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Enabling Stress Resistance (ESR)

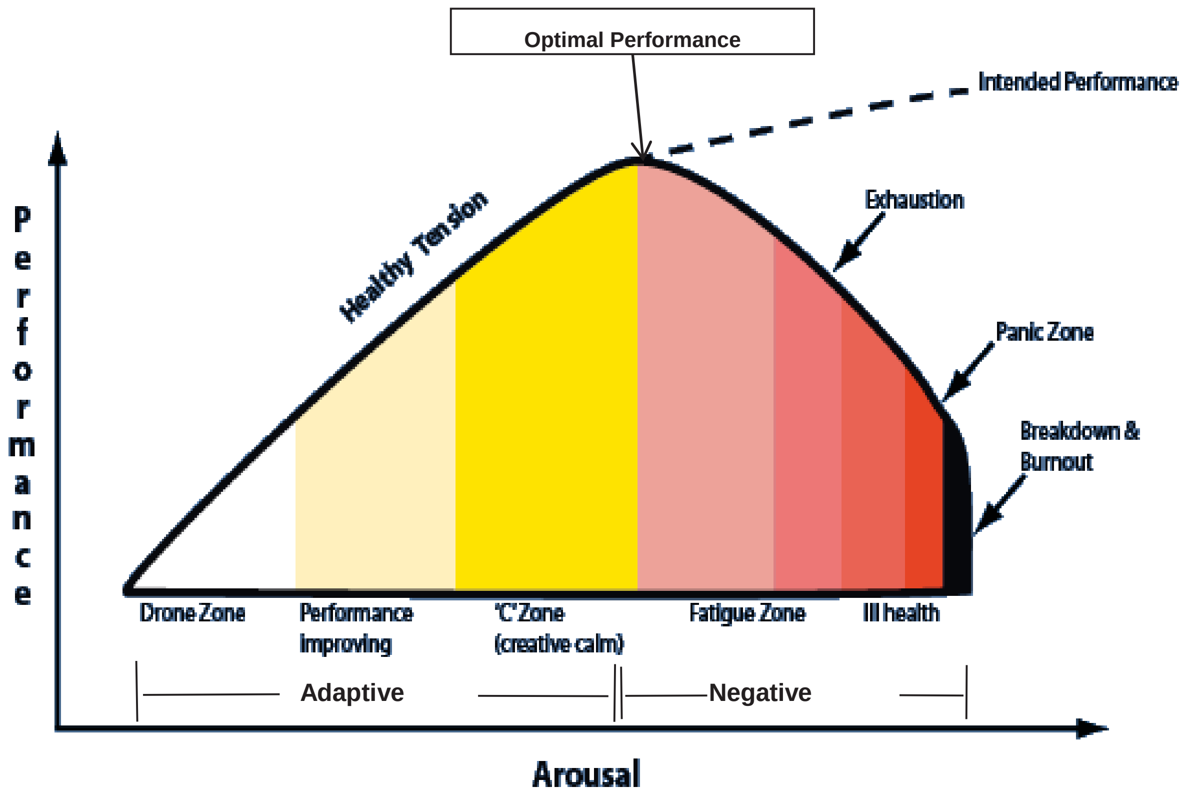
COL Christian Macedonia

Program Manager, DARPA/DSO

ESR Overview

Current State-of-the-Art: Reactive Stress Research and Skyrocketing PTSD Rates

A substantive understanding of what moderates the stress response is a prerequisite to promoting resilience and preventing stress-provoked cognitive dysfunction.



The Yerkes-Dodson Law (1908)

Examples of fundamental questions that should drive these investigations are:

1. *What are the mechanisms involved that afford an initial resistance to stress induced cognitive dysfunction*
2. *What is the underlying mechanism behind the breaking point from an adaptive response to stress to a maladaptive response?*
3. *What are the mechanisms involved behind the various negative effects of stress on cognition, memory, attention, etc.?*

Why is ESR Important?

- The negative impact that stress has on the cognitive, emotional and physical well-being of our warfighters is irrefutable
- Current government funding is supporting **reactive** research
- A multi-disciplinary approach is necessary to identify networks involved in response to stress
- ESR will explore the problem, and identify solutions towards providing **defense**, **resistance** and **resilience** to stressful situations

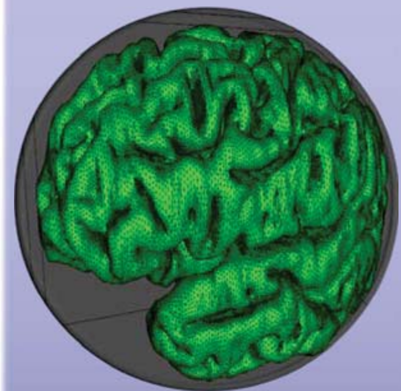
ESR Vision

Program Vision: Create a comprehensive, quantitative description of the impact of stress on the brain

- There is a critical need to understand stress from a fundamental viewpoint (i.e. what networks are altered in response to stress?)
- This program will:
  - Leverage cutting-edge technologies in existing animal models of acute and chronic stress for development of a comprehensive understanding of the complex effects of stress on the animal
  - Develop pharmaceutical, cognitive and behavioral preventative interventions for eventual translation

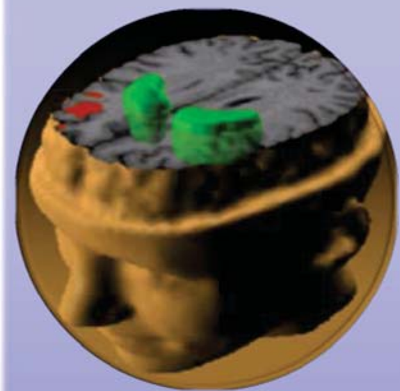
From a Comprehensive Neurobiological Understanding of Stress to Concrete Interventions

ESR Teams Span Key Technical Areas



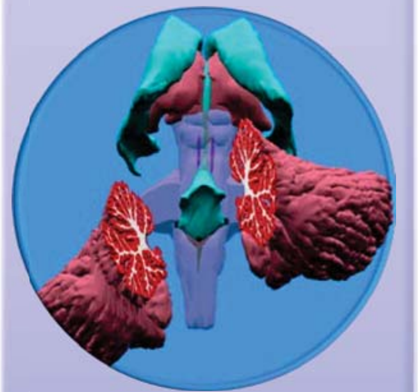
UC-Boulder

- **Cortical** response to stress with continuous cortical EEG recording, heart rate, mean arterial pressure, core body temperature and spontaneous activity
- Potential behavioral interventions



MIT

- Determination of novel genetic, functional and structural correlates of stress-induced dysfunction in the **striatum and amygdala**
- Focus on stress-induced dysregulation of emotional circuits



Children's Hospital of Philadelphia

- Optogenetic modulation of neuronal populations in the **locus coeruleus** during chronic stress
- Identification of changes in neural arousal systems in adaptive rats

Northwestern

- Exploratory approach with high throughput **genetic and genomic** screening of genetically variable animals
- Correlation and pathway analysis statistics

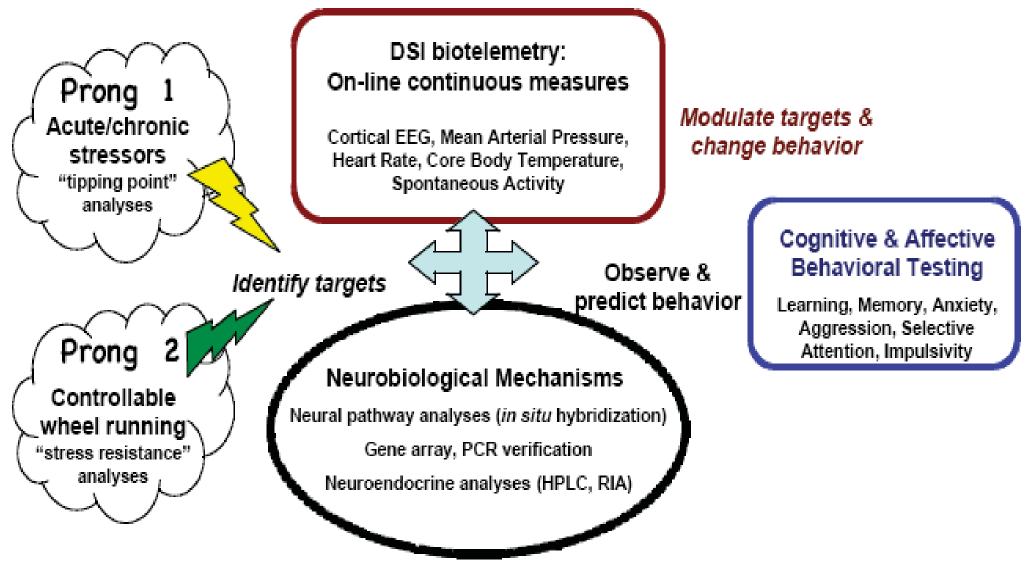
Selected teams feature complementary but distinct approaches targeting known stress pathways

University of Colorado

UNIQUE APPROACH: Employs both a tipping point analysis as well as a stress resistance analysis to determine mechanisms involved in coping

Technical Approach:

- Identification of targets at coping points and point where stress response becomes maladaptive using real-time cortical EEG, neurophysiological stress response and behavior along the entire stress continuum
- Focus on stress-buffering effects of voluntary wheel running by analyzing the controlled exercise component as well as the perceived control component
- Comprehensive analysis of stressed animals including genetics, neural pathway approaches and neuroendocrine analysis
- Inclusion of behavior and coping strategies as a way forward, potential for immediate use
- Stressors include: Conditioned fear, Sleep disturbance, uncontrollable tailshock, mandated exercise



Team: ***Monika Fleshner*** (UC-Boulder), Benjamin Greenwood (UC-Boulder), Daniel Barth (UC-Boulder), Mark Opp (University of Michigan), Matt McQueen (UC-Boulder), Tiejun Tong (UC-Boulder), Robin Johnson (Advanced Brain Monitoring)

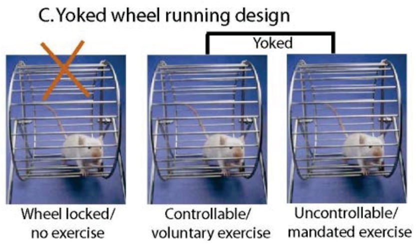
UCB Experimental Overview

Novel Two Prong Approach:

1. **<u>Tipping point analysis:</u>** Identify targets that are dysregulated at the tipping point, when stress consequences cross-over from adaptive to maladaptive.

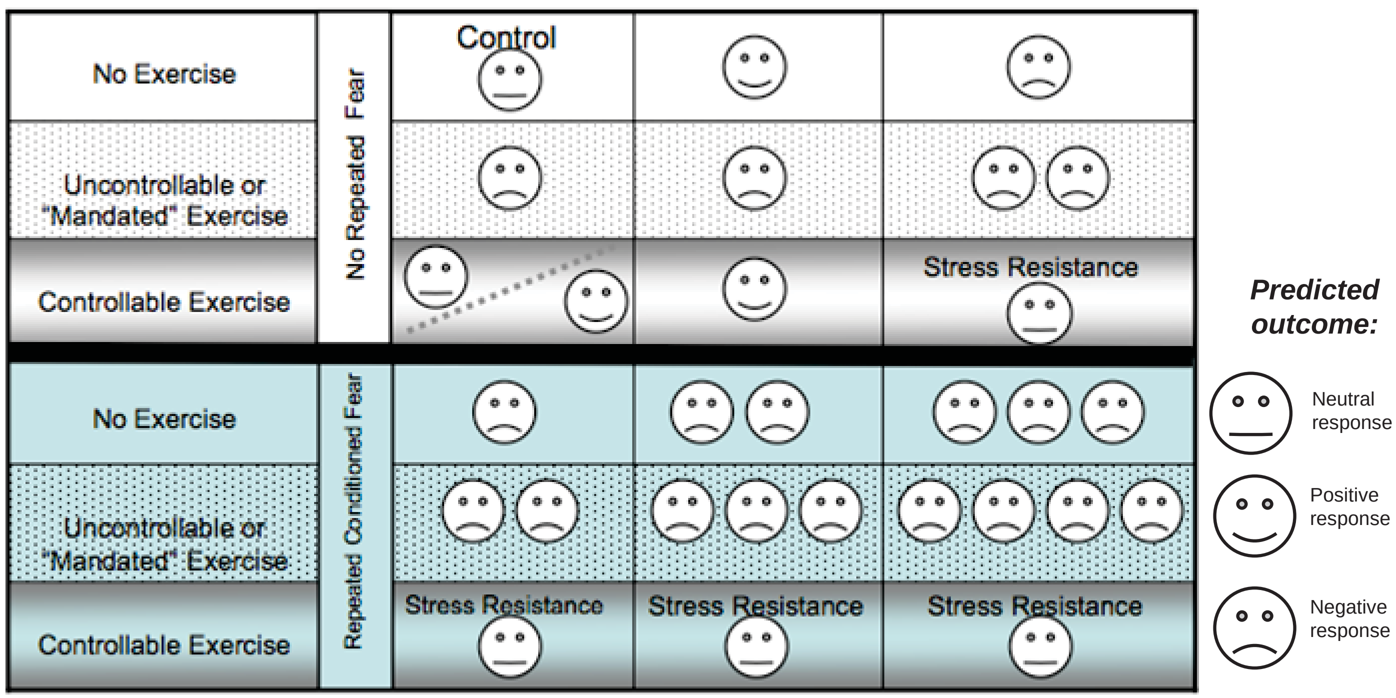
2. **<u>Stress resistance analysis:</u>** Identify targets involved in active stress coping and stress resilience.

Exercise design:



Exercise Condition ***Acute Stressor Exposure***

No acute stress *Mild (10 shocks)* *Severe (100 shocks)*



****Experiment will be completed once for online and neurobiological measures, and once for cognitive and affective behavioral measures.***

UCB Technical Approach

Independent and Dependent Variables

Proposed Stressors Relevant to Warfighters

1) Affective (conditioned fear, repeated/chronic)
2) Sleep disturbance (repeated/chronic)
3) Physical with a psychological component (mild or severe shock, acute)
4) Uncontrollable / mandated exercise (repeated/chronic)

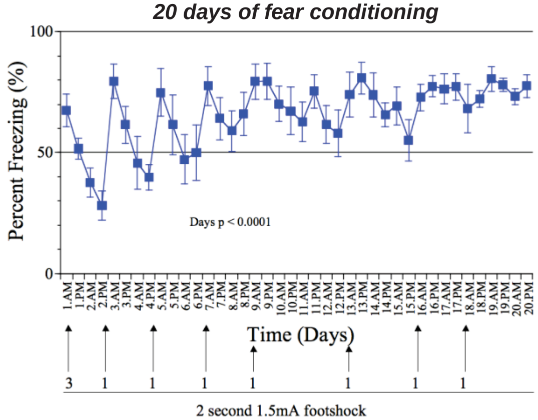
Online Telemetry and Neurobiological Measures

Online / Neurobiological Variable	Method of Measurement	Dependent Measure
Physiological response	Online DSI biotelemetry	MAP, HR, CBT, spontaneous activity, body weight
Cortical EEG	Online DSI biotelemetry	Cortical EEG during wake, stress, sleep
Gene expression	Affymetrix gene chip analysis and qRT-PCR verification	Whole-genome analysis (~27,000 genes)
Neural pathway analysis	*In situ* hybridization	Mapping of immediate early genes (fos / zif) in the stress-responsive neural pathway.
Neuroendocrine	HPLC, RIA	Corticosterone, corticosterone-binding globulin, plasma and tissue norepinephrine

Cognitive and Affective Behavioral Measures

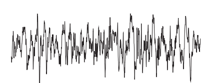
Cognitive / Affective Variable	Animal Behavioral Test	Dependent Measure
Anxiety / fear learning, memory and extinction	Conditioned fear (acquisition, expression and decay)	Freezing
Anxiety	Social discrimination	Social exploratory behaviors
Hippocampus-dependent learning and memory of traumatic event	Context-dependent object recognition	Object exploration – (preference for novel object / context pairing)
Selective attention	Social discrimination	Social exploration (novelty preference)
Instrumental learning	Shuttle box escape	Latency to escape foot shock
Aggression	Social discrimination	Social aggressive behaviors
Impulsivity	Shuttle box escape	Number of spontaneous shuttle crossings

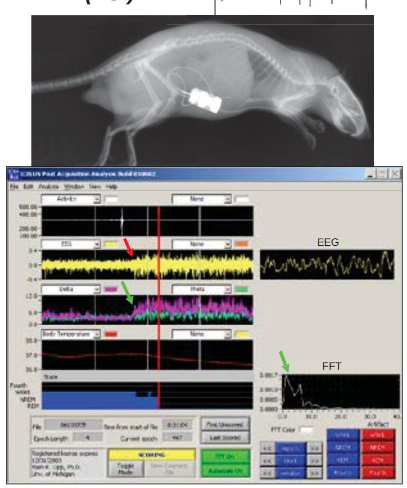
Chronic affective stressor: 20 days of fear conditioning

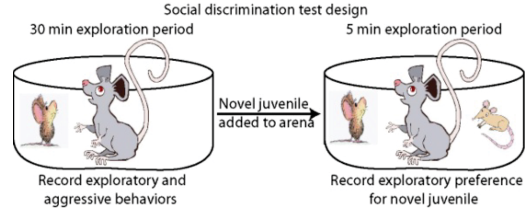


Rats exposed to fear conditioning maintain high level of fear for 20 days

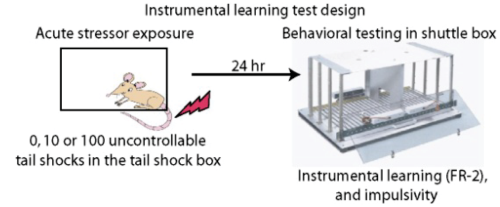
Online biotelemetry (DSI)





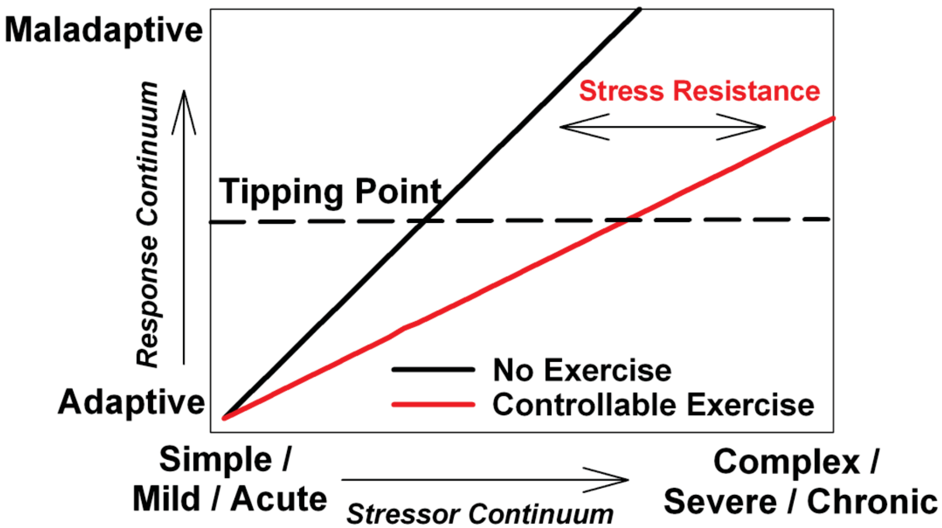


Anxiety, aggression, selective attention



Instrumental learning, impulsivity

UCB – Impact



Tipping point initiates changes in cortical EEG, neurophysiological stress response, gene expression, and neural pathway activation

Perception of "control" plus exercise actively engages stress-coping mechanisms to shift the tipping point (physiological and behavioral)

- **Two prong approach will rapidly allow identification of targets of stress resistance**
- **Vast genetic, neural pathway activation, and behavioral data will lead to a valid predictive model of stress resistance, thereby opening the door to novel approaches for enabling stress resistance in warfighters**
- **Completion of Phase I goals will allow rapid progression into Phase II modulation of stress resistance targets**
- **Optimal stress resistance protocols will be developed in male and female rats for optimal transference to humans**

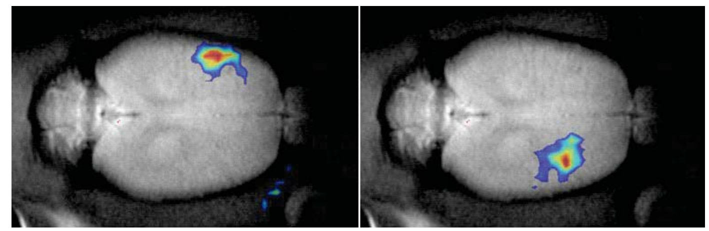
Massachusetts Institute of Technology

UNIQUE APPROACH: Identification of morphological, neural circuit firing and genetic correlates of changes in reward circuits in response to stress

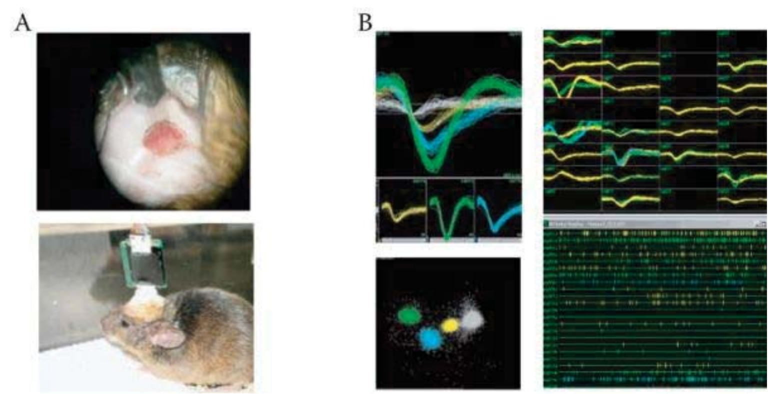
Technical Approach:

- Explore structural and functional changes in fear and reward circuits following multiple stressors
- Capture neural data using structural and functional magnetic resonance imaging as well as in vivo single unit recording
- Develop 'reward titration curves' from stressed and control animals to detect stress-related modulation of subjective reward perception
- Determine biomarkers of dysregulation following four chronic human-relevant stressors
- Statistical contrasts of gene expression across specific brain regions
- Stressors include: Immobilization stress, social defeat, triadic stress paradigm (shock), forced exercise

Team*:* *Ki Goosens* (MIT), Ann Graybiel (MIT), Alan Jasanoff (MIT), Rodrigo Cunha (University of Coimbra)



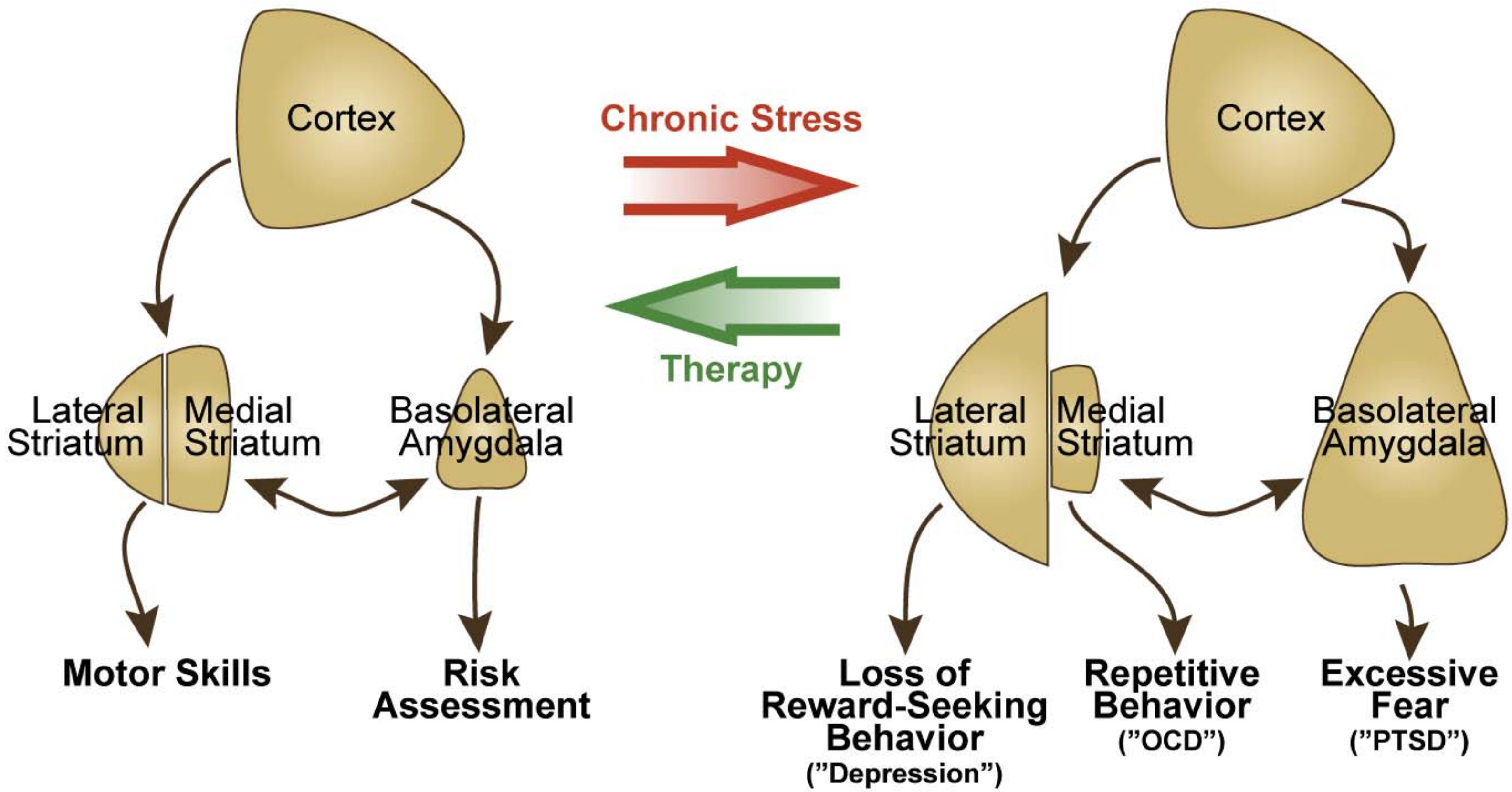
fMRI in a mouse brain



Single unit recording in a mouse

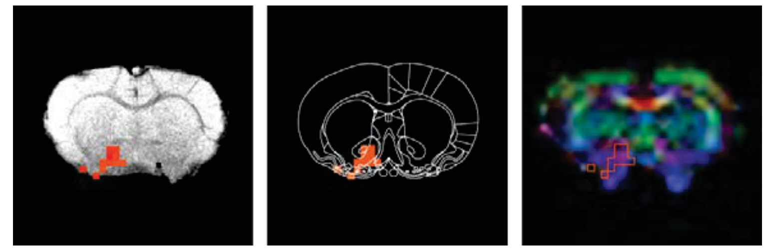
MIT Hypothesis

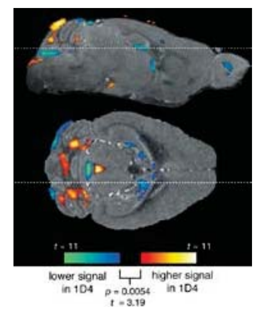
- **Chronic stress facilitates function in circuits for aversion and habit, and impairs function in circuits mediating reward**

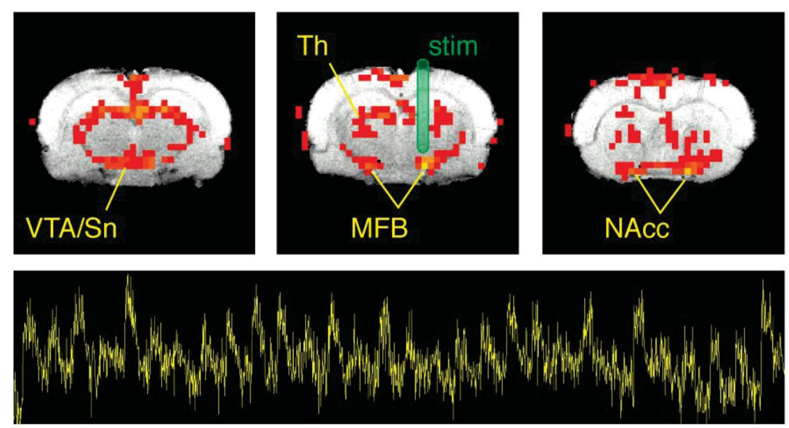


MIT Phase I – Overview

- Goal 1: Identify a small number of novel genes regulated by chronic stress in circuits regulating reward, aversion, and habit learning
- Goal 2: Measure structural and functional changes in these circuits across chronic stressors using structural and functional neuroimaging, and single-unit recording techniques
- Goal 3: Determine the impact of multiple stressors on cognitive function using behavioral measures relevant to stress-induced mental illness (fear conditioning, reward learning, and stereotypy)







MIT Technical Approach

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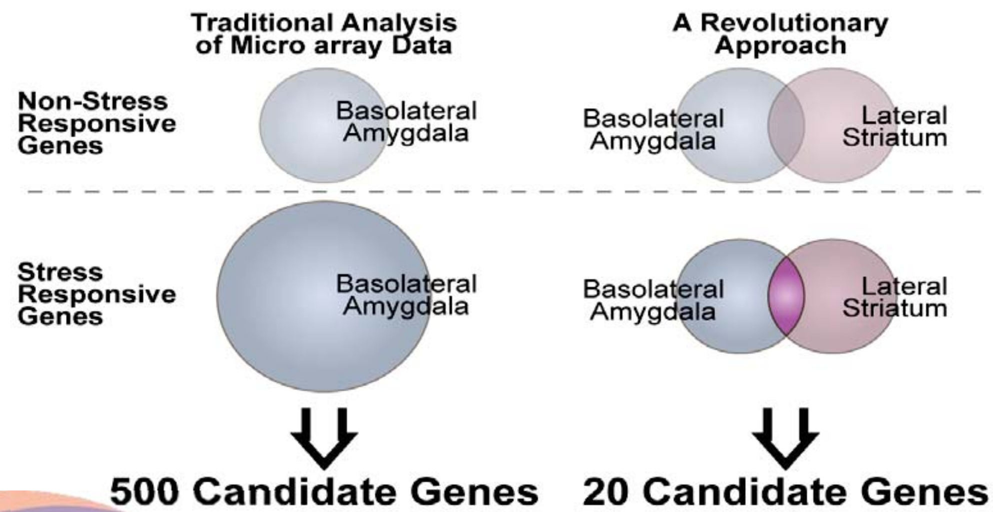
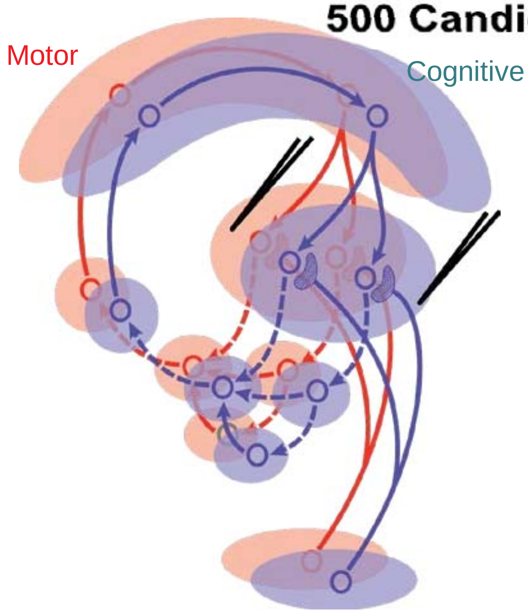
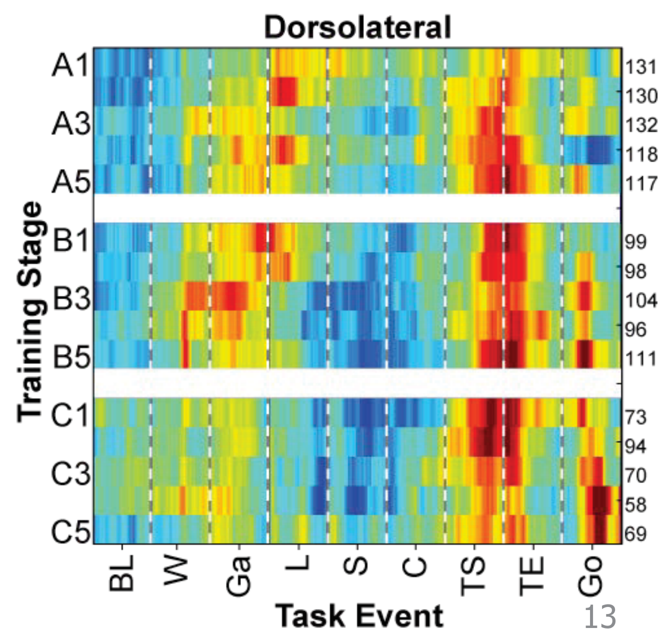
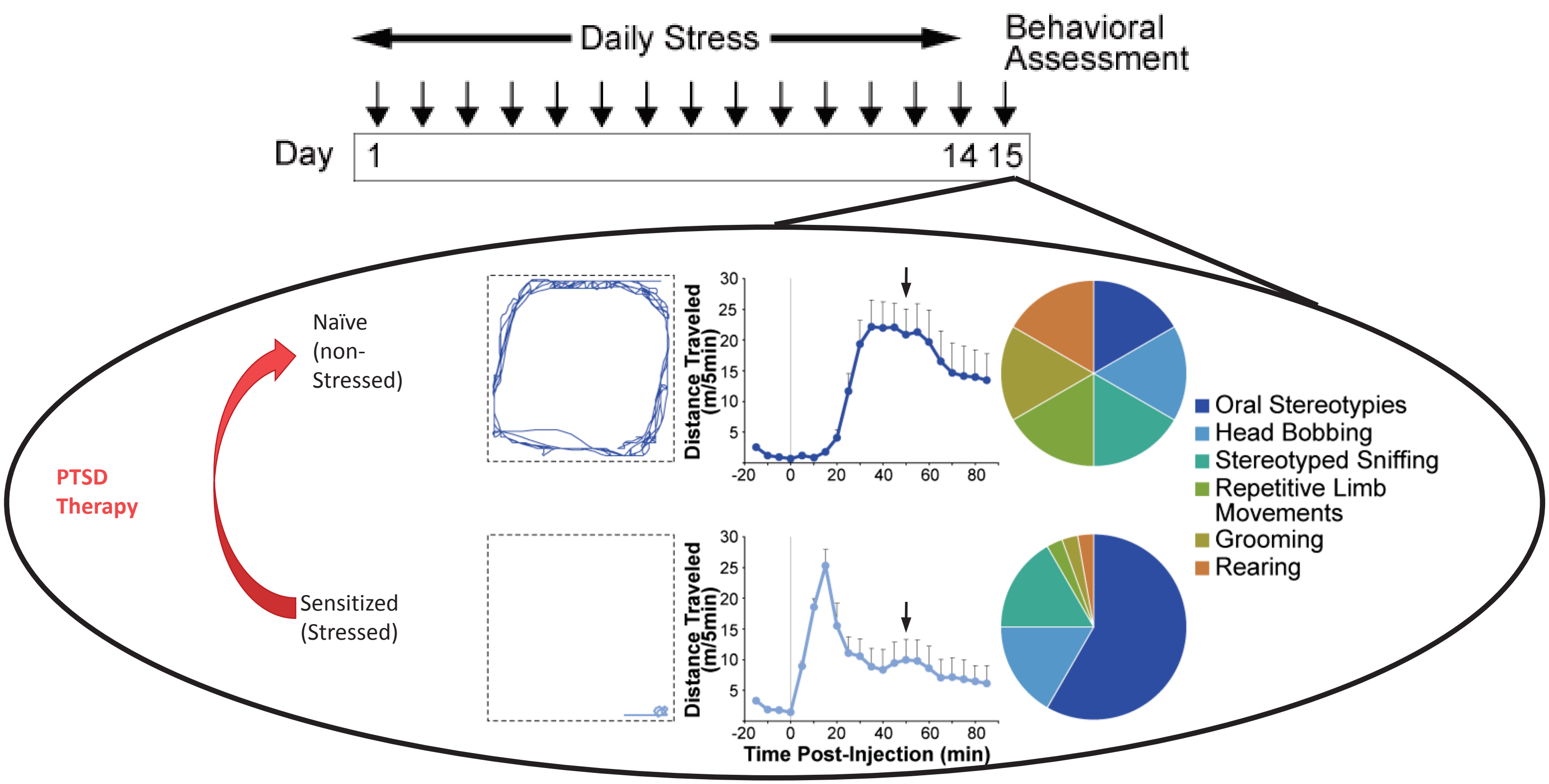






MIT Technical Approach

- Goal 3: Determine the impact of multiple stressors on cognitive function using behavioral measures relevant to stress-induced mental illness (fear conditioning, reward learning, and stereotypy)

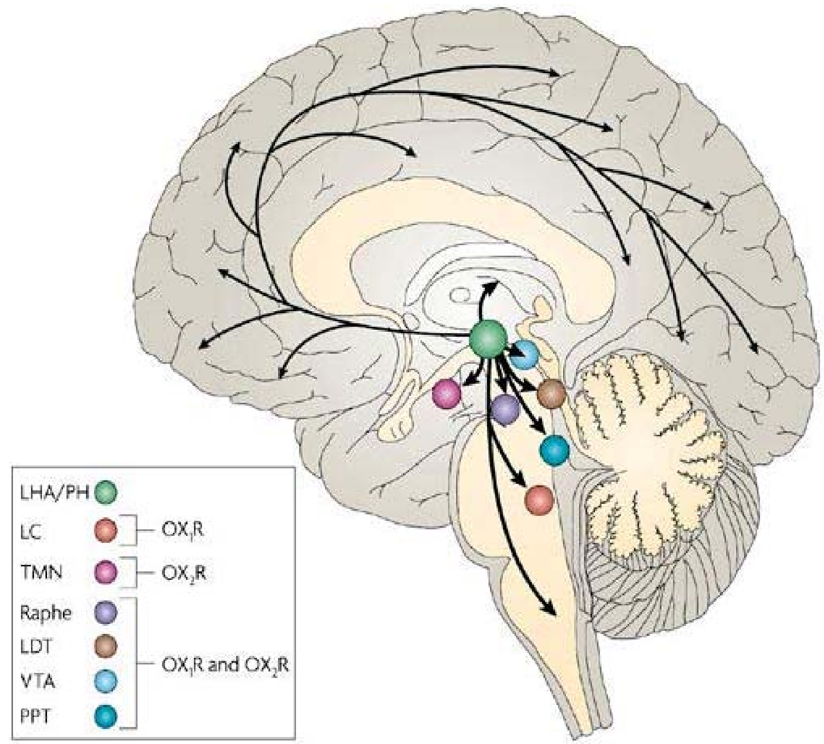


Children's Hospital of Philadelphia

UNIQUE APPROACH: Identification of changes in neural arousal systems associated with adaptive biological responses to stress

Technical Approach:

- Diagnostics approach with diffusion tensor imaging and manganese NMR for characterization of neuromorphology and its link to stress
- Quantify changes in neural structures, intracellular mediators and transmitters using optogenetic technologies to simulate stress activation or inhibition of arousal systems
- Determine intracellular and molecular mechanisms activated by chronic stress in the locus coeruleus using in vivo electrophysiology
- Identify epigenetic modifications induced by stress and the genes targeted by these modifications
- Stressors include: social defeat, sleep deprivation, restraint, forced swim



Team: ***Seema Bhatnagar*** (CHOP), Cheryl Beck (CHOP), Ted Abel (University of Pennsylvania), Luis DeLecea (Stanford), James Gee (University of Pennsylvania), Rita Valentino (CHOP)

CHOP Approach

OBJECTIVES
Phase 1a

TASKS

STRESS

DISCOVERY SCIENCE

1. Identify changes in white matter connectivity and gray matter microstructure by diffusion tensor magnetic resonance imaging.

2. Identify pathways and structures by manganese enhanced magnetic resonance imaging activated by the interaction of chronic and acute stress.

3. Identify epigenetic modifications induced by stress and the genes targeted by these modifications.

TARGETED SYSTEMS APPROACH

4. Quantify locus coeruleus activity using *in vivo* electrophysiology during behavior in the cortical-mediated set-shifting test.

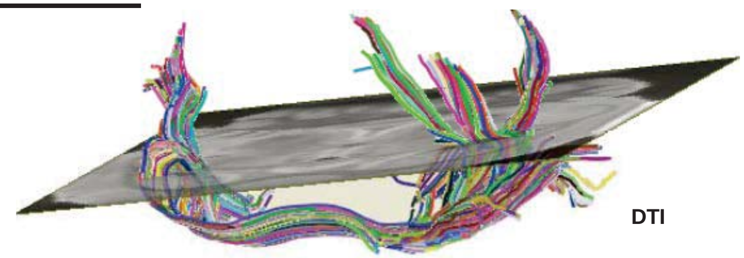
5. Determine intracellular and molecular mechanisms activated by chronic stress in the locus coeruleus using *in vitro* electrophysiology.

6. Quantify effects of optogenetic activation of Orexin cell bodies on behavior, physiology, neuronal activity and markers of plasticity in stress-regulatory regions.

CHOP Technical Approach

DISCOVERY SCIENCE

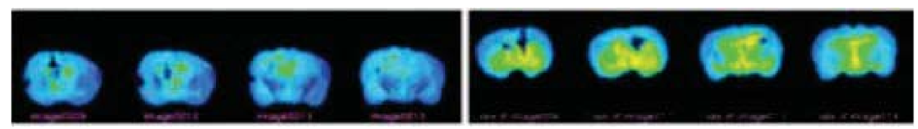
- DTI to determine white matter connectivity in stressed animals
- MN enhanced MRI following stress to determine neural substrates of stress
- Identify epigenetic modifications following stress



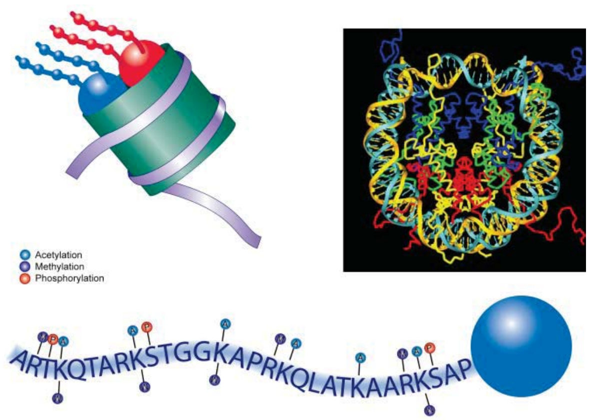
DTI

48 hours following forced swim

Control Stress



MN MRI

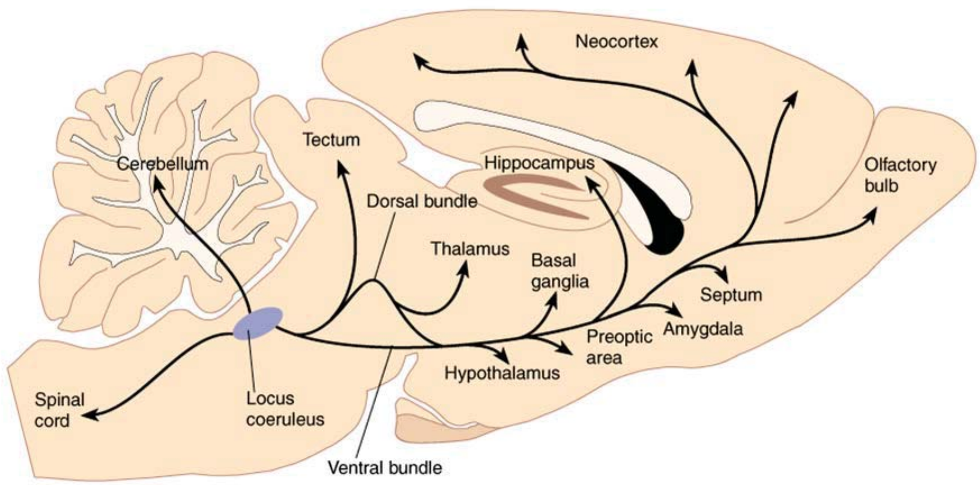


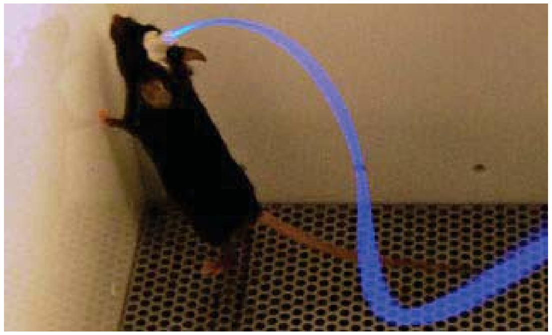
Histone proteins

CHOP Technical Approach

TARGETED SYSTEMS APPROACH

- Focus on the Locus Coereleus
- In vivo electrophysiology to determine firing patterns associated with behavior
- Effect of optogenetic activation on targeted cell populations





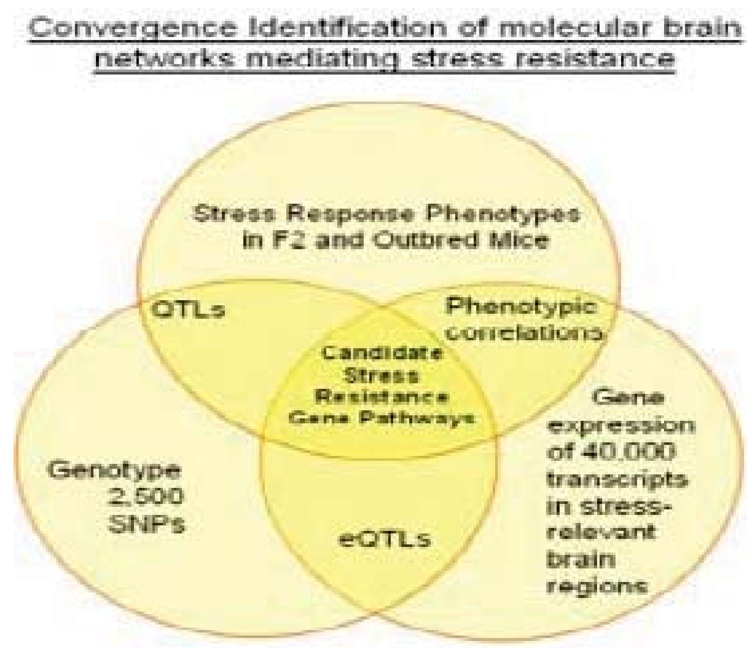
Optogenetics

Northwestern University

UNIQUE APPROACH: Exploits the genetic and genomic basis of stress resistance with high throughput genetic screening of genetically variable animals

Technical Approach:

- Use of both B6 and A/J parental strains as a platform for promoting genetic diversity within the animal population
- Use of correlation and pathway analysis to link phenotype, genotype (QTL) and gene expression levels (eQTL)
- Extensive use of state-of-the-art bioinformatics allowing for predictive modeling of stress pathway interactions
- Involvement of commercial pharmaceutical partners from Phase 1
- Investigate multiple stressors including social isolation, restraint stress, cold, sleep deprivation, forced swim, fear conditioning and social defeat under both acute and chronic conditions



Team: ***Fred Turek*** (Northwestern), Martha Vitaterna (Northwestern), David Johnson (Pinnacle Technologies), George Wilson (University of Kansas), Christopher Winrow (Merck)

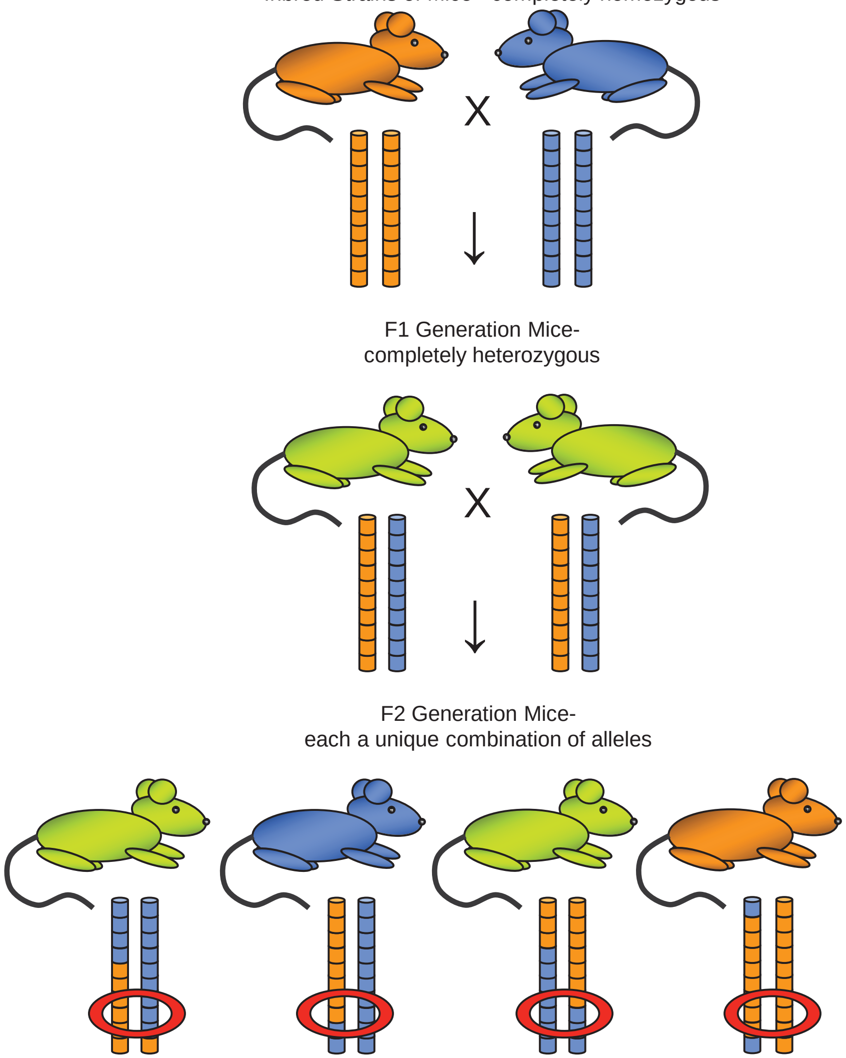
Phase 1a Experiments

- Experiment 1: Comparison of eight different acute stresses
- Experiment 2: Comparison of real-time brain glutamate and glucose levels in response to two acute stressors
- Experiment 3: Interaction of sleep deprivation with repeated stress

Phase 1b Experiment

- Experiment 4: Identify genetic loci (genes and networks of genes) involved in Enabling Stress Resistance to multiple stressors using 300 F2 offspring from two genetically and phenotypically diverse strains of mice.

Northwestern Experiments



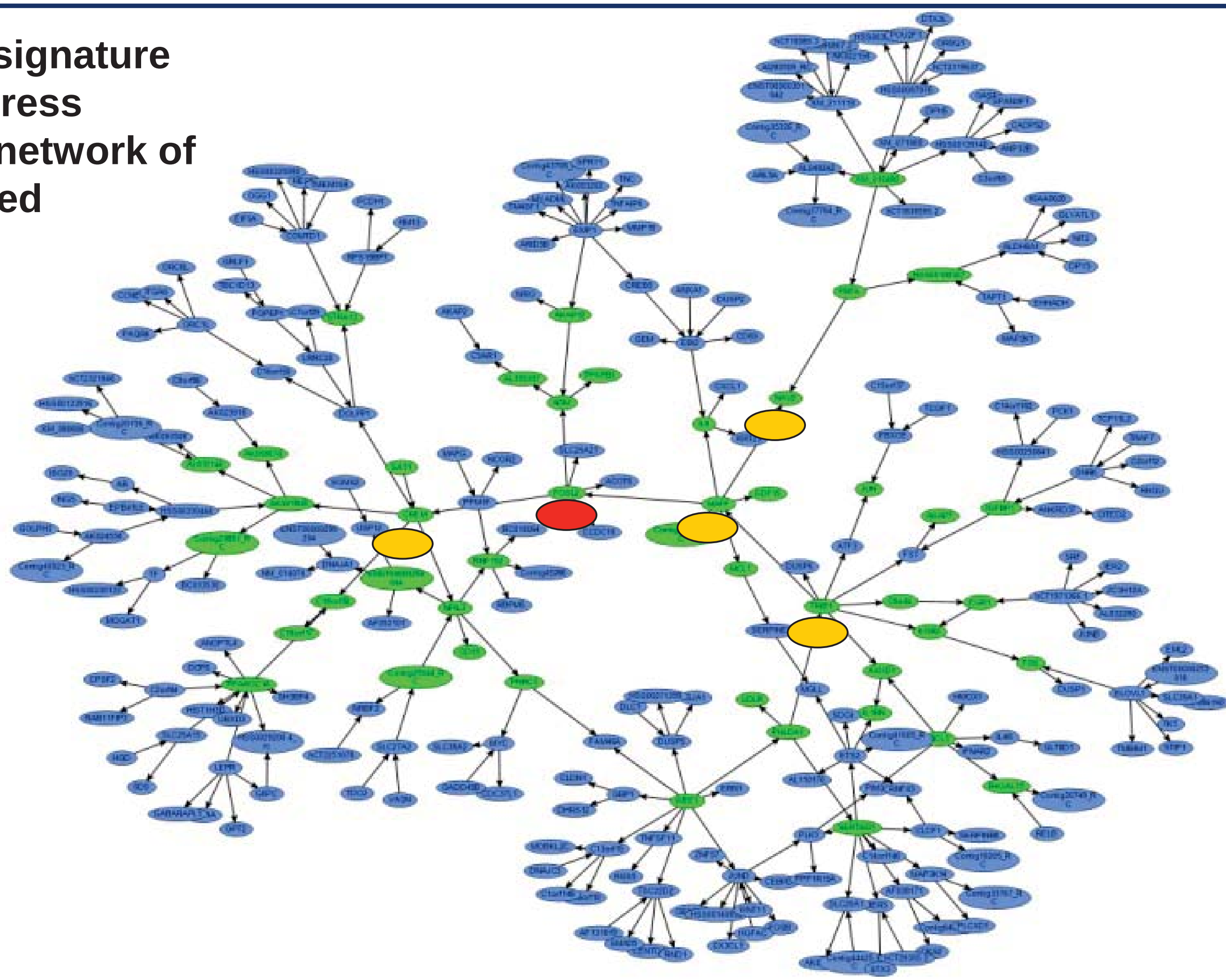
Identify target regions using 2,500 genetic markers for QTL analysis

Chronic Stress Groups			
A/J	**B6**	**F1**	**F2**
20	20	20	300

Stressor	Measurements of Stress Responses
Social Isolation	Elevated Plus Maze, Open Field Behavior, Blood pressure
Restraint Stress	4 time points for glucose and corticosterone
Cold Exposure	Body temperature, Blood pressure
Porsolt Forced Swim Test	Swimming vs. Floating behavior, one time point for glucose and corticosterone
Foot shock & Fear Conditioning	Fear (freezing) behavior during training & again 24 hours later, Blood pressure
Social Defeat	Latency to display submissive behavior, Elevated Zero maze, 1 time point for glucose & corticosterone
Dexamethasone Suppression	Fear behavior (freezing) retention test, 4 time points for ACTH and corticosterone; Feeding rhythms
Sleep Deprivation	48 hr EEG/EMG recording, 1 biosensor channel (to be determined by phase Ia)
Sleep Deprivation & Restraint	48 hr EEG/EMG recording (24 hr baseline)
Necropsy	Serum glucose, corticosterone, T3, T4, TSH, cytokines, telomerase, tissues dissected and frozen for gene expression analysis of 40,000 expressed transcripts for e-QTL analysis

Northwestern Anticipated Findings

Overlay key signature drivers for stress response to network of genes involved



Targets to Enable Stress Resistance