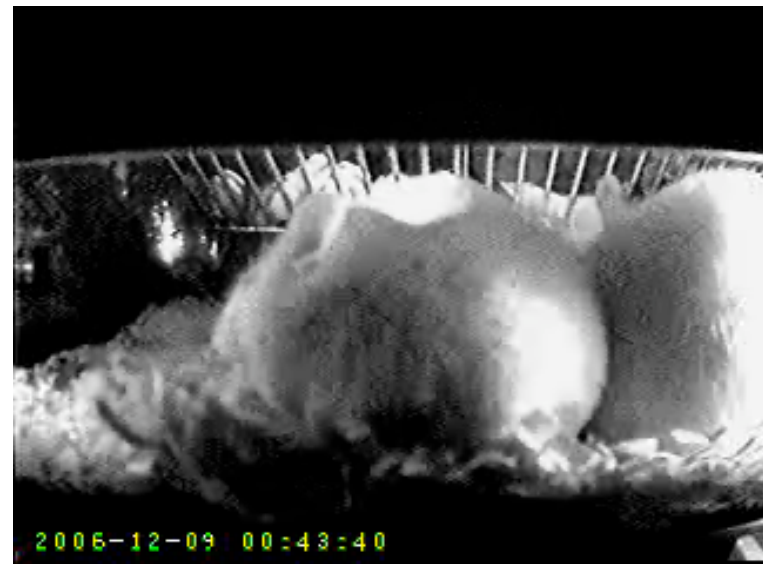
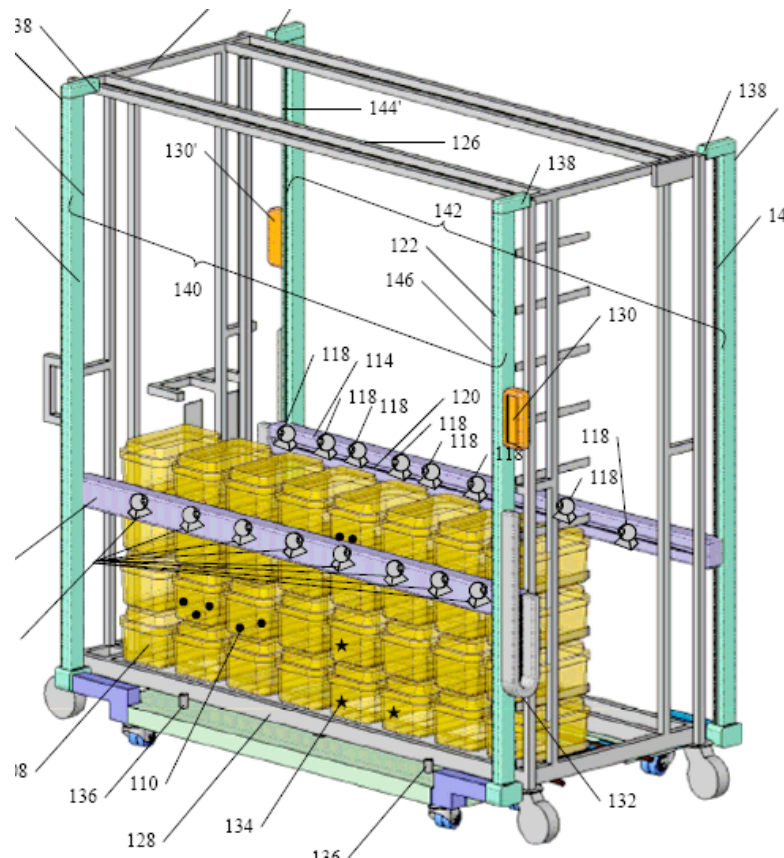
# Abnormal Activity Metrics (3)

- Gantry-mounted cameras scan all cages on a rack 24/7, images web-accessed

## MyMice<sup>®</sup>



- Duration of recording intervals programmable, web-based controls



# Sounds as Endpoints

Open access, freely available online PLOS BIOLOGY

## Ultrasonic Songs of Male Mice

Timothy E. Holy\*, Zhongsheng Guo

Department of Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, Missouri, United States of America

Previously it was shown that male mice, when they encounter female mice or their pheromones, emit ultrasonic vocalizations with frequencies ranging over 30–110 kHz. Here, we show that these vocalizations have the characteristics of song, consisting of several different syllable types, whose temporal sequencing includes the utterance of repeated phrases. Individual males produce songs with characteristic syllabic and temporal structure. This study provides a quantitative initial description of male mouse songs, and opens the possibility of studying song production and perception in an established genetic model organism.

Citation: Holy TE, Guo Z (2005) Ultrasonic songs of male mice. PLoS Biol 3(12): e386.

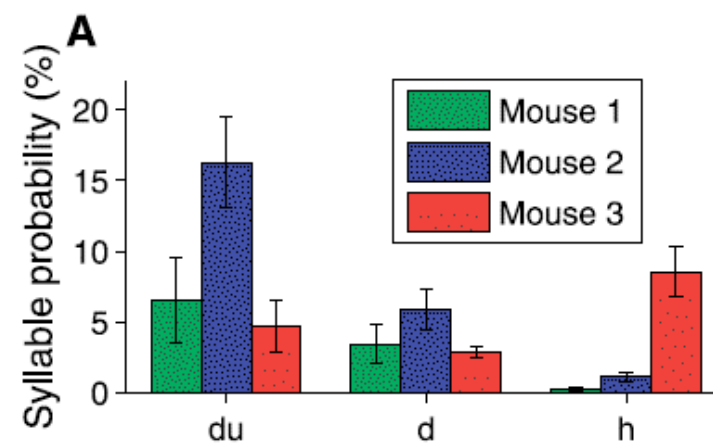
**Table 1.** The Most Common Syllable Types in Mouse Ultrasonic Vocalizations, Labeled by Pitch Jump

Syllable Type <sup>a</sup>	Number (Trial A)	Percent (Trial A)	Percent (Population)
SS	360	48	70–93
du	97	13	2–15
h	132	18	0–12
d	16	2	1–4
hdu	89	12	0–2
u	5	1	0–2
hd	20	3	0–1
Remainder	31	4	1–4

<sup>a</sup>“Trial A” refers to the trial analyzed in Figure 2B. “Population” refers to a single trial from each of 45 males; the ranges indicate the boundaries of the first and last quartiles.

<sup>b</sup>Pitch jumps are arranged in their temporal order, so that “hd” refers to a syllable with an “h” jump followed by a “d” jump.

DOI: 10.1371/journal.pbio.0030386.t001



# Saliva and Corticosteroids

## Biomarkers Core Laboratory Yerkes National Primate Research Center

[http://www.research.yerkes.emory.edu/biomarkers\\_core/assay/index.html](http://www.research.yerkes.emory.edu/biomarkers_core/assay/index.html)

Hormone	Assay Method	Assay Sensitivity	Species	Sample Type	Minimum Sample Volume
Cortisol	EIA	0.1 µg/dl	Monkey, human	Saliva	75 µl
Cortisol "Free"	RIA	0.5 µg/dl	Monkey, human	Urine	375 µl



Groundbreaking discoveries  
*ADVANCING SCIENCE AND IMPROVING HEALTH*

Worldwide collaboration  
Unique resource  
Worldwide Translational research





# A Benign Oriented Approach

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Observations without handling



Non-invasive sampling (sounds, voided urine & feces, expired air)



Non-painful/non-distressful sampling  
(saliva, tears, hair)



Temporarily stressful (bleed, anesthetize, euthanize)

# Measuring Emotional Responses to Toxicants?

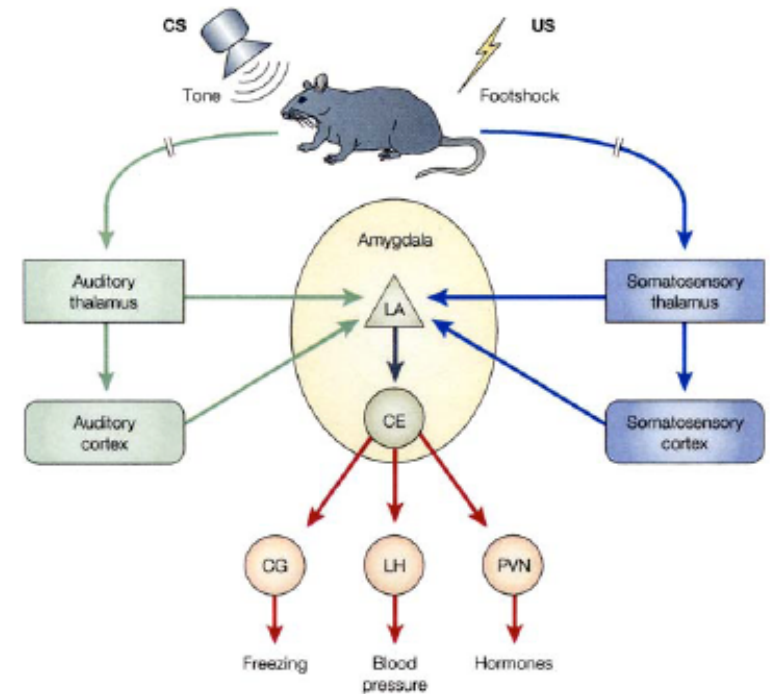
## Contributions of the Amygdala to Emotion Processing: From Animal Models to Human Behavior

Neuron, Vol. 48, 175–187, October 20, 2005, Copyright ©2005 by Elsevier

Elizabeth A. Phelps<sup>1,\*</sup> and Joseph E. LeDoux<sup>2</sup>

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New York University  
4-6 Washington Place  
New York, New York 10003



# Brain SPECT Imaging

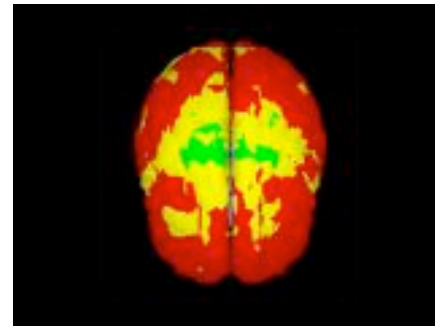
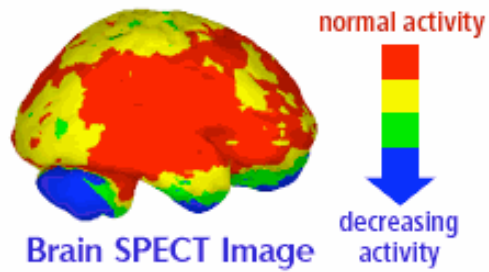
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- *Single Photon Emission Computed Tomography*
- Provides a “snapshot” of cerebral blood flow that can be imaged later
- Indicates areas of normal vs. abnormal activity
- Used to Dx, and monitor Rx of, human CNS lesions

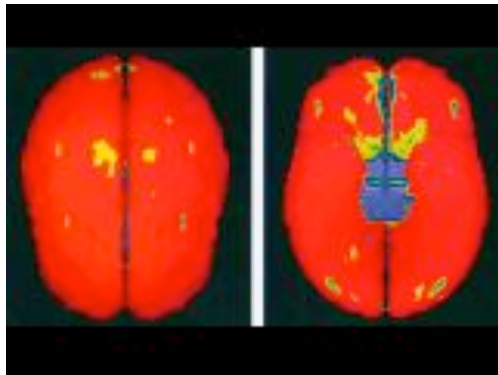


# Brain SPECT Images 1

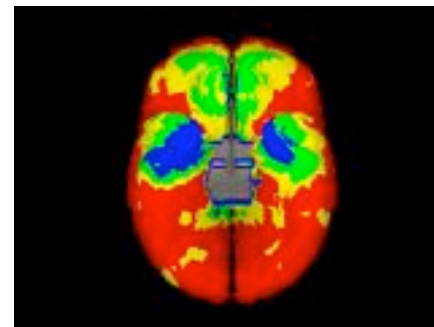
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DEPRESSION,  
SURFACE VIEWS

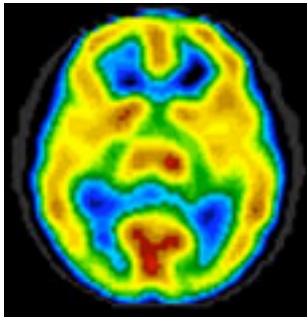


NORMAL,  
SURFACE  
VIEWS

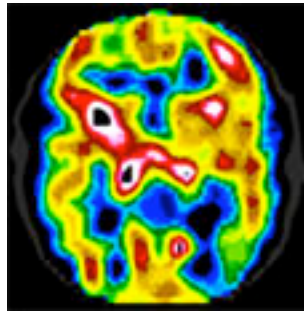


# Brain SPECT Images 2

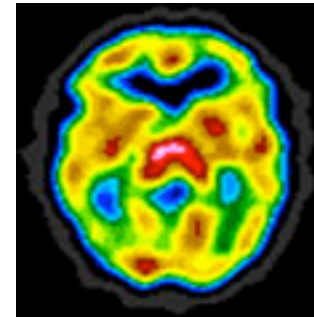
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NORMAL,  
TRANSVERSE  
VIEW

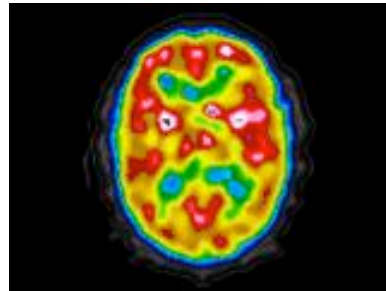


ANXIETY AND  
PANIC DISORDER

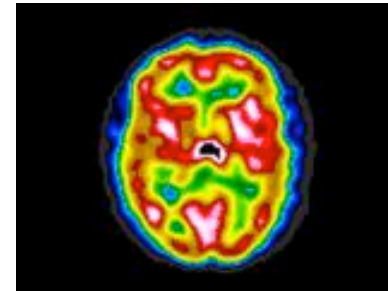


DEPRESSION

<http://www.brainmattersinc.com/>



ANXIETY



DEPRESSION

<http://braininspect.com/>

# How It Could Work

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Groups of animals dosed together



Serially anesthetize, administer reagents, scan



Look for patterns indicative of anxiety

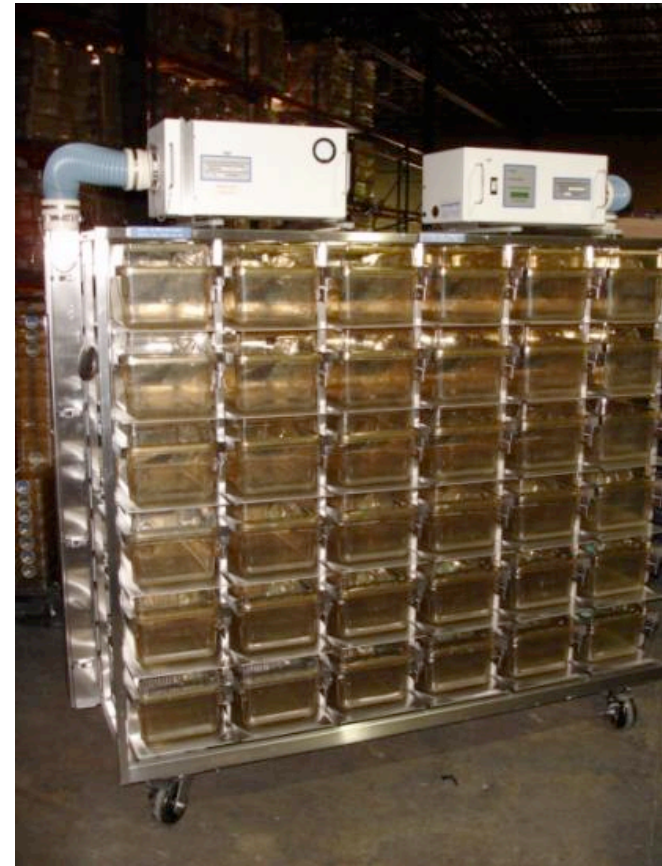
# If This Sounds Too Far Fetched...

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Rodent housing 30  
years ago



Rodent housing today



# Opportunities for Detecting Pain and Distress Associated with Acute Systemic Toxicity

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