Enabling Stress Resistance (ESR)

COL Christian Macedonia
Program Manager, DARPA/DSO
A substantive understanding of what moderates the stress response is a prerequisite to promoting resilience and preventing stress-provoked cognitive dysfunction.

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 Proactive Approach

Multi-Disciplinary Approach

Wide-Ranging Results

Opportunity for More Rapid Interventions
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.
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)
**Novel Two Prong Approach:**

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

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

### Exercise design:

**C. Yoked wheel running design**
- Wheel locked/ no exercise
- Controllable/ voluntary exercise
- Uncontrollable/ mandated exercise

### Acute Stressor Exposure

<table>
<thead>
<tr>
<th>Exercise Condition</th>
<th>No acute stress</th>
<th>Mild (10 shocks)</th>
<th>Severe (100 shocks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Exercise</td>
<td>Control</td>
<td>☹</td>
<td>☹</td>
</tr>
<tr>
<td>Controllable Exercise</td>
<td>No Repeated Fear</td>
<td>☹</td>
<td>☹</td>
</tr>
<tr>
<td>Uncontrollable or &quot;Mandated&quot; Exercise</td>
<td>☹</td>
<td>☹</td>
<td>☹</td>
</tr>
<tr>
<td>Stress Resistance</td>
<td>Stress Resistance</td>
<td>Stress Resistance</td>
<td>Stress Resistance</td>
</tr>
</tbody>
</table>

### Predicted outcome:

- Neutral response
- Positive response
- Negative response

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

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

Cognitive and Affective Behavioral Measures

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

Online biotelemetry (DSI)

Chronic affective stressor: 20 days of fear conditioning

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

Anxiety, aggression, selective attention

Instrumental learning, impulsivity
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.
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)
Chronic stress facilitates function in circuits for aversion and habit, and impairs function in circuits mediating reward.
• 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)
• 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).
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

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.

STRESS


TARGETED SYSTEMS APPROACH
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

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
**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

Inbred Strains of Mice - completely homozygous

F1 Generation Mice - completely heterozygous

F2 Generation Mice - each a unique combination of alleles

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

Chronic Stress Groups

<table>
<thead>
<tr>
<th></th>
<th>A/J</th>
<th>B6</th>
<th>F1</th>
<th>F2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>300</td>
</tr>
</tbody>
</table>

Stressor

- Social Isolation
- Restraint Stress
- Cold Exposure
- Porsolt Forced Swim Test
- Foot shock & Fear Conditioning
- Social Defeat
- Dexamethasone Suppression
- Sleep Deprivation
- Sleep Deprivation & Restraint
- Necropsy

Measurements of Stress Responses

- Elevated Plus Maze, Open Field Behavior, Blood pressure
- 4 time points for glucose and corticosterone
- Body temperature, Blood pressure
- Swimming vs. Floating behavior, one time point for glucose and corticosterone
- Fear (freezing) behavior during training & again 24 hours later, Blood pressure
- Latency to display submissive behavior, Elevated Zero maze, 1 time point for glucose & corticosterone
- Fear behavior (freezing) retention test, 4 time points for ACTH and corticosterone; Feeding rhythms
- 48 hr EEG/EMG recording, 1 biosensor channel (to be determined by phase Ia)
- 48 hr EEG/EMG recording (24 hr baseline)
- 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

Identify target regions using 2,500 genetic markers for QTL analysis
Overlay key signature drivers for stress response to network of genes involved

Targets to Enable Stress Resistance