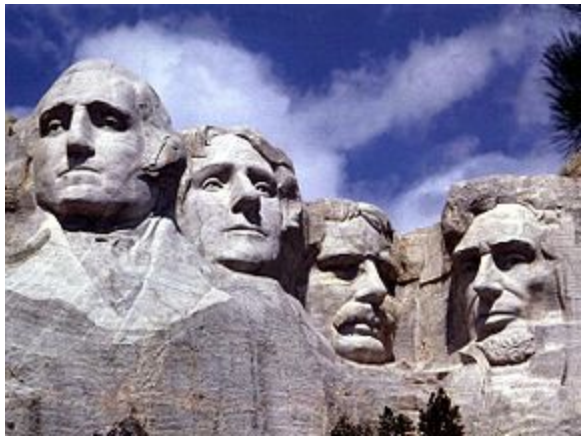
New Approaches for Detecting Pain and Distress

Center for Comparative Medicine
Massachusetts General Hospital

American Association for Laboratory Animal Science
59th Annual Meeting, Indianapolis IN
November 12, 2008

Starting Points



- √ Avoid (prevent) pain or distress
- √ Minimize (reduce) pain or distress
- √ Alleviate (remove) pain or distress

Getting Beyond the Basics

- Most pain/distress is unintended
  = unnecessary
  = unwanted

- Current “humane” endpoints
  = inaccurate
  = misleading

- Pain and distress may occur during animal care as well as animal use

“Real World” Acute Toxicity

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

Common Clinical Signs of Poisoning

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

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

Common Symptoms of Poisoning

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

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

A Common Early Symptom

Malaise ≡ initial, general pain response

"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 measure malaise or discomfort in animals?

Animal Models at MGH

Allergy
Alzheimer's Disease
Anesthesiology
Artificial Organs
Atherosclerosis
Behavioral Neuroscience
Biomaterials
Bone Marrow Transplants
Burn Injury
Cancer
Congestive Heart Failure
Crohn's Disease
Deafness
Diabetes Mellitus
Drug Addiction
Gene Therapy
Genomics
Hepatitis
HIV/AIDS
Huntington's Disease
Inflammation
Laser Therapy
Limb Regeneration
Nerve Regeneration
Obesity
Organ Transplants
Orthopedics
Parkinson's Disease
Pharmacology
Plastic Surgery
Pneumonia
Radiation Therapy
Renal Failure
Sepsis
Shock
Skin Grafting
Stem Cells
Stroke
Trauma
Ulcerative Colitis
Vaccines
Vascular Surgery

Abnormal Behavior Cues

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

Abnormal Activity Metrics (1)

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 (1)

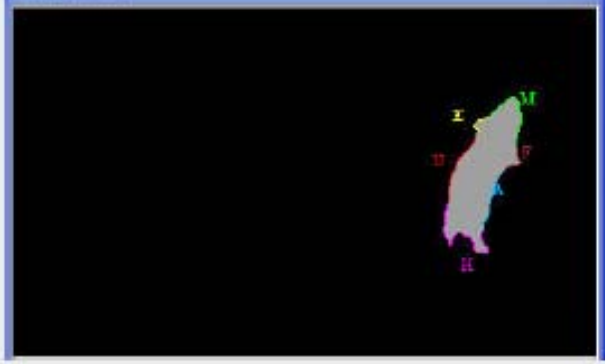
Clever Sys Inc.

The Behavior Recognition Company

Behaviors HomeCageScan can detect:

- Rear Up
- Come Down
- Walk/Run
- Hang
- Jump
- Land
- Turn
- Eat
- Drink
- Chew
- Sniff
- Dig
- Forage
- Circle
- Groom
- Stretch
- Sleep
- Twitch
- Pause
- Awaken
- Urinate

Illustration



Behavior Sequence

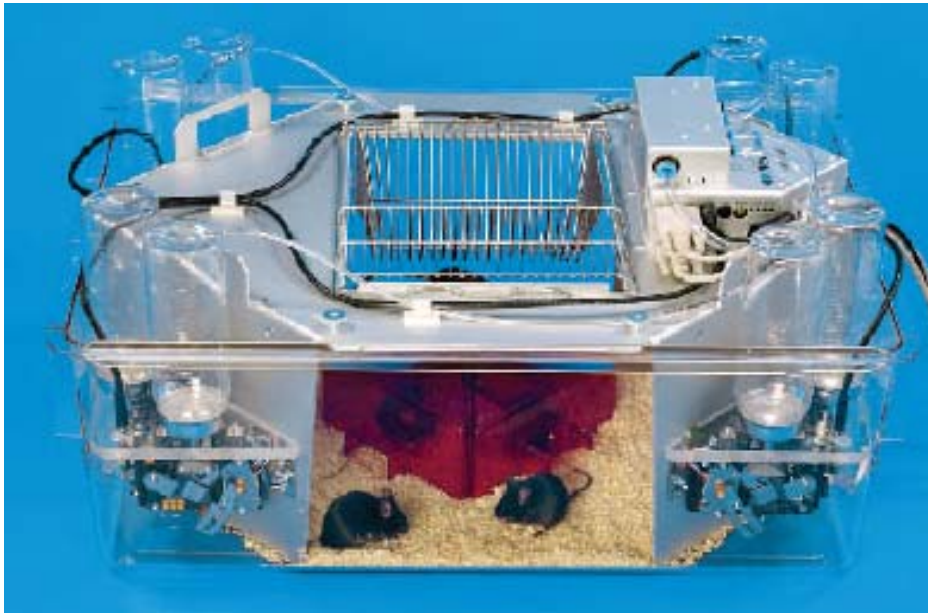
	From	To	Length	Behavior	Comment
1	0"	0"	0.80	Sniff	
2	0"	1"	0.60	Remain RearUp	
3	1"	1"	0.20	Come Down To Partially Reared	
4	1"	1"	0.20	Come Down From Partially Reared	
5	1"	2"	0.20	Remain Low	
6	2"	2"	0.20	Walk Slowly	
7	2"	2"	0.20	Remain Low	
8	2"	2"	0.20	Rear Up	
9	2"	3"	0.40	Eat	Zone 1
10	3"	4"	1.80	Sniff	

Abnormal Activity Metrics (2)

NewBehavior

IntelliCage®

http://www.newbehavior.com/

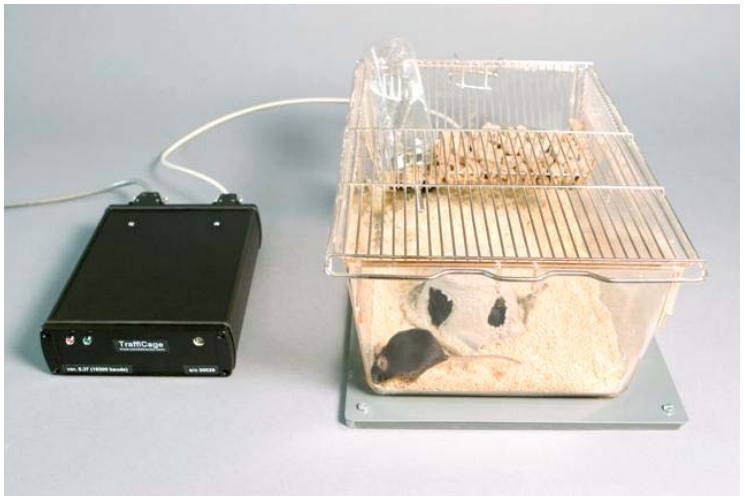


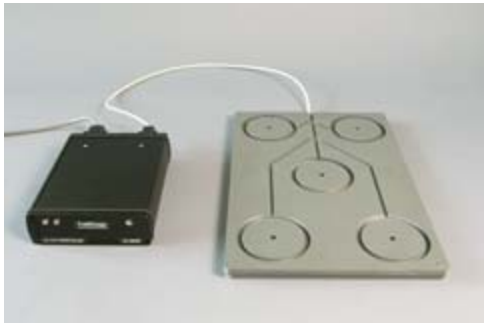
Abnormal Activity Metrics (2)

NewBehavior

TraffiCage®

http://www.newbehavior.com/





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.

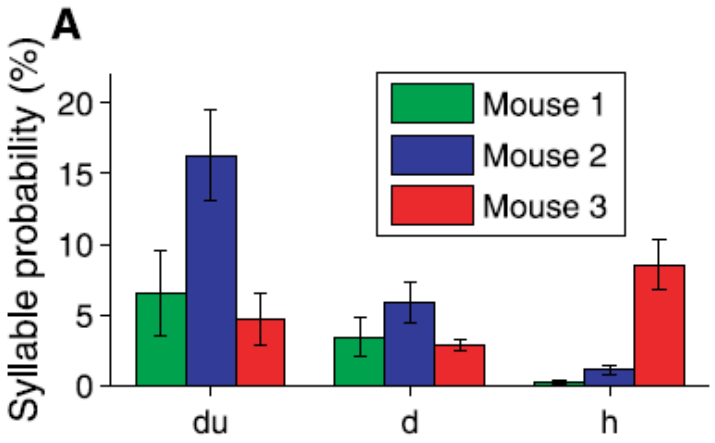
Table 1. The Most Common Syllable Types in Mouse Ultrasonic Vocalizations, Labeled by Pitch Jump

Syllable Type[a]	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

"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.

[a]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



Odors as Endpoints

Olfactory Dysfunction As a Predictor of Neurodegenerative Disease

Mark W. Albers, MD, PhD, Matthias H. Tabert, PhD, and D. P. Devanand, MD

Department of Neurology, Columbia University
College of Physicians and Surgeons, 710 West 168th Street,
New York, NY 10032, USA.
E-mail: mwa28@columbia.edu

Current Neurology and Neuroscience Reports 2006, 6:379–386
Current Science Inc. ISSN 1528-4042

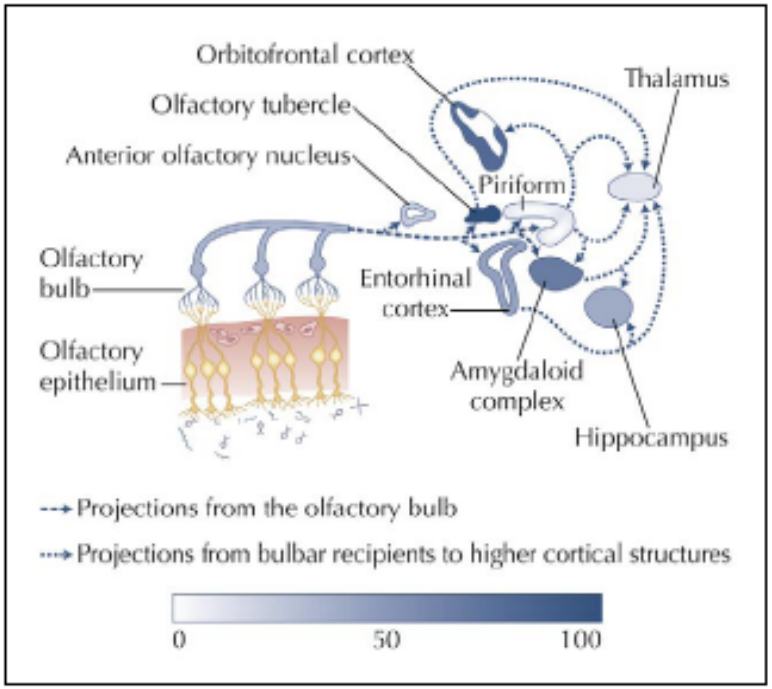


Figure 2. Density of amyloid plaques in the olfactory circuit.

Saliva and Corticosteriods

Biomarkers Core Laboratory
Yerkes National Primate Research Center

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

YERKES NATIONAL PRIMATE RESEARCH CENTER
EMORY

Hair and Corticosteriods

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General and Comparative Endocrinology 147 (2006) 255–261

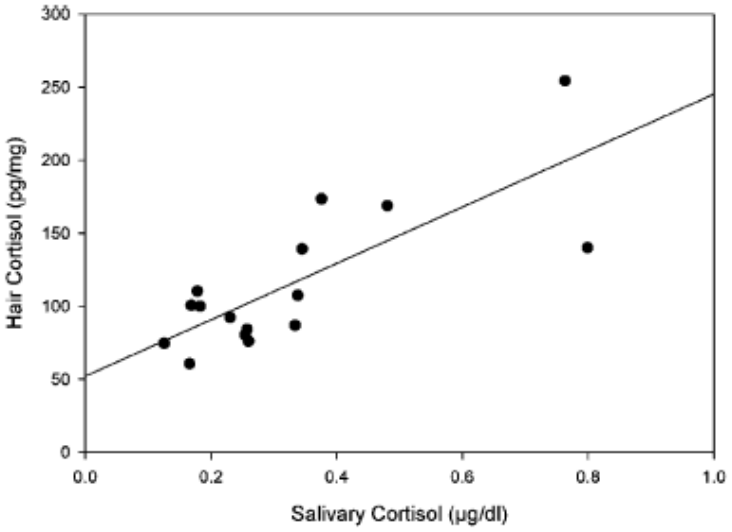
GENERAL AND COMPARATIVE ENDOCRINOLOGY

www.elsevier.com/locate/ygcen

Analysis of endogenous cortisol concentrations in the hair of rhesus macaques

Matthew D. Davenport [a,b], Stefan Tiefenbacher [a], Corrine K. Lutz [a], Melinda A. Novak [a,b,c], Jerrold S. Meyer [b,c,*]

[a] *Division of Behavioral Biology, New England Primate Research Center, Harvard Medical School, 1 Pine Hill Drive, Southborough, MA 01772, USA*
[b] *Neuroscience and Behavior Program, University of Massachusetts, Amherst, MA 01003, USA*
[c] *Department of Psychology, University of Massachusetts, Amherst, MA 01003, USA*

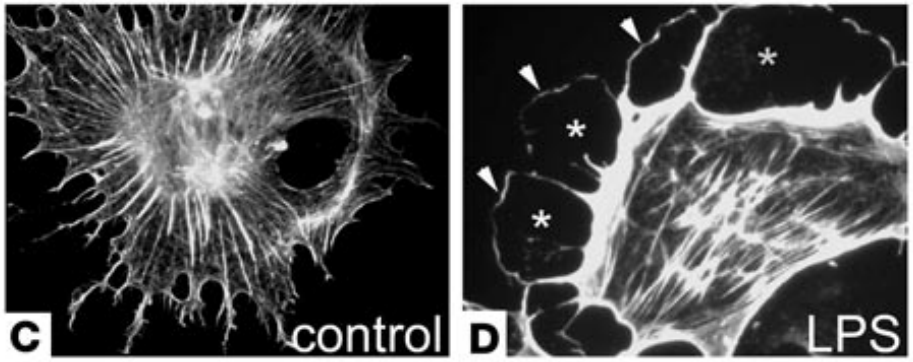


Other Saliva-Based Endpoints?

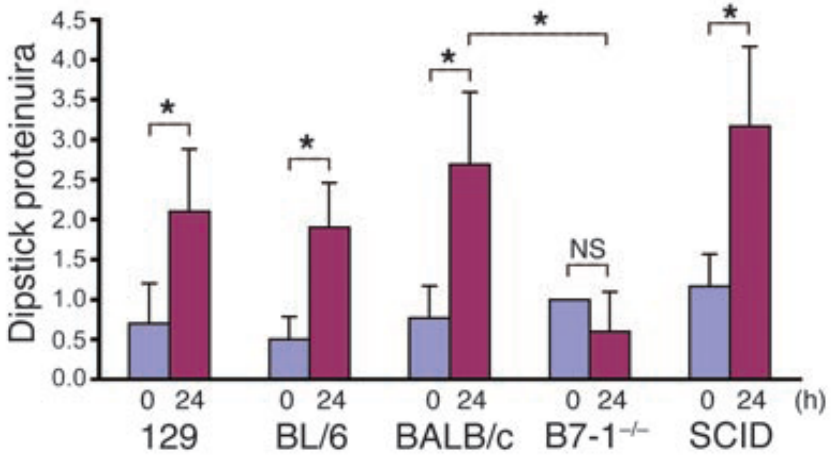
- C-reactive protein quantification in porcine saliva: A minimally invasive test for pig health monitoring. A. M. Gutierrez, et al. *Veterinary Journal* (In press, 2008).

- Elevated salivary dehydroepiandrosterone-sulfate but normal cortisol levels in medicated depressed patients: Preliminary findings. J. Assies, et al. *Psychiatry Research 128* (2004): 117-122.

Better Sepsis Endpoints



- 200 μg LPS in 200 μl PBS IP
- Measure urine protein at t_0 and 24 hours



(also demonstrated for genetic, drug-induced, and immune-mediated kidney disease models)

Reiser, et al., JCI 11(10):1390-7, 2004

Early Symptom of Poisoning ≈ Fear

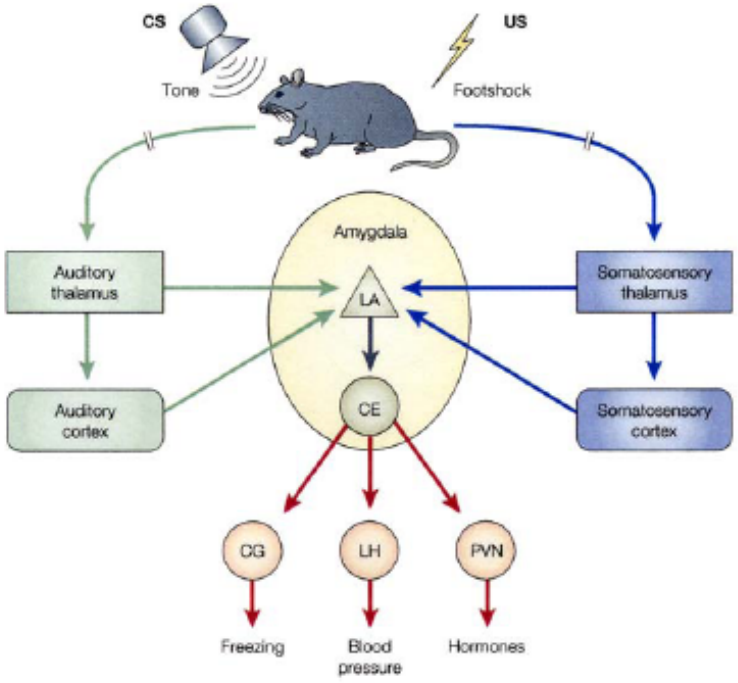
Apprehension/Fear/Anxiety ≡ initial, general distress responses

"Anxiety - an abnormal and overwhelming sense of apprehension and fear often marked by physiological signs (as sweating, tension, and increased pulse), by doubt concerning the reality and nature of the threat, and by self-doubt about one's capacity to cope with it feeling of general discomfort or uneasiness." (http://www.merriam-webster.com)

Can one measure anxiety/apprehension in animals?

A Neurobiological Continuum

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

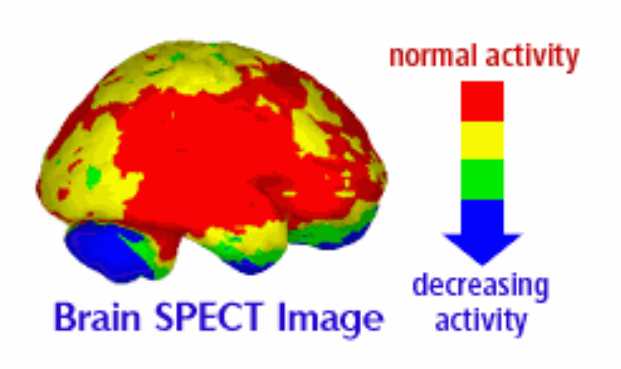


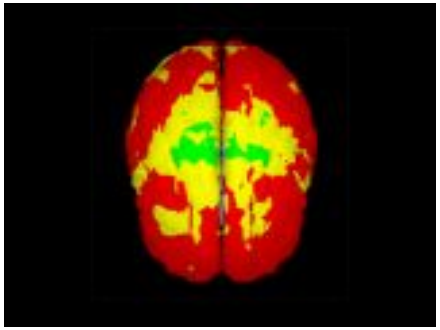
Phelps and LeDoux, Neuron 48:175-87, 2005

Brain SPECT Imaging

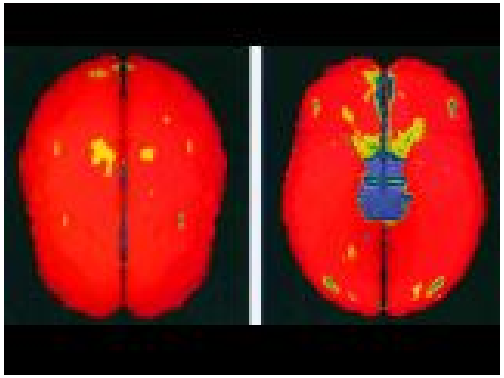
- *Single Photon Emission Computed Tomography*
- Provides a “snapshot” of cerebral blood flow that can be imaged later
- Indicates areas of normal vs. abnormal activity
- Used to Dx and monitor Rx of human psychiatric conditions

Brain SPECT Images 1

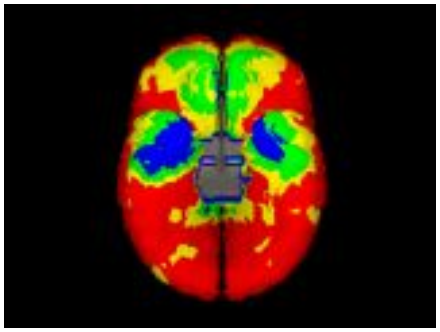




DEPRESSION, SURFACE VIEWS

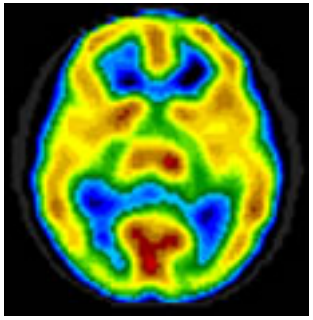


NORMAL, SURFACE VIEWS

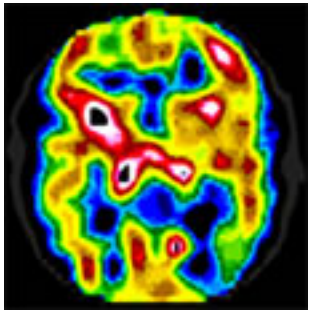


http://braininspect.com/

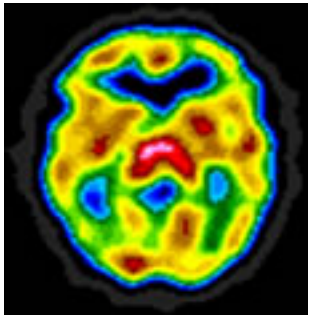
Brain SPECT Images 2



NORMAL, TRANSVERSE VIEW

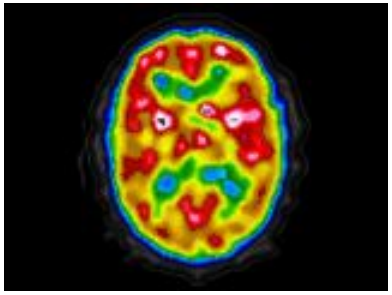


ANXIETY AND PANIC DISORDER

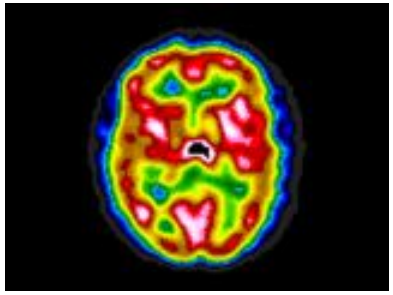


DEPRESSION

http://www.brainmattersinc.com/



ANXIETY



DEPRESSION

http://braininspect.com/

Idiot-Proof Distress Markers?

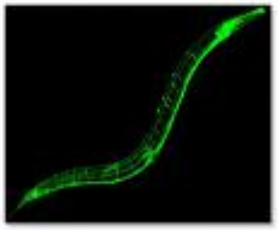
"Heat-shock induction of T-cell lymphoma/leukaemia in conditional Cre/lox-regulated transgenic zebrafish"
(Feng, et al., Brit J Haematol, 138(2):169-75, 2007)

Transgenic cassette for stress + marker genes?

↓

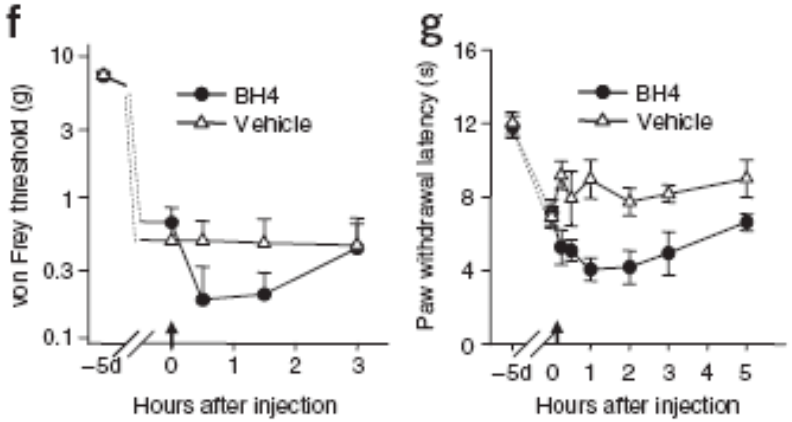
Apply various environmental or behavioral stressors

↓

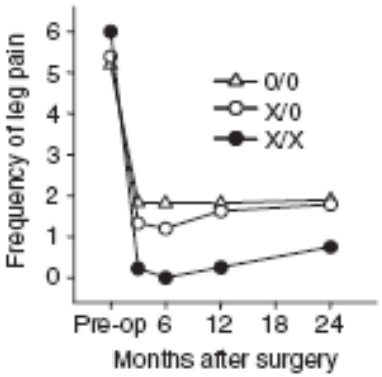


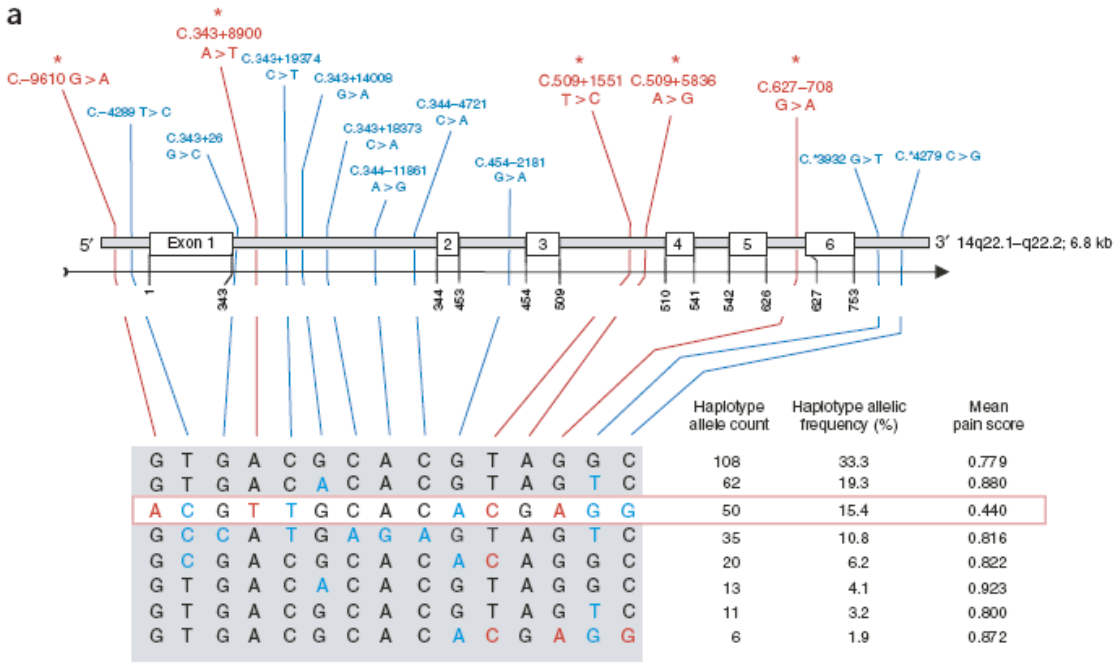


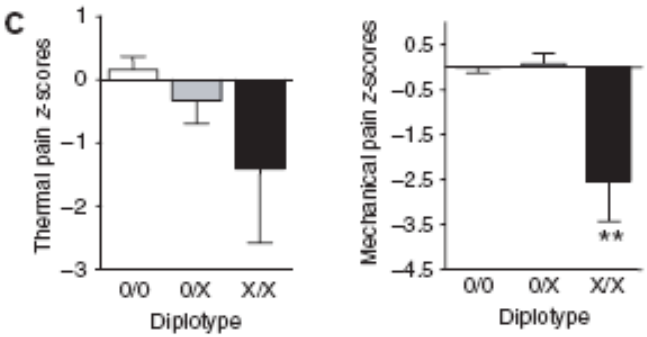
Differential Predictors of Pain



GCH1 ⟶ ↑ or ↓ BH4 ⟶ ↑ or ↓ pain







Tegeder, et al., Nature Medicine 12 (11):1269-77, 2006

Truly Humane Endpoints

1. Observations without handling

↓

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

↓

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

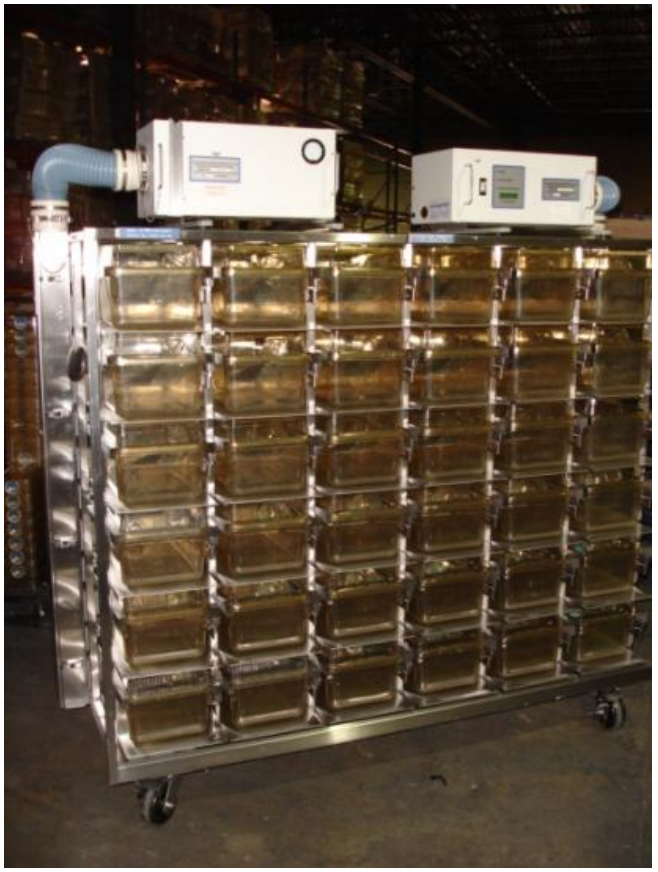
↓

4. Temporarily stressful (bleed, anesthetize, euthanize)

If This Sounds Too Far Fetched…

Rodent cages 30 years ago

Rodent cages today



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