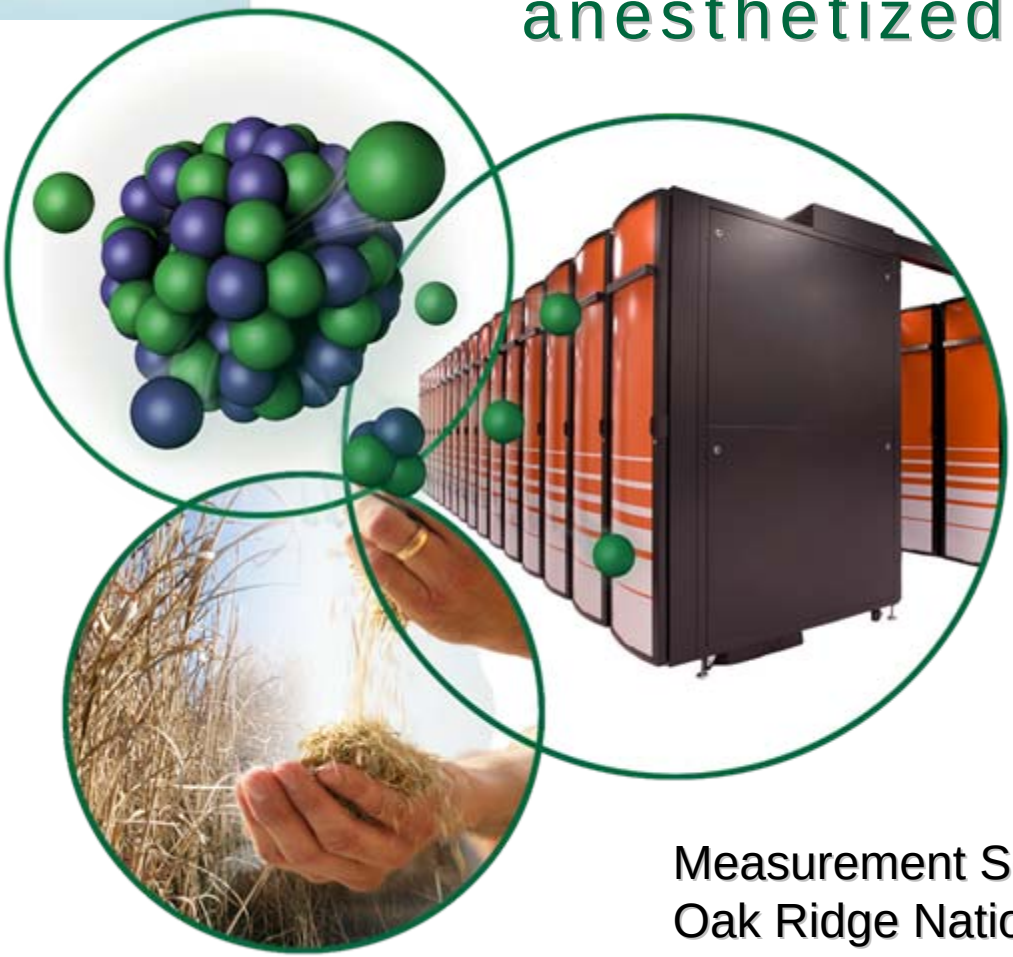
U.S. Department of Energy
Office of Science
Office of Biological & Environmental Research

A Case for Non-invasive, Awake, Non-anesthetized, Unrestrained Imaging



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Motivation

- **Understanding Addictions**

"The age of first encounter with psychoactive compounds is critical, since a higher probability to shift from use to abuse and to develop addiction is associated with an early approach (Robins and Przybeck, 1985; for tobacco, see Taioli and Wynder, 1991; Anthony and Petronis, 1995; Breslau and Peterson 1996)."

- **e.g. Nicotine Addiction**

"Nearly one-third of worldwide adults are smokers, and the majority started this habit when adolescents (Mansvelder and McGehee, 2002)."

"Adolescents also express symptoms of nicotine dependence after only few cigarettes (DiFranza et al, 2000)."

Adriani et al. Neuropsychopharmacology 29: 869–878 (2004).

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Neuropsychopharmacology (2007) 32, 1760–1773
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www.neuropsychopharmacology.org

Protracted 'Pro-Addictive' Phenotype Produced in Mice by Pre-Adolescent Phenylpropanolamine

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For decades, the sympathomimetic phenylpropanolamine (PPA; ±-norepinephrine) was an active ingredient found in popular children's over-the-counter (OTC) cold, cough, and allergy medications. To examine the possibility that pre-adolescent PPA exposure may induce neuroadaptations that influence behavioral and neurochemical responding to cocaine, C57BL/6J mice were pretreated with PPA (0–40 mg/kg) during postnatal days 21–31. The behavioral and neurochemical responses to acute and repeated cocaine (4 × 15 mg/kg)

"Phenylpropanolamine (PPA; 7-norephedrine) is a nonselective adrenergic receptor agonist and norepinephrine reuptake inhibitor (Hoffman, 2001) that was widely available for decades in popular children's OTC cold, cough, and allergy medications (eg, Alka-Seltzers Plus Children's Cold Elixer and Orange, Cherry, Berry, and Yellow Triaminics 3D preparations)."

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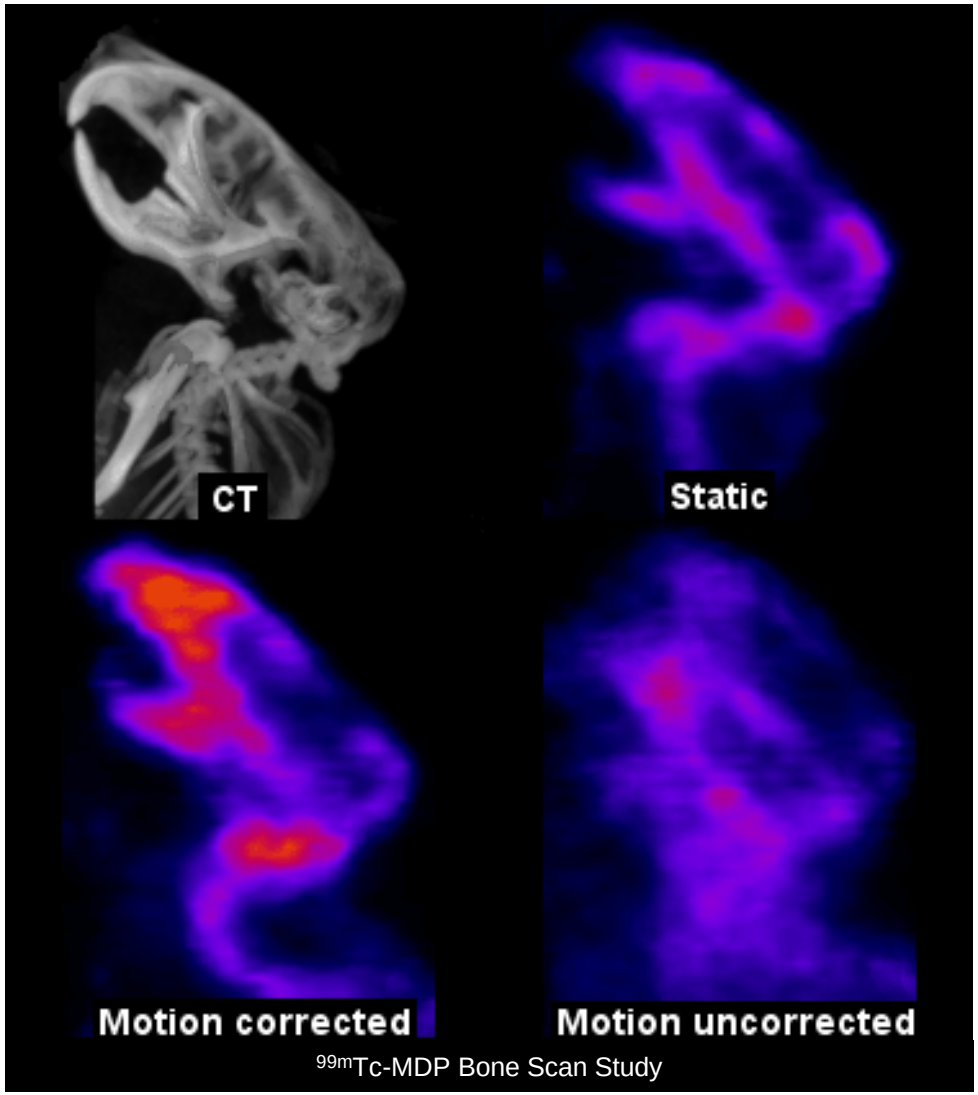
Relevant Applications

- **Behavioral Research**
  - **Brain development**
    - **Early childhood**
    - **Preadolescent**
    - **Adolescent**
- **Clinical Motion-Corrected Diagnostic Imaging**
  - **Parkinson's and other motor control pathologies**
  - **Late stage Alzheimer's**
  - **Children**
  - **Babies**
  - **Elderly**
- **Pharmacology and Drug Development**
  - **Neural agents and therapies**
  - **Brain cancer therapies**
  - **Anesthetics**
- **Patho-physiology Research**
  - **Monitoring and understanding brain tumor dynamics**
  - **TBI**

Awake Animal Imaging

GOAL: Motion corrected functional imaging of awake, un-anesthetized, unrestrained, untrained small animals



^{99m}Tc-MDP Bone Scan Study

Collaborative Work

Oak Ridge National Laboratory (ORNL)- optical imaging based motion tracking

Thomas Jefferson National Accelerator (JLab)- high resolution detector development

ORNL/JLab-motion corrected SPECT functional image reconstruction

Johns Hopkins University- animal studies

Why Small Animal (Mouse) Focus

- **Most challenging-cannot tell them to stay still**
- **Transgenic mouse models- available for various disease pathologies**
- **Longitudinal studies-use each as its own control**
- **Dedicated imaging systems-available preclinical systems -no competition with clinical use**
- **Physiologically relevant melieu- AIS enables "normal state" studies**

Why Non-Anesthetized Imaging

Anesthesia introduces measurement and process artifacts for brain functional imaging

- **Anesthesia knowns:**
  - **affects blood flow/metabolism based on dose and type of anesthetic used**
  - **perturbs cerebrovascular reactivity to CO_2**
  - **suppresses neural activity**
- **Anesthesia unknowns ???:**
  - **how anesthetics modulate global vs. regional cerebral blood flow (CBF)**
  - **why different species/strains/individuals have varying sensitivities to a particular anesthetic**

Effects of Some Common Anesthetics on CBF

Anesthetic	Effect on CBF
isoflurane	↑↑
chloral hydrate	=
α- chloralose	↓
propofol	↓
sevoflurane	↓
pentobarbital	↓↓

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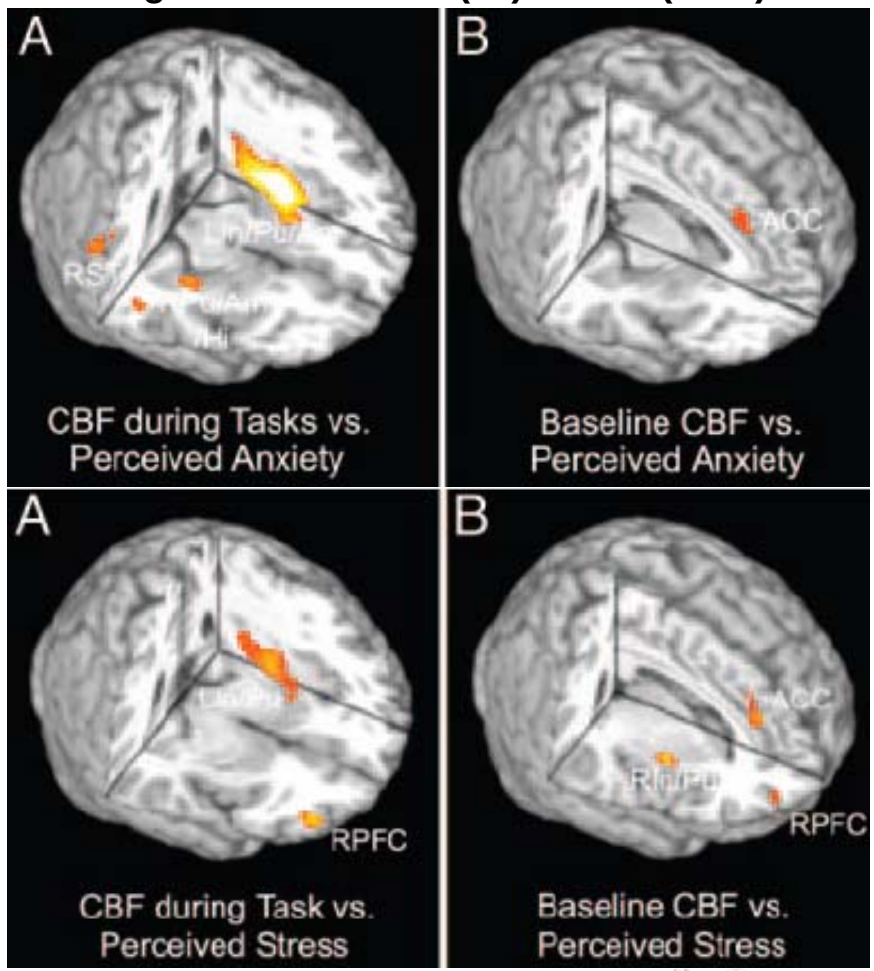
Why Non-Restrained Imaging

Restraining introduces measurement artifacts for brain functional imaging, restricting analysis to qualitative assessments

Wang et al. PNAS 102 (49):17804. (2005)



- **Restraint effects:**
  - **elevates stress hormones with corresponding blood flow/metabolism change in BF (fight or flight response activation)**
  - **perturbs cerebrovascular reactivity to CO2**
  - **activates and increases neural activity**

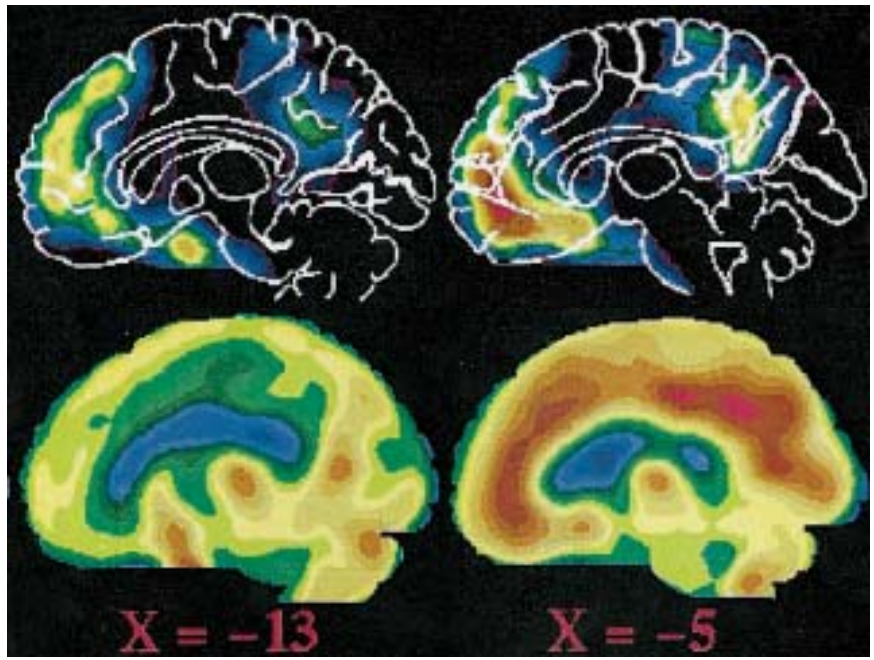
RatCap

Why Awake Imaging

Awakeness/alertness necessary for determining brain functionality

- **Awakeness/alertness enables:**
  - **mimicking normal state**
  - **determining cognitive and motor pathways**
  - **studying behavior pathways and physiology to better understand**
    - **addictions**
    - **early brain development**
    - **adolescent brain development**

Raichle et al. PNAS 98 (2):676. (2001)



Cerebral blood flow (CBF) for:

Row 1: awake and alert cognitive tasks

Row 2: awake and resting with eyes closed

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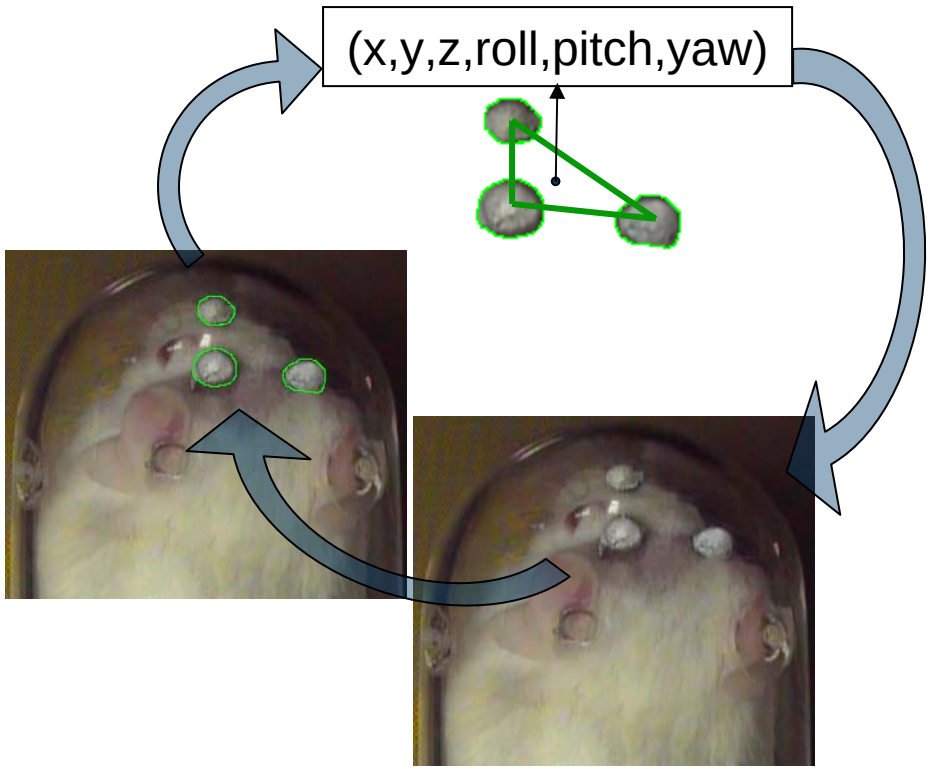
Challenges to Accomplishing Objective

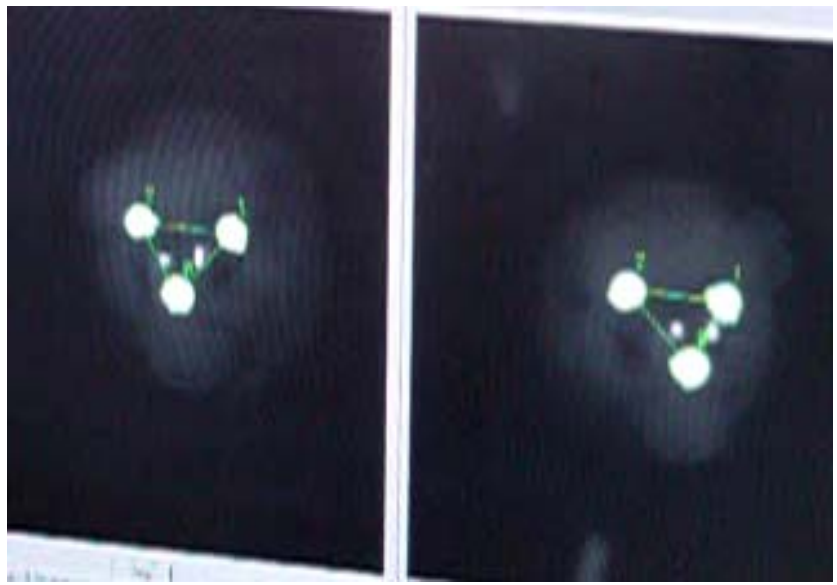
Correcting for subject motion during imaging scans

- **Our approach**
  - **Fast stop action equivalent imaging because of required long integration times for Positron Emission Tomography (PET) / Single Photon Emission Tomography (SPECT) scans**
    - **Count and time stamp photons while simultaneously recording time stamped position of moving subject being imaged**
    - **Use corresponding subject position coordinates to transform all detected photons to a fixed reference coordinate axis**
    - **Reconstruct to get un-blurred image of subject**

Example of Extrinsic Marker Based Motion Tracking

- **Random voluntary motion of mouse subject**
- **2D near infrared imaging (780nm)**
- **Acquisition rate of 6.5 frames/sec for 3 camera system**





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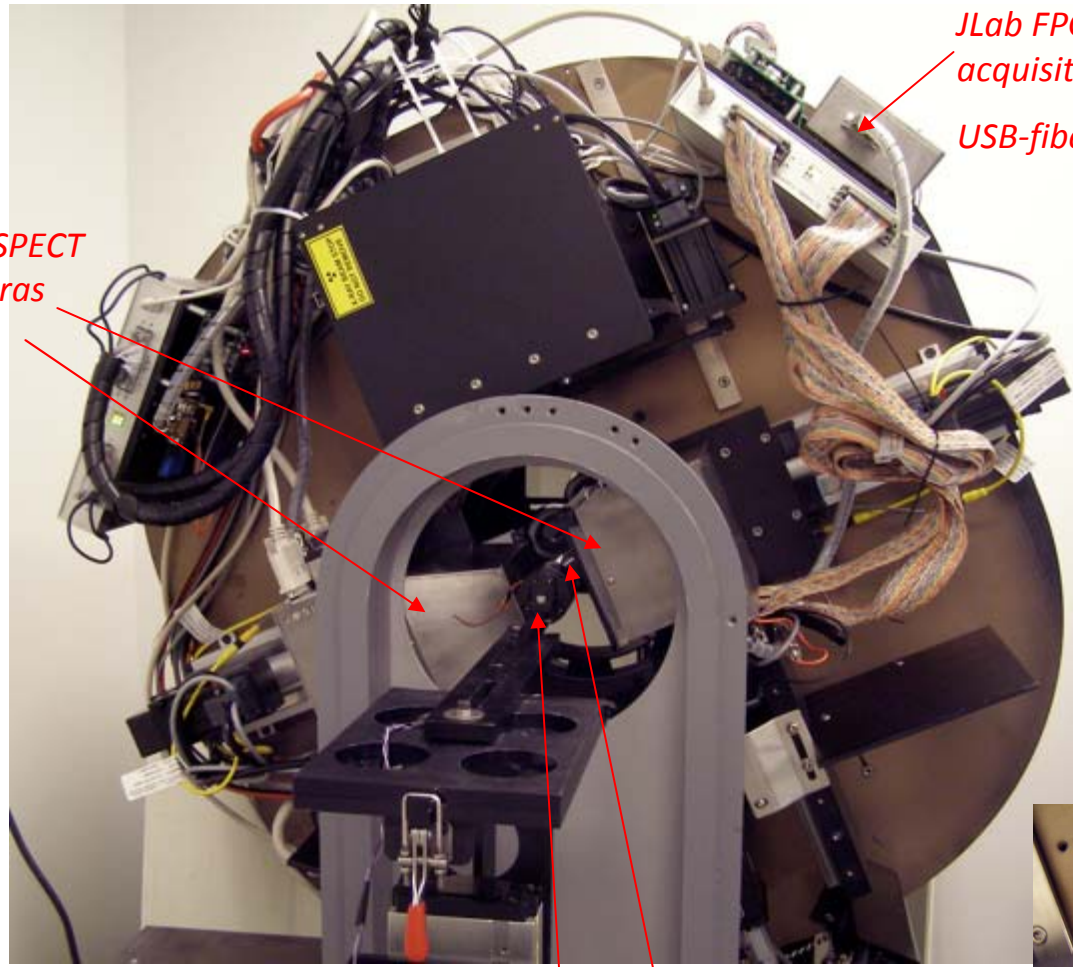
Awake Animal SPECT-CT Imaging System Implementation

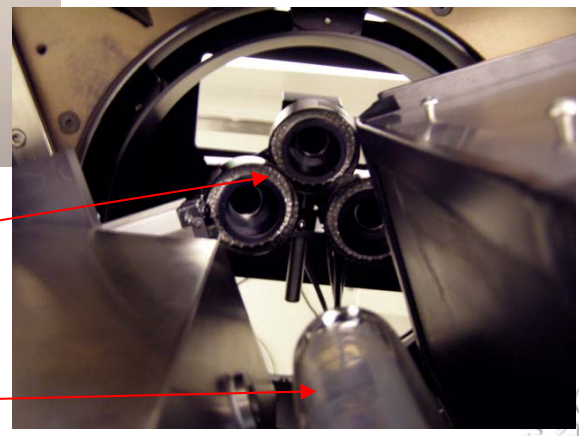


JLab FPGA data acquisition system

USB-fiber optic

JLab SPECT cameras

An awake mouse with infrared reflectors for head tracking in imaging burrow



ORNL Infrared Motion Tracking System

ORNL mouse burrow

SPECT Cameras:
10 cm x 20 cm active area
2x4 array of flat panel PSPMTs
pixelized NaI scintillator ~ 1.25 mm step
Pinhole & parallel hole collimators

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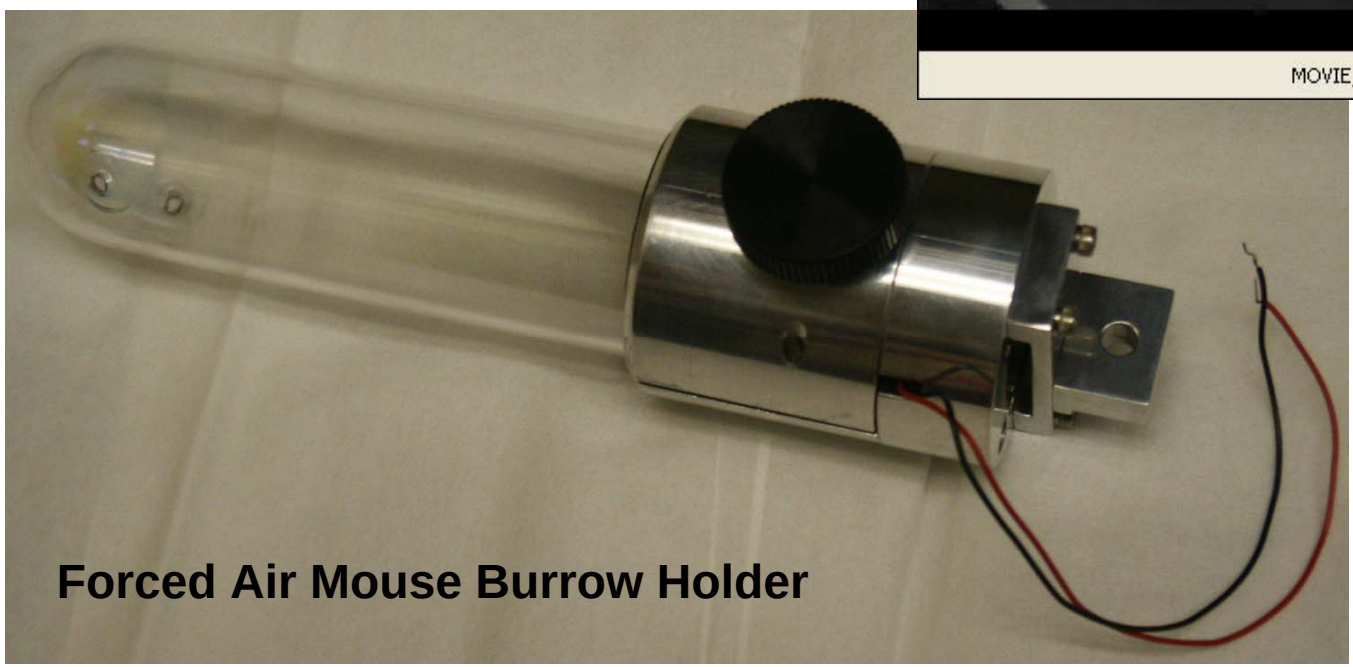
Awake Animal Imaging Validation Studies Issues

- **Mouse Viability**
- **Mouse Behavior**



Forced Air Mouse Burrow Holder

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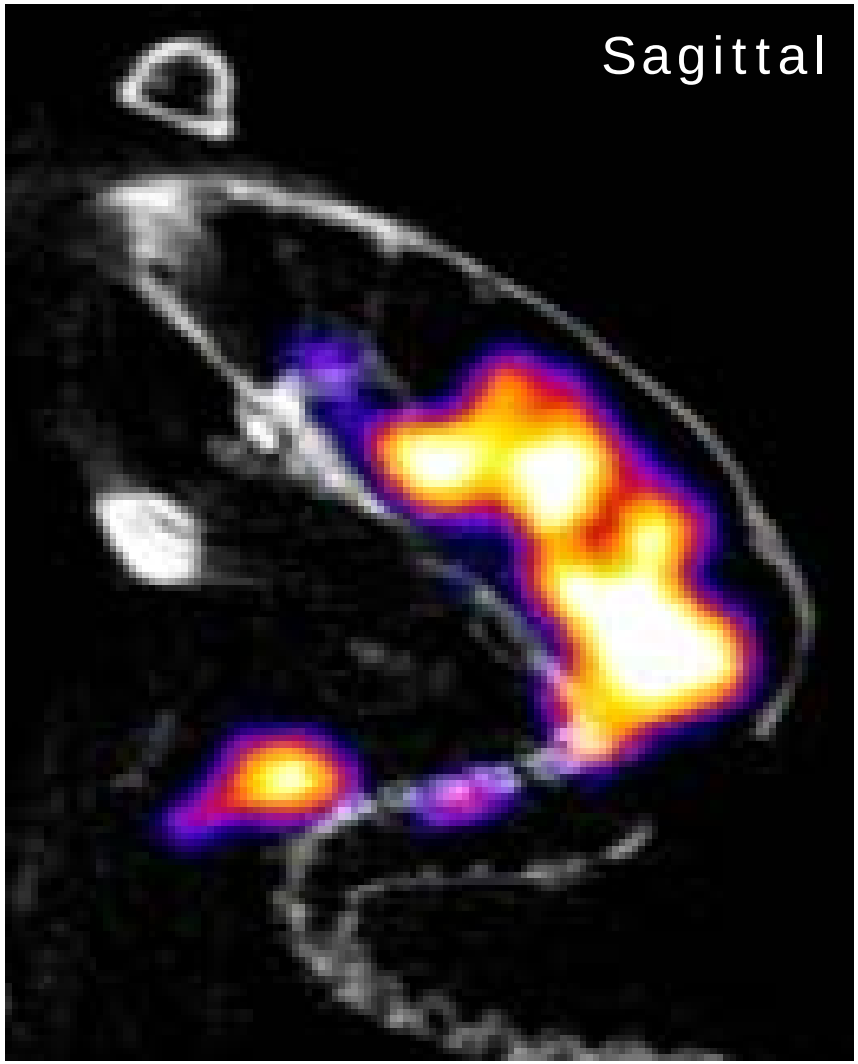
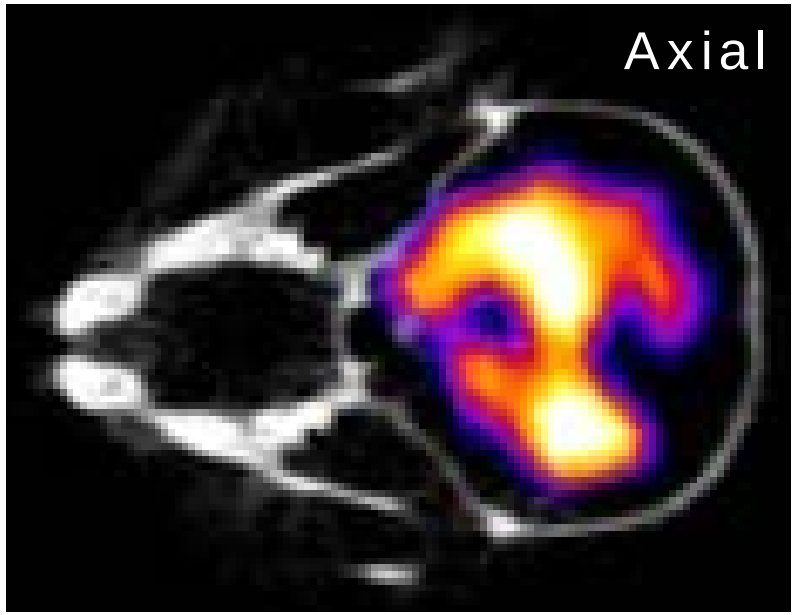
Still Need to Address:

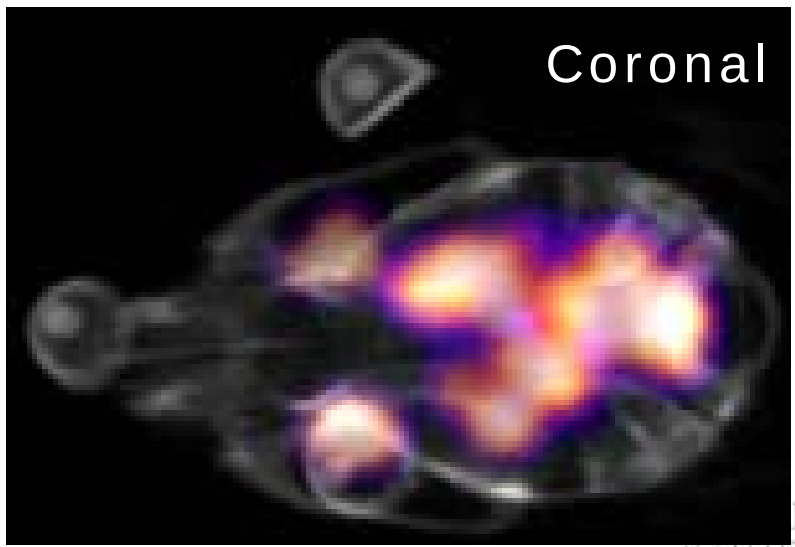
- **Mouse Temperature Regulation Control and Monitoring**
- **Non-Invasive Mouse Physiological State Monitoring**
- **Quantification of Mouse Stress Due to Burrow Constriction of Movement**
- **Intrinsic Feature Based Motion Tracking**

Recent Results-DatSCAN 15 min Awake Scan (Jan. 2009)

DatSCAN is an 123I cocaine analog useful for imaging of dopamine receptors







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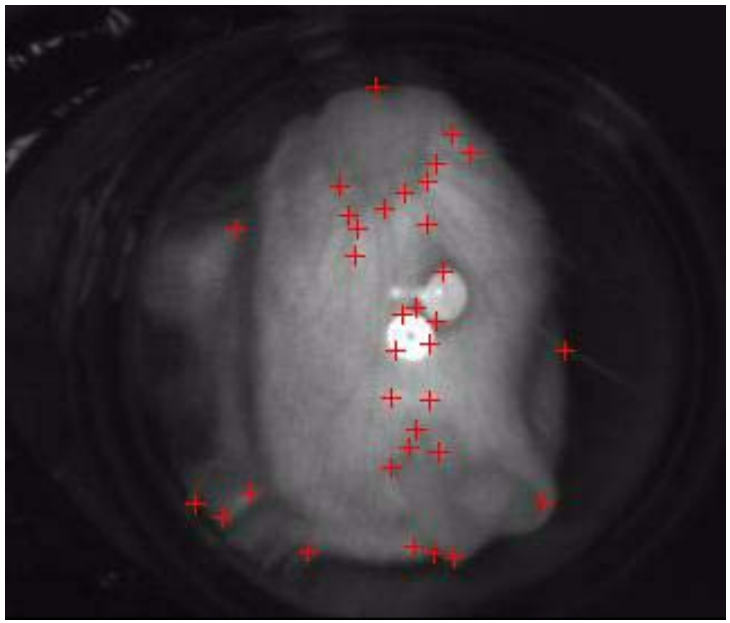
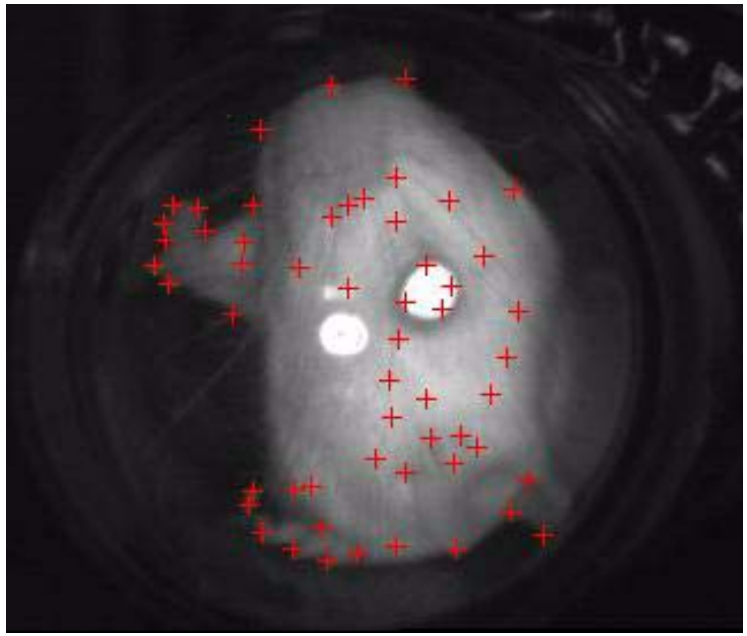
Preliminary Intrinsic Marker Based Motion Tracking

Natural Feature Extraction from Live Mouse Stereo Images





Left **Right**

Features are matched for each stereo pair to calculate 3D location.

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Acknowledgements

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