Transcriptional and Epigenetic Mechanisms of Depression

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The Friedman Brain Institute

1

Use of Genome-Wide Datasets to Gain Unique Insight Into the Biology of Depression

Today's talk:

1. Brief overview of transcriptional and epigenetic mechanisms of gene regulation.

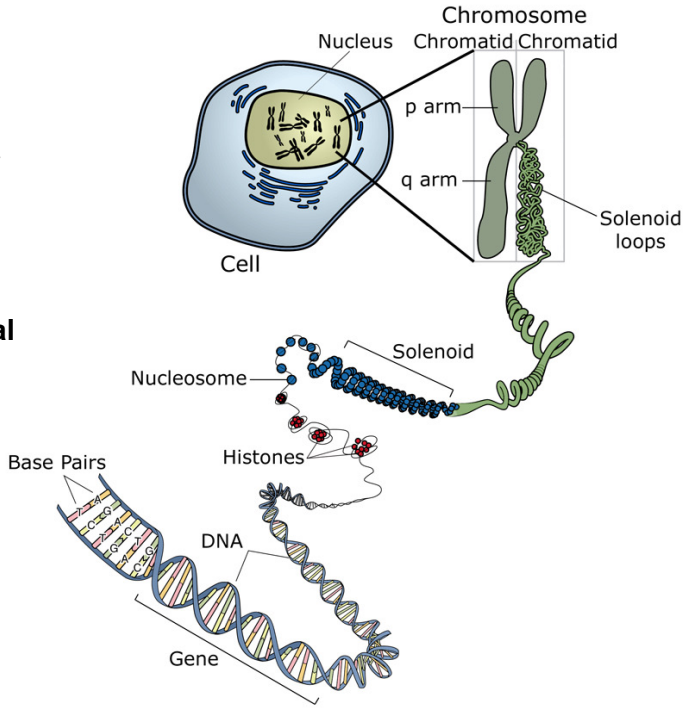
2. Insight into the molecular basis of sex differences in depression.
- **Depression is >2-fold more common in women than men.**
- **Yet, little is known about the underlying molecular mechanisms involved.**

3. Insight into the molecular basis of stress resilience vs. susceptibility.
- **The mechanisms that govern an individual's inherent vulnerability to different forms of chronic stress.**
- **Two examples:**
  - **LINC00473: a long noncoding RNA and proresilience factor in females only.**
  - **OTX2: a transcription factor that mediates the effects of early life stress.**

2

Chromatin Studies Offer Major Advances

- **Help identify genes affected by stress in mice or depression in humans, or those associated with stress resilience.**
- **First ever look at transcriptional mechanisms *in vivo*.**
- **Unique mechanisms of long-lasting adaptations.**



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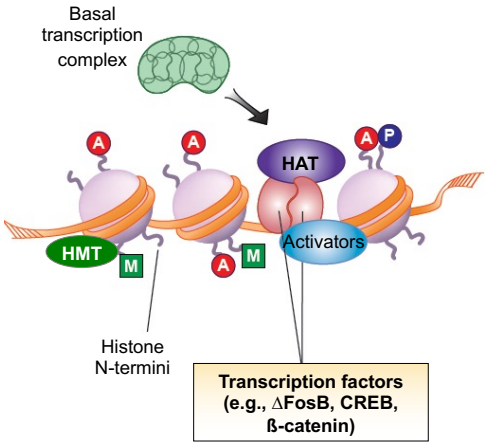
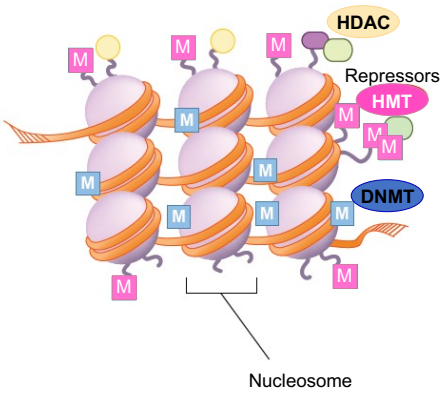
Regulation of Gene Expression is Reflected at the Chromatin Level

Active chromatin (open)



Inactive chromatin (condensed)



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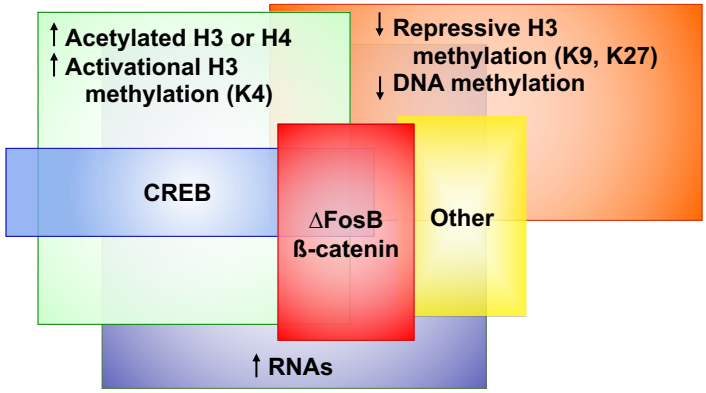
Identifying Transcriptional and Epigenetic Mechanisms of Stress Susceptibility & Resilience in Mice and Humans

Overlaying ChIP-seq and related analyses of chromatin modifications and transcription factors onto RNA-seq measures of gene expression:



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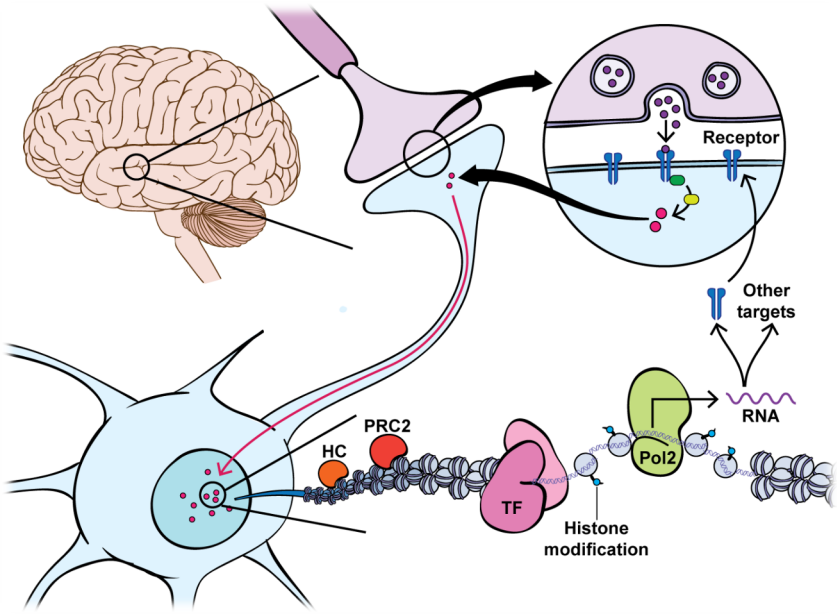
Template for Understanding Disease Pathophysiology and Advancing New Treatments

These unbiased studies provide an unprecedented look at genes, proteins, and biochemical pathways that are crucial for stress susceptibility vs. resilience, and will guide drug discovery efforts beyond the synapse.

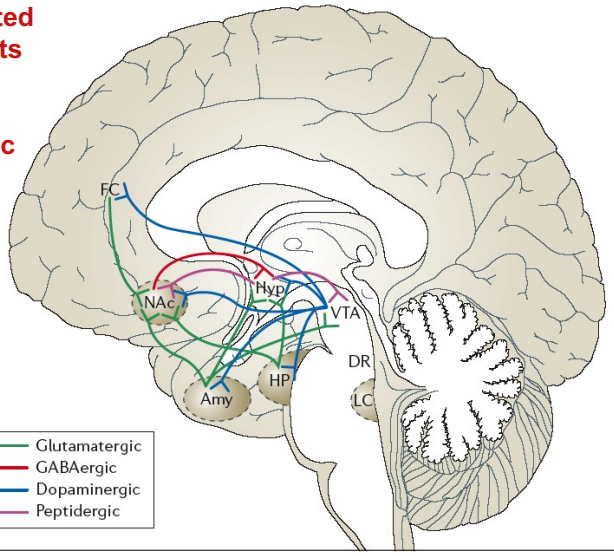


Mews et al., *CSHSQB*, 2019

6

Neural Substrates of Stress Susceptibility and Resilience

Highly integrated "limbic" circuits innervated by brainstem monoaminergic systems.



7

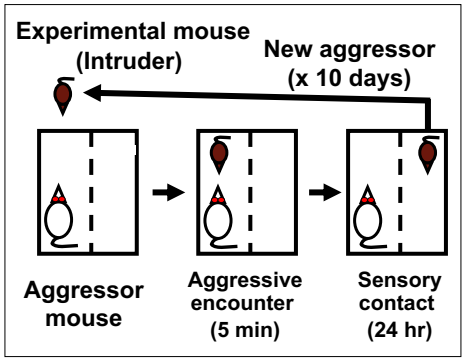
Rodent Models of Stress Susceptibility vs. Resilience?

Happy | Sad | Humorful | Hopeless | Optimistic

Suicidal | Lustful | Frustrated | Worried | Confused

8

Social Defeat Model of Stress Susceptibility vs Resilience



Berton et al., *Science*, 2006
Tsankova et al., *Nat Neurosci*, 2006
Krishnan et al., *Cell*, 2007
Chuang et al., *Biol Psychiatry*, 2010
Covington et al, *Neuron*, 2011

Chronic social defeat stress causes:

- **Anhedonia-like symptoms (decreased interest in sucrose, sex, etc.)**
- **Anxiety-like symptoms**
- **Hyperactivity of HPA axis**
- **Disrupted circadian rhythms**
- **Increased addiction liability**
- **Metabolic syndrome**
- **Profound social avoidance**
- **Reversal of symptoms with chronic ADT**

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Features of the Social Defeat Paradigm

1. While most mice are "susceptible," a smaller subset, despite equivalent levels of stress, are "resilient."

- **They avoid anhedonia, social avoidance, and metabolic impairments, but still display anxiety-like symptoms.**

2. Some of these symptoms of susceptibility (e.g., social avoidance and metabolic impairments) are essentially life-long.

3. The anhedonia, social avoidance, and metabolic impairments in susceptible mice are reversed by chronic (not acute) treatment with standard antidepressant medications.

- **Anxiolytic drugs are without effect.**
- **Single doses of ketamine are also effective.**

These findings are consistent with the observation that a majority of humans are resilient in the face of horrendous stress.

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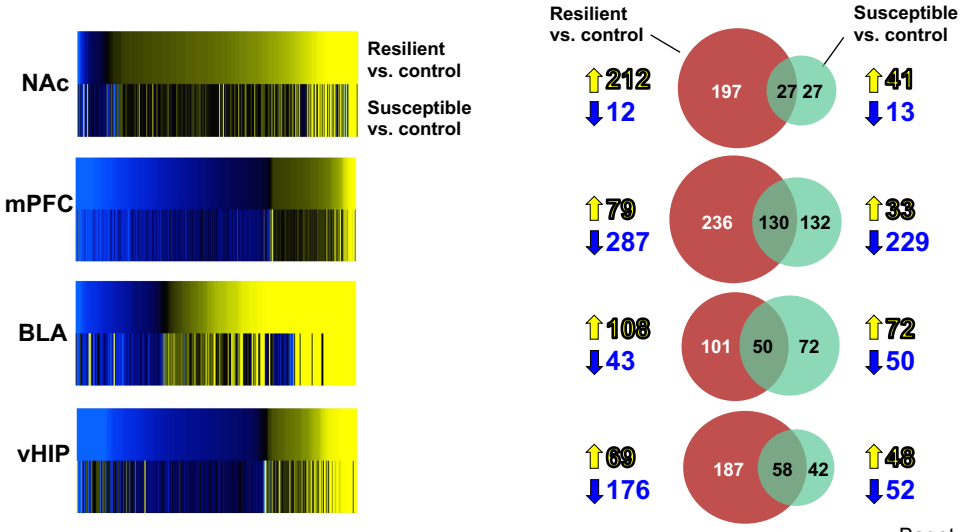
Insight Into Susceptibility vs. Resilience

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



Bagot et al., *Neuron,* 2016

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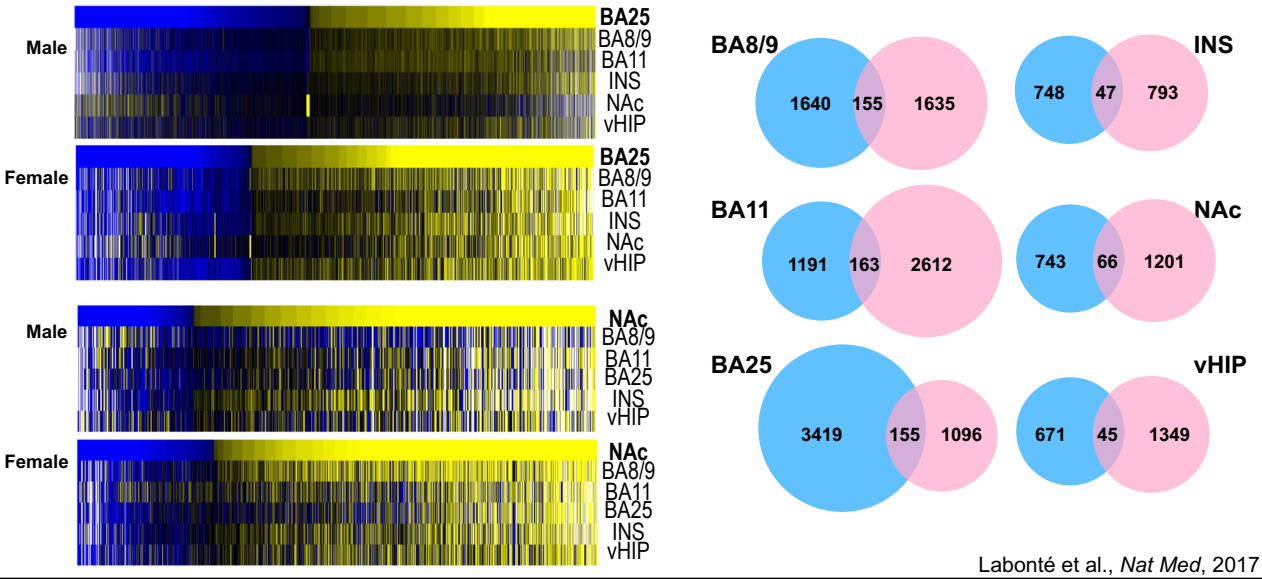
Insight Into Human Depression

RNA-seq analysis in depressed humans (n=100):



Labonté et al., *Nat Med*, 2017

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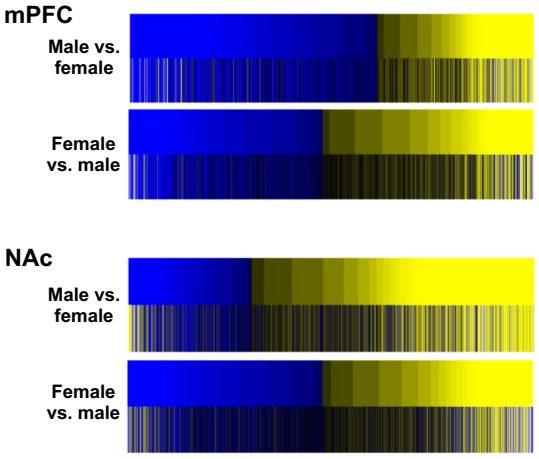
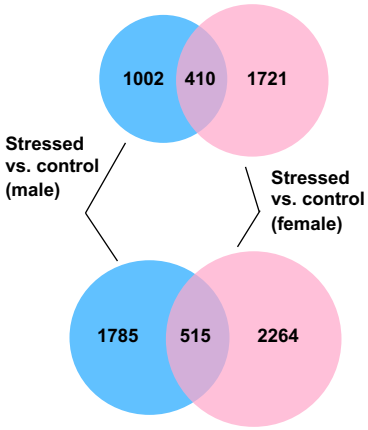
Gene Expression Differences in Chronically Stressed Mice: Males vs. Females

Chronic (21 days) variable stress paradigm produces equivalent behavioral abnormalities in male vs. female mice but very different changes in gene expression:





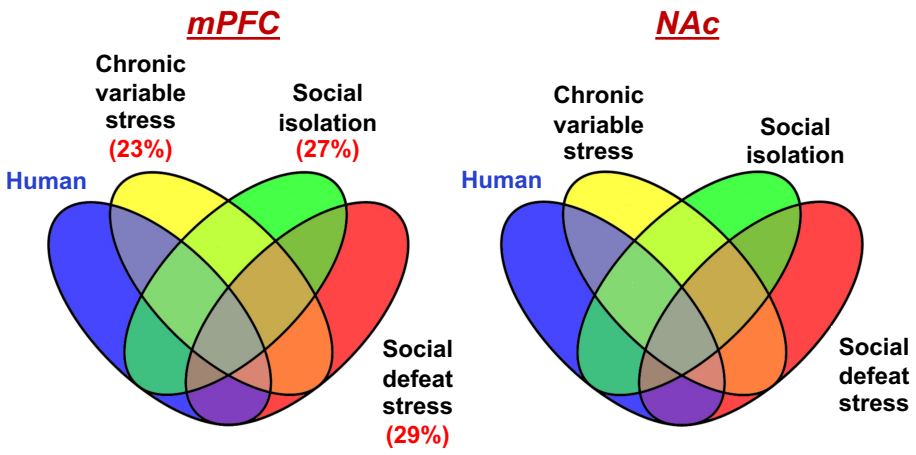
Similar lack of convergence seen in other chronic stress models, for example, chronic social defeat stress, in which males and females show similar susceptibility.

Labonté, Hodes et al., *Nat Med*, 2017

13

Comparing Transcriptional Signatures in Humans and Animal Models

Molecular validation of an animal model:



Labonte et al., *Biol Psychiatry*, 2020

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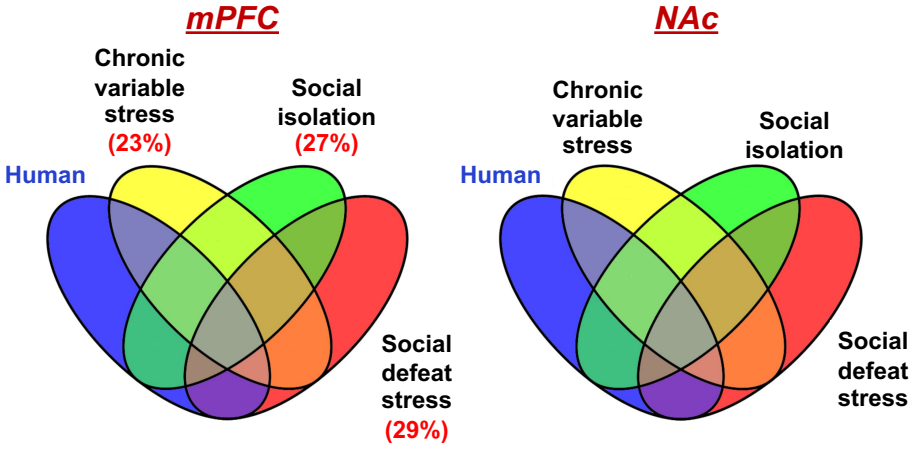

This convergence between chronic stress models in mice and human depression is not seen for other human syndromes (e.g., schizophrenia, bipolar disorder, autism).

Labonte et al., *Biol Psychiatry*, 2020

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Long Non-Coding RNAs: Novel Mediators of Epigenetic Regulation

1. Long non-coding RNAs (lncRNAs) are a recently discovered class of regulatory RNAs that play pivotal roles in epigenetic regulation.

2. They are defined as containing more than 200 nucleotides and generally have similar structural properties as transcripts of protein-coding genes (PCGs).

3. LncRNAs play regulatory roles as decoys, scaffolds, or guides, and are classified into several biotypes according to their genomic proximity to PCGs:
- **Those with no overlap with PCGs are long intergenic lncRNAs (lincRNAs).**
- **Those encoded by the opposite strand of a PCG are antisense RNAs.**

4. There are more lncRNAs than PCGs in the human genome, with a third of lncRNAs having arisen within the primate lineage.
- **~40% of lncRNAs are brain-specific.**

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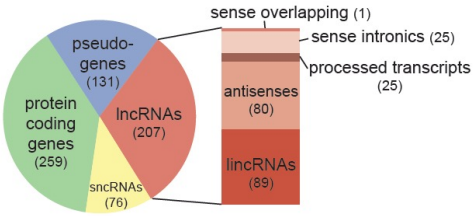
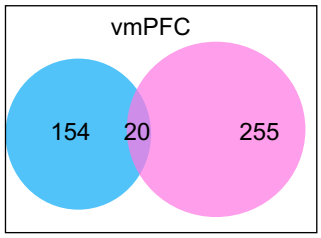
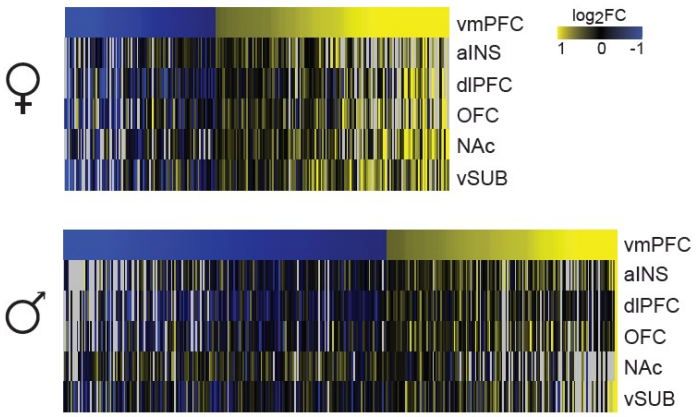
Prominent Regulation of lncRNAs in Human Depression

More lncRNAs than PGCs are altered in human depression:





Regulation of lncRNAs across brain regions:



Issler et al., *Neuron*, 2020

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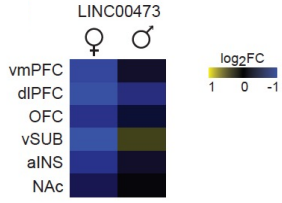
Suppression of LINC00473 in vmPFC of Depressed Females

Identifying LINC00473:

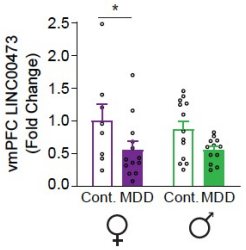
	♀	♂
Correlation with PCGs	931	757
Higher abundance	616	551
Differentially expressed	20	0
Neuronal	2	0
Expressed in NPCs	1	0

LINC00473

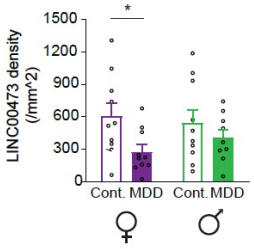
Suppression of LINC00473 in depressed females only:

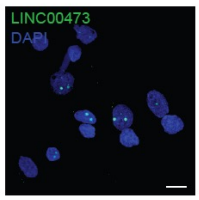


Replication of RNA-seq findings in vmPFC of second human cohort:



Downregulation of LINC00473 in vmPFC of depressed females, but not males, as determined by RNA-Scope (Chunfeng Tan & Carol Tamminga):





Issler et al., *Neuron*, 2020

18

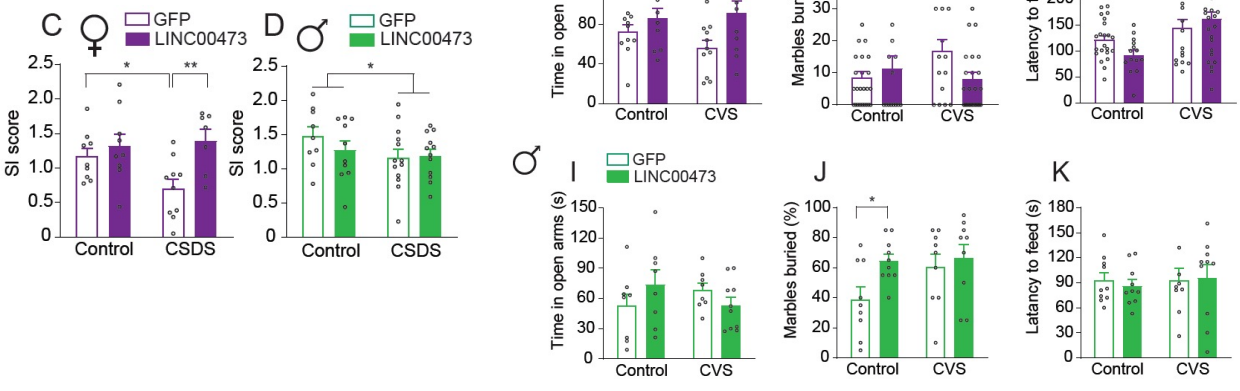
How Does One Study the Actions of a lncRNA That is Primate-Specific?

Issler et al., *Neuron*, in review

19

Sex-Specific Effects of LINC00473 Expression in vmPFC of Female vs. Male Mice

Viral-mediated expression of LINC00473 in vmPFC neurons promotes resilience in female but not male mice:



Issler et al., *Neuron*, 2020

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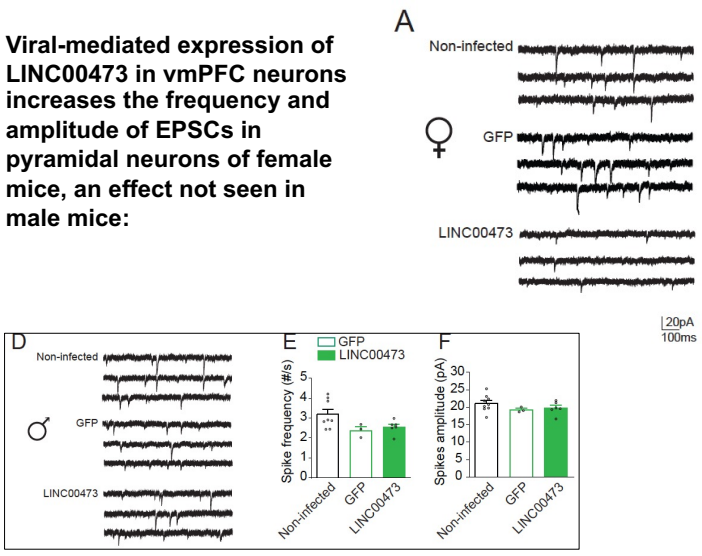
Sex-Specific Effects of LINC00473 Expression on Excitability of vmPFC Pyramidal Neurons of Female vs. Male Mice

Viral-mediated expression of LINC00473 in vmPFC neurons increases the frequency and amplitude of EPSCs in pyramidal neurons of female mice, an effect not seen in male mice:



Issler et al., *Neuron*, 2020

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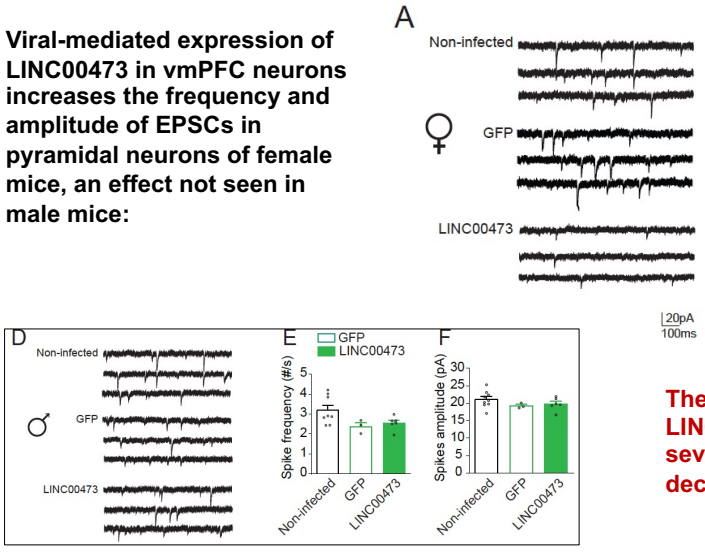


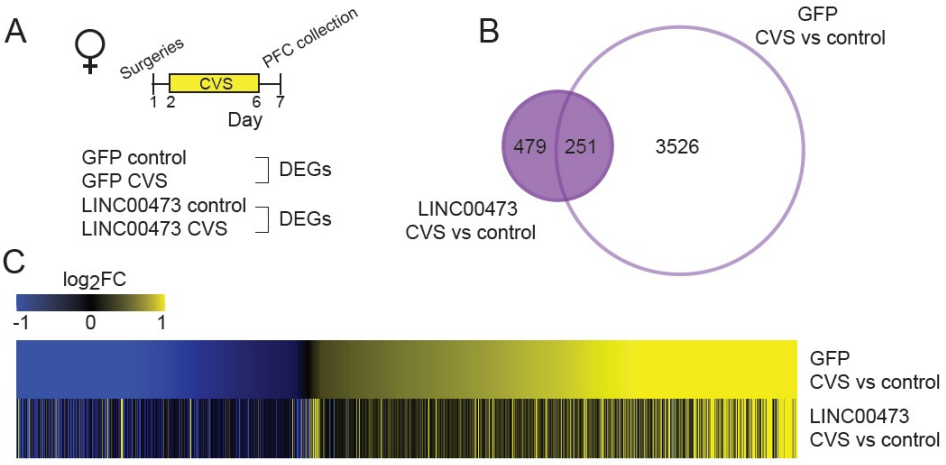
The reduced vmPFC excitability seen upon LINC00473 expression is consistent with several other manipulations which show that decreasing vmPFC excitability is proresilient.

Issler et al., *Neuron*, 2020

22

Effect of LINC00473 Expression in vmPFC Neurons on Gene Expression in Female Mice

Viral-mediated expression of LINC00473 in vmPFC neurons dramatically suppresses the ability of chronic variable stress (CVS) to alter gene expression:



Issler et al., *Neuron*, 2020

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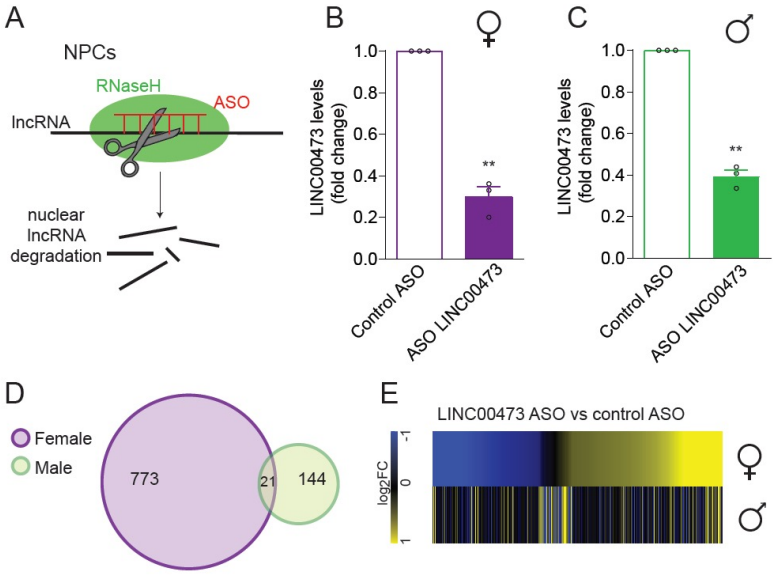
Studies of LINC00473 in Human Neuroprogenitor Cells

Knockdown of LINC00473 in neuroprogenitor cells (NPCs) from control human subjects regulates the expression levels of 5-fold more genes in female NPCs than in male NPCs.



Issler et al., *Neuron*, 2020

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Ongoing Studies of LINC00473 in Depression

1. We are currently using CHIRP (Chromatin Isolation by RNA Purification) to identify the direct binding partners for LINC00473.

- **Early evidence is identifying both genomic and protein targets.**

2. We are studying the mechanisms underlying the selective deficit in LINC00473 in female depression and the more dramatic effects of LINC00473 in female cells:

- **Chromosomal mechanisms?**
- **Hormonal mechanisms?**

3. Studies of LINC00473 exemplify striking sex differences in transcriptional abnormalities associated with depression, which raises the more general question of why the behavioral presentations and treatments of depression are relatively similar if the molecular pathology is so different?

- **Many commonly affected genes and gene pathways.**
- **Despite the existence of many sex-specific factors, some produce equivalent functional effects in both sexes.**

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How Does Early Life Stress Increase Lifelong Stress Susceptibility?

1. Early life stress is one of the strongest known risk factors for depression and related syndromes in humans.

- **4-fold increase in lifetime risk.**
- **Increased susceptibility to subsequent stressful events in adulthood.**
- **Associated with more severe illness and treatment resistance in adulthood.**
- **Underlying molecular mechanisms poorly understood.**

2. There is a large literature on early life stress in rodents, focused primarily on forms of maternal separation.

- **However, it has been difficult to develop models of "latent" risk (no behavioral changes until subsequent stress) as seen in most humans.**

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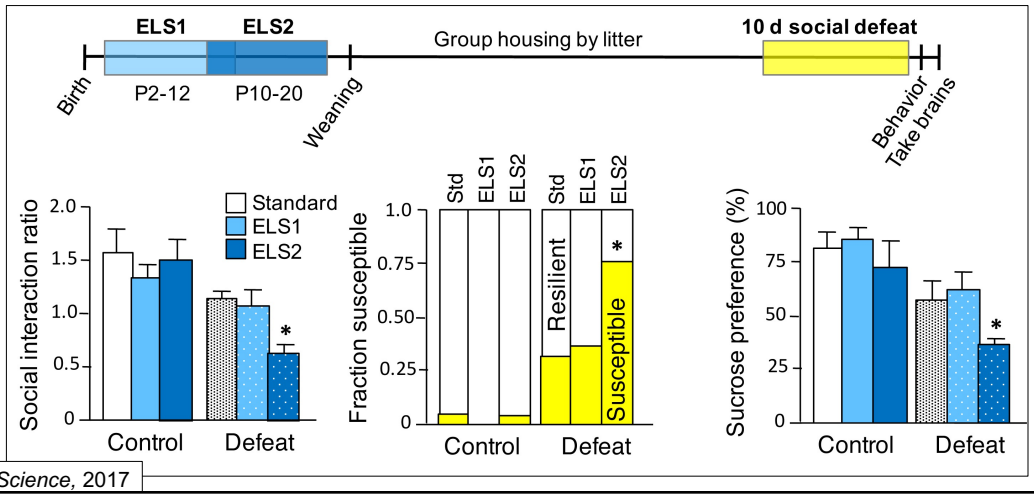
Early Life Stress Enhances Stress Susceptibility for a Life Time

Exposure of C57BL6 mice to early life stress (ELS) at a particular window in development increases susceptibility to adult stress:



Peña et al., *Science*, 2017

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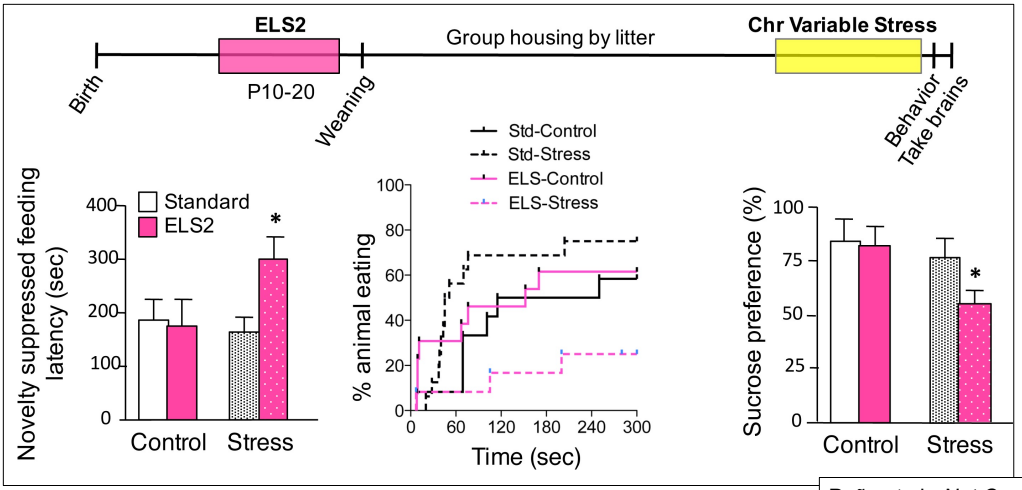
Early Life Stress Enhances Stress Susceptibility for a Life Time

Early life stress (ELS) induces a similar increase in stress susceptibility in female mice:



Peña et al., *Nat Commun*, 2019

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Early Life Stress "Primes" Gene Expression Toward a Stressed-Like State

RNA-seq data for adult mice after ELS2 or standard rearing:

Peña et al., *Science*, 2017; *Nat Commun*, 2019

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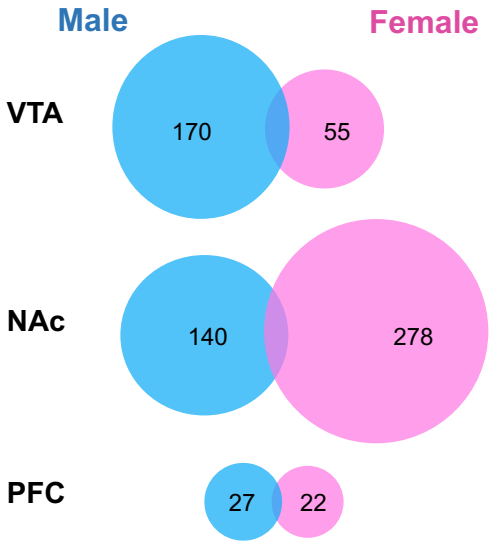
Dramatic Sex Differences in Gene Expression Changes Induced by Early Life Stress

Small (<5%) overlap in gene expression changes induced by ELS2 in males vs females despite similarity in behavioral effects:

This is consistent with a recent RNAseq study of six brain regions of depressed vs. healthy humans (Labonté et al., *Nat Med*, 2017).



Peña et al., *Nat Commun*, 2019

30

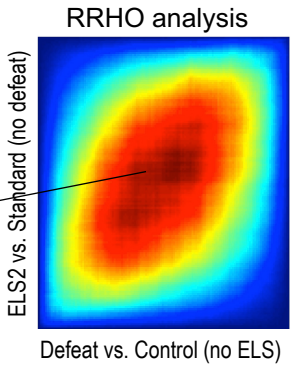
Early Life Stress "Primes" Gene Expression Toward a Stressed-Like State

OTX2 is a key mediator of this priming in the male VTA, but not in females and not in any other brain region studied:

Male

VTA

ELS2 vs Standard
Stress vs. Control



- OTX2, a transcription factor important for VTA dopamine neuron development <u>but never before implicated in stress responses or depression</u>, partly mediates these lifelong changes in gene expression and stress susceptibility.
- Transient suppression of OTX2 induces "chromatin scars" at its target genes which alter their expression for a lifetime.
- We are now studying the epigenetic basis of these chromatin scars.

Peña et al., *Science,* 2017

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Mechanisms By Which Early Life Stress Increases Lifelong Stress Susceptibility

1. Early life stress—at a particular timepoint—induces a pattern of gene expression that resembles the effects of adult stress.
- **This may prime animals for increased stress susceptibility.**
- **Dramatic sex differences in underlying mechanisms.**

2. In males, transient suppression of OTX2 expression induces a lasting reduction in target gene expression in VTA.
- **Even though OTX2 binding at those genes recovers.**

3. These findings suggest the involvement of "chromatin scars" that mediate the lasting suppression of OTX2 target genes.
- **Now using ChIP-seq to identify those chromatin changes.**

4. Model system for how early life experience changes the brain for a lifetime through epigenetic mechanisms.

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Summary and Future Directions

1. Studies of molecular changes in limbic brain regions reveal novel mechanisms of stress susceptibility and resilience in mouse models and depressed humans.

- **Mechanisms of natural resilience.**
- **Dramatic sex differences.**
- **Early life stress programs lifelong changes in stress susceptibility.**
- **Antidepressant action.**

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

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

- **Developing new treatments to recapitulate mechanisms of natural resilience.**
- **Ongoing clinical trials now testing this hypothesis.**

Icahn School of Medicine at Mount Sinai

NIH National Institute of Mental Health

HOPE FOR DEPRESSION RESEARCH FOUNDATION

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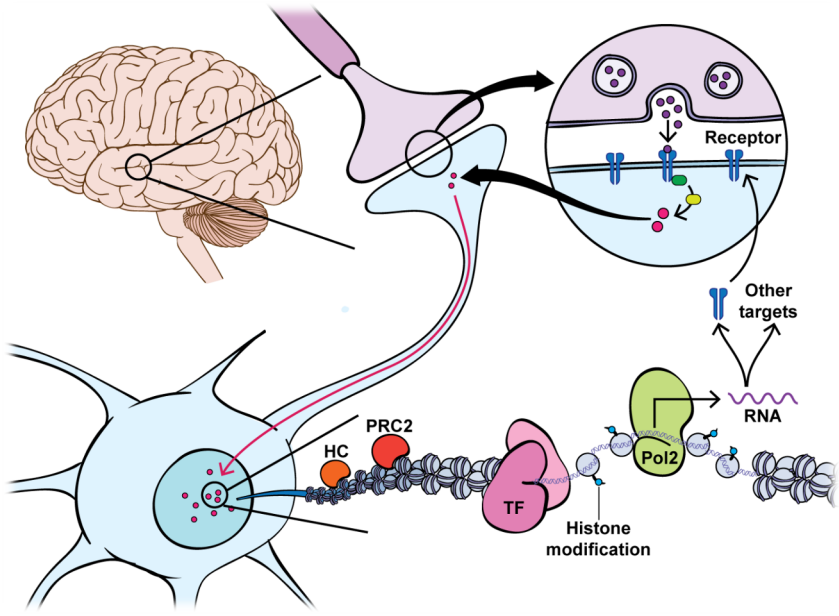
Template for Drug Discovery

These unbiased studies provide an unprecedented look at genes, proteins, and biochemical pathways that are crucial for susceptibility versus resilience to chronic stress, and will guide drug discovery efforts beyond conventional drug targets (neurotransmitter receptors, transporters).

It is even conceivable that transcriptional factors and epigenetic modulators underlying depression could themselves be effective targets.



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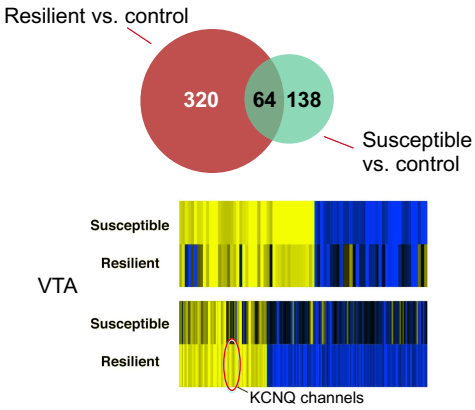
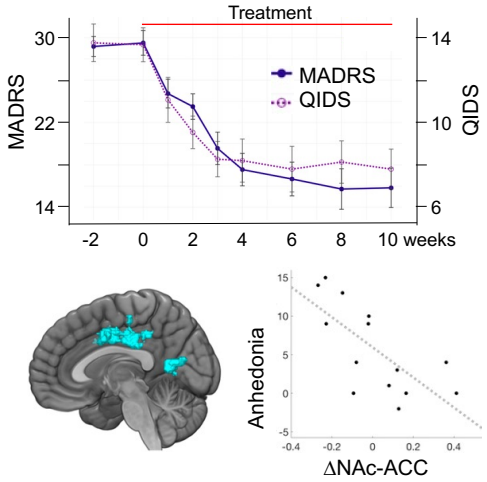
Example: Induction of KCNQ Channels in Resilient VTA

Induction of KCNQ channels in the resilient VTA, first observed with these gene discovery approaches, suggested that KCNQ channel potentiators may be antidepressant.



KCNQ potentiator, ezogabine, is antidepressant in a small N, open-label clinical study.



Krishnan, Han, et al., *Cell*, 2007

Tan … Murrough, *Mol Psychiatry*, 2018

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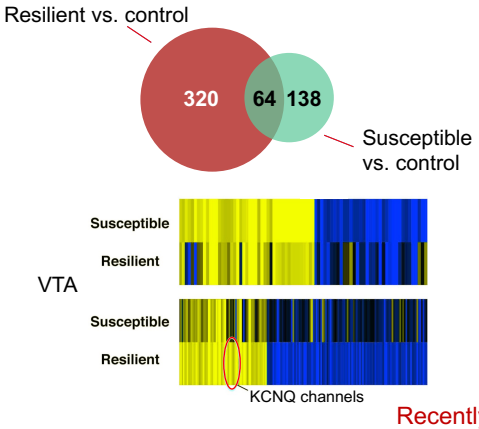
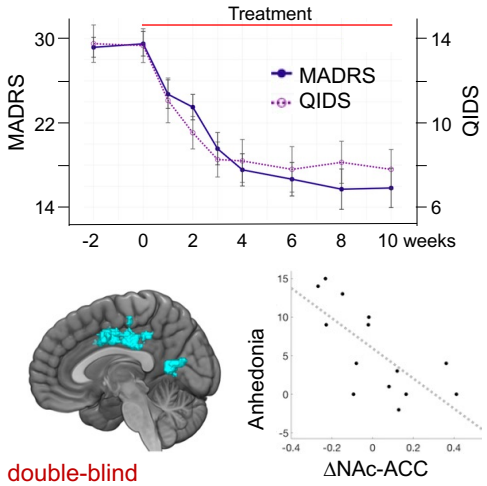


KCNQ potentiator, ezogabine, is antidepressant in a small N, open-label clinical study.



Recently replicated in double-blind study (Costi et al., *Am J Psychiatry).*

Krishnan, Han, et al., *Cell*, 2007

Tan … Murrough, *Mol Psychiatry*, 2018

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