Transcriptional and Epigenetic Mechanisms of Depression

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Depression

Incidence
- 2-5% severe, 20% mild.
- One of the top causes of morbidity and mortality worldwide (World Health Organization).

Causes
- Roughly 50% of the risk is genetic, but specific causative genes have not yet been found.
- Role for chronic stress implicated in some people.
- Syndromic: a heterogeneous collection of many illnesses.

Treatment
- Very effective treatments, but only half of all patients show full remission.
## Diagnosis of Depression

### Diagnostic criteria for major depression

**Mood:**
- Depressed mood
- Irritability, anxiety in some patients

**Cognition:**
- Low self esteem
- Feelings of hopelessness, worthlessness, and guilt
- Decreased ability to concentrate and think
- Recurrent thoughts of death and suicide

**Neurovegetative function:**
- Decreased or increased appetite
- Weight loss or weight gain
- Insomnia or hypersomnia
- Low energy, fatigue or increased agitation
- Anhedonia: decreased interest in pleasurable stimuli (e.g., sex, food, social)

Based on the Diagnostic and Statistical Manual (DSM, 2013)
Highly integrated “limbic” circuits innervated by brainstem monoaminergic systems.
Deep brain stimulation of sg25 or of nucleus accumbens elevates mood and is antidepressant in humans.
Rodent Models of Depression?
Animal Models of Depression

“Despair”-based tests

- Forced swim, tail suspension, & learned helplessness tests.
- Rapid, easy to perform.
- Most widely used screens for antidepressant action.
- But involve acute stress and acute antidepressant effects in normal animals.
- Lack etiological (construct) and face validity.
Social Defeat Model of Depression

Chronic social defeat causes:

- Anhedonia-like symptoms (decreased interest in sucrose and sex)
- Anxiety-like symptoms
- Hyperactivity of HPA axis
- Disrupted circadian rhythms
- Increased addiction liability
- Metabolic syndrome
- Profound social avoidance

Berton et al., *Science*, 2006
Social Defeat Induced Avoidance

Long-lasting social avoidance:

Berton et al., Science, 2006
Reversal of Social Avoidance by Antidepressant Treatment

Long-lasting social aversion is reversed by chronic, but not acute, antidepressants. The aversion generalizes to all mice, making it maladaptive (pathologic).

Social Defeat Model of Depression: Susceptibility vs. Resilience

Roughly one-third of defeated mice are “resilient”:

Resilience for social avoidance is associated with resilience for other symptoms of chronic social defeat (e.g., anhedonia, metabolic syndrome), but not all symptoms (e.g., anxiety).

Krishnan et al., Cell, 2007
Social Defeat Model of Depression: Metabolic Syndrome

Weight gain associated with:

- Elevated plasma glucose, insulin, and leptin
- Elevated LDL cholesterol
- Abnormal liver metabolism

Reversed by antidepressants

Chuang … Lutter, Biol. Psychiatry, 2010
What Human Syndrome Is Modeled by Chronic Social Defeat Stress in Mice?

1. It is impossible to know at present, mainly because of very limited knowledge of the biology of human depression and anxiety syndromes, which are highly comorbid.

2. Social defeat produces a mixed picture of depressive- and anxiety-like symptoms, but clearly separates the two: susceptible mice show both, while resilient mice show anxiety only.

3. We thus believe that the social defeat paradigm is useful in helping us understand the biology of stress-related illness (including depression, anxiety, post-traumatic stress, etc.).

4. Ultimately, only a better understanding of the human syndromes will allow us to relate a particular illness with this or any other animal model.
Chromatin Studies Offer Major Advances

- Help identify stress-regulated genes.
- First ever look at transcriptional mechanisms *in vivo*.
- Unique mechanisms of long-lasting adaptations.
- New approaches to treatment.
Regulation of Gene Expression is Reflected at the Chromatin Level

Active (open)
- Basal transcription complex
- HDAC
- HAT
- Co-activators
- Transcription factors (e.g., CREB, ∆FosB)
- Histone N-termini

Inactive (condensed)
- HDAC
- HMT
- DNMT
- Repressors
- Nucleosome
Overlay RNA-seq and ChIP-seq data to identify genes regulated by stress and the underlying epigenetic mechanisms involved.

- Acetylated H3 or H4
- Activational H3 methylation (K4)
- Repressive H3 methylation (K9, K27)
- DNA methylation
- CREB (pro-susceptibility)
- ΔFosB (pro-resilience)
- Other
- mRNAs
**Insight Into Susceptibility vs. Resilience**

RNA-seq analyses 48 hr after chronic social defeat stress:

<table>
<thead>
<tr>
<th>Region</th>
<th>Resilient vs. control</th>
<th>Susceptible vs. control</th>
</tr>
</thead>
<tbody>
<tr>
<td>NAc</td>
<td>↑ 212</td>
<td>↓ 12</td>
</tr>
<tr>
<td>PFC</td>
<td>↑ 79</td>
<td>↓ 287</td>
</tr>
<tr>
<td>BLA</td>
<td>↑ 108</td>
<td>↓ 43</td>
</tr>
<tr>
<td>vSUB</td>
<td>↑ 69</td>
<td>↓ 176</td>
</tr>
</tbody>
</table>
Correlation of Expression Levels of Genes Differentially Regulated by Social Defeat

**NAc correlations**

- **vSUB**
  - Control: 0.8 ± 0.1
  - Resilient: 0.9 ± 0.2
  - Susceptible: 0.8 ± 0.1
- **PFC**
  - Control: 0.9 ± 0.1
  - Resilient: 0.9 ± 0.1
  - Susceptible: 0.8 ± 0.1
- **BLA**
  - Control: 0.9 ± 0.1
  - Resilient: 0.9 ± 0.1
  - Susceptible: 0.8 ± 0.1

**PFC correlations**

- **vSUB**
  - Control: 0.8 ± 0.1
  - Resilient: 0.9 ± 0.2
  - Susceptible: 0.8 ± 0.1
- **NAc**
  - Control: 0.9 ± 0.1
  - Resilient: 0.9 ± 0.1
  - Susceptible: 0.8 ± 0.1
- **BLA**
  - Control: 0.9 ± 0.1
  - Resilient: 0.9 ± 0.1
  - Susceptible: 0.8 ± 0.1

**BLA correlations**

- **vSUB**
  - Control: 0.8 ± 0.1
  - Resilient: 0.9 ± 0.2
  - Susceptible: 0.8 ± 0.1
- **PFC**
  - Control: 0.9 ± 0.1
  - Resilient: 0.9 ± 0.1
  - Susceptible: 0.8 ± 0.1
- **NAc**
  - Control: 0.9 ± 0.1
  - Resilient: 0.9 ± 0.1
  - Susceptible: 0.8 ± 0.1

**vSUB correlations**

- **NAc**
  - Control: 0.8 ± 0.1
  - Resilient: 0.9 ± 0.2
  - Susceptible: 0.8 ± 0.1
- **PFC**
  - Control: 0.9 ± 0.1
  - Resilient: 0.9 ± 0.1
  - Susceptible: 0.8 ± 0.1
- **BLA**
  - Control: 0.9 ± 0.1
  - Resilient: 0.9 ± 0.1
  - Susceptible: 0.8 ± 0.1
## Predicted Upstream Regulators in NAc of Resilience vs. Susceptibility

<table>
<thead>
<tr>
<th>Upstream regulator</th>
<th>NAc</th>
<th>PFC</th>
<th>BLA</th>
<th>vSUB</th>
</tr>
</thead>
<tbody>
<tr>
<td>ESR1 (estrogen receptor 1)</td>
<td>Activated</td>
<td>ns</td>
<td>Activated</td>
<td>Inhibited</td>
</tr>
<tr>
<td>IL6 (interleukin 6)</td>
<td>ns</td>
<td>Inhibited</td>
<td>ns</td>
<td>Inhibited</td>
</tr>
<tr>
<td>E. coli b4 LPS (lipopolysaccharide)</td>
<td>Activated</td>
<td>ns</td>
<td>Activated</td>
<td>Inhibited</td>
</tr>
<tr>
<td>Calmodulin</td>
<td>Activated</td>
<td>Inhibited</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Adcyap1 (adenylyl cyclase activating peptide)</td>
<td>Activated</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>WNT3a</td>
<td>Activated</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>β-Catenin</td>
<td>Activated</td>
<td>ns</td>
<td>ns</td>
<td>Activated</td>
</tr>
</tbody>
</table>
Concerted regulation of WNT–DVL–GSK3β–β-catenin signaling in NAc.

Pathway down in susceptible, up in resilient, mice; and down in depressed humans.

Color code depicts different modes of regulation on gene and chromatin arrays.
Suppression of DVL-\(\beta\)-Catenin Signaling in NAc Promotes Susceptibility

Viral-mediated overexpression of dominant negative DVL or \(\beta\)-catenin (or wildtype GSK3\(\beta\)) in NAc increases susceptibility.

- Equivalent effects seen in sucrose preference.

Wilkinson et al., *J. Neurosci.*, 2011; Dias et al., unpublished
Upregulation of DVL-βCatenin Signaling in NAc Promotes Resilience

Viral-mediated overexpression of DVL or β-catenin (or dominant negative GSK3β) in NAc increases resilience.

Chronic (10 day) defeat

- Equivalent effects seen in sucrose preference.

Wilkinson et al., J. Neurosci., 2011; Dias et al., unpublished
Pharmaceutical experience is that GSK3β is not a good drug target.

Now focusing on genes downstream of β-catenin (by ChIP-Seq) as a way to identify better targets.
Identifying Novel Targets of β-Catenin in NAc

ChIP-seq reveals β-catenin targets

Control

Susceptibility

Resilience

Promoter

Proximal promoter
1 kb promoter
3 kb promoter
Gene body
Gene desert
Other intergenic
Pericentromere

β-catenin

Nucleus

Cytoplasm

Plasma Membrane

Extracellular Space

b-Catenin targets

Promoter types:
- Proximal promoter
- 1 kb promoter
- 3 kb promoter
- Gene body
- Gene desert
- Other intergenic
- Pericentromere
Examples of Target Genes: Induction of KCNQ Channels in Resilient VTA

Induction of several $K^+$ channel subunits, including KCNQ channels, in the VTA of resilient but not susceptible mice.

Induction of $K^+$ channels in the resilient VTA, first observed with these gene discovery approaches, suggested that susceptibility vs. resilience is related to alterations in VTA neuronal excitability.

Krishnan, Han, et al., *Cell*, 2007
Dopaminergic hyperactivity is selective for the VTA-NAc circuit, with hypoactivity seen in the VTA-mPFC circuit.

Dopamine neurons that project from the VTA to the NAc show hyperactivity:

VTA-NAc Circuit

Dopamine neurons that project from the VTA to the mPFC show hypoactivity:

VTA-mPFC Circuit

Krishnan, et al., Cell, 2007; Cao et al., J. Neurosci., 2010; Chaudhury ... Han, Nature, 2013
Hyperactivity of the VTA-NAc Circuit Mediates Social Defeat Stress

Bidirectional optogenetic control of the VTA-NAc circuit.

Selective targeting of VTA-NAc circuit

Subthreshold defeat

Chronic defeat

Equivalent effects seen in sucrose preference.

Chaudhury … Han, Nature, 2013
Lasting Effects of Susceptibility vs. Resilience

RNA-seq analysis 4 weeks after chronic social defeat stress:

Log2 fold change

NAc

PFC

BLA

vSUB

Resilient vs. control

Susceptible vs. control

Resilient vs. control

Susceptible vs. control

140

106

122

74

154

64

208

157

115

167

126

156

291

49

141

199

551

454

296

389

176

450

115

127

142

100
Lasting Effects of Susceptibility vs. Resilience

RNA-seq analysis 4 weeks after social defeat + acute defeat:

- NAc
  - Resilient vs. control: 32
  - Susceptible vs. control: 226
- PFC
  - Resilient vs. control: 200
  - Susceptible vs. control: 67
- BLA
  - Resilient vs. control: 96
  - Susceptible vs. control: 110
- vSUB
  - Resilient vs. control: 22
  - Susceptible vs. control: 60
Epigenetic Modifications Further Define Stress-Related Genes in NAc

ChIP-seq for histone modifications and transcription factors in NAc that control resilience vs. susceptibility.

All changes shown persist at 1 month, and are filtered by altered mRNA expression.

- Imipramine reverses most of the lasting effects of susceptibility.

- Most lasting changes seen in susceptibility are not seen in resilience and vice versa.

Wilkinson et al., *J Neurosci*, 2009; unpublished data
Antidepressants work in part by inducing some of the same chromatin changes that occur naturally in resilience.
What Mechanisms Underlie Vulnerability vs. Resilience?

1. **Genetic** factors are important:
   - Inbred mouse strains show different rates of vulnerability.

2. **Environmental** factors are important:
   - Prolonged social isolation increases vulnerability.
   - Maternal separation increases vulnerability.

3. **Epigenetic** factors?
   - Great variability seen despite constant genetics, constant environment (rearing, dominance, etc.), and no difference in acute stress responses.
   - Stochastic, epigenetic changes that occur during development that contribute to further variability in a species.
Preliminary Evidence for True Epigenetic Transmission of Stress Vulnerability

Offspring of defeated mice are more vulnerable to defeat.

Sons of defeated mice exhibit greater baseline anxiety:

Sons of control mice
Sons of defeated mice

Elevated plus maze

Social interaction time (sec)

Corner time (sec)

Episodes of social defeat

Dietz et al., Biol. Psychiatry, 2011
1. Studies of molecular changes in limbic brain regions reveal novel mechanisms of stress susceptibility and resilience as well as antidepressant action.

2. Parallel studies underway on:
   - Life-long increases in susceptibility caused by early life stress; or increases in resilience caused by other stimuli.
   - Postmortem brain tissue from depressed humans vs. control subjects.
   - Other chronic stress models and blood biomarkers.

3. Insight into long-lasting mechanisms from chromatin studies.

4. Use this vast dataset to develop fundamentally new diagnostic tests and treatments for depression and related syndromes.