Sex-specific Integrative Genomics of Human Brain in Posttraumatic Stress Disorder

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Posttraumatic Stress Disorder

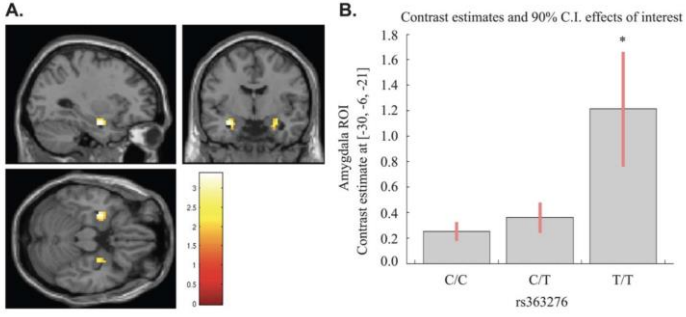
- **Characterized by**
  - uncontrollable states of fear
  - persistent unavoidable re-experiencing of traumatic memories
  - inability to extinguish fear
  - hyperarousal

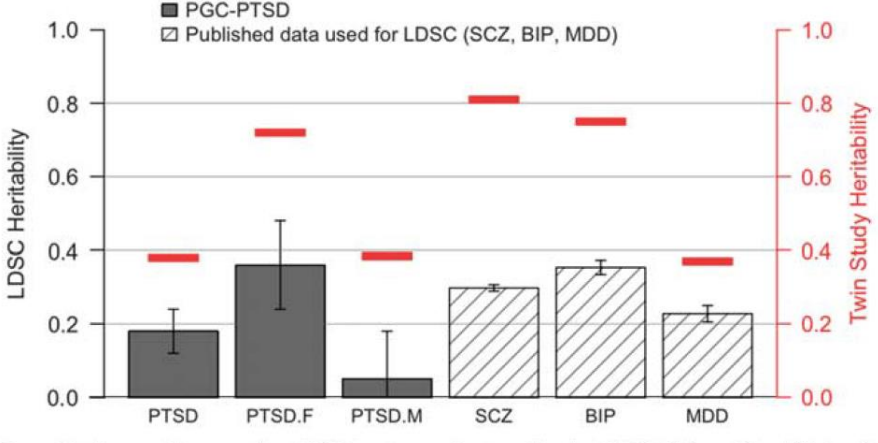
- As many as 30% of returning war fighters are found to have PTSD 1 year after deployment.

- In the general population the rates are close to 8%.

- While triggered by some traumatic event (Environmental) there is likely a heritable (Genetic) component as well.

- **About 10% of women will develop PTSD in their lifetime and woman are twice as likely as men to develop PTSD.**





PGC=psychiatric genomics consortium, PTSD=posttraumatic stress disorder, LDSC=Linkage Disequilibrium Score Regression, SCZ=schizophrenia, BIP=bipolar disorder, MDD=major depressive disorder

Neurobiology of PTSD

<u>Prefrontal Cortex</u>: Modulation and extinguishing fear memory

<u>In PTSD:</u>

- Decreased WM density
- Decreased responsiveness

to emotional stimulus

<u>Amygdala</u>: Fear learning

<u>In PTSD:</u>

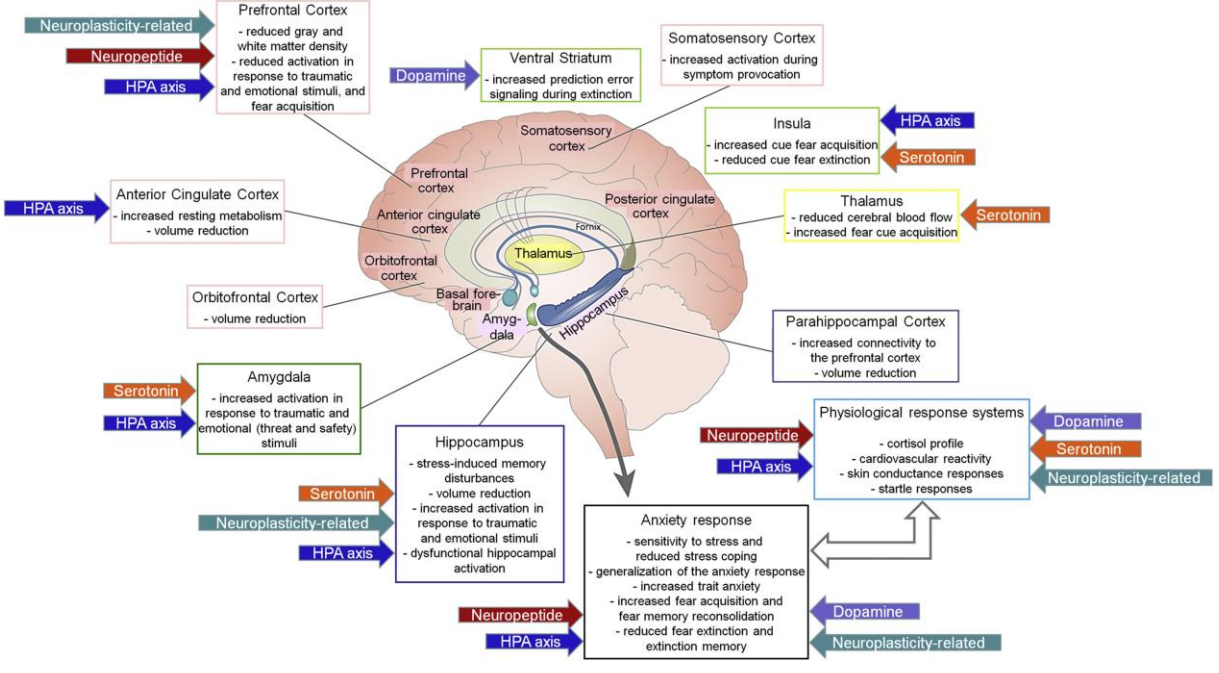
- Increased responsiveness to

traumatic events

<u>Hippocampus</u>: Fear memory storage

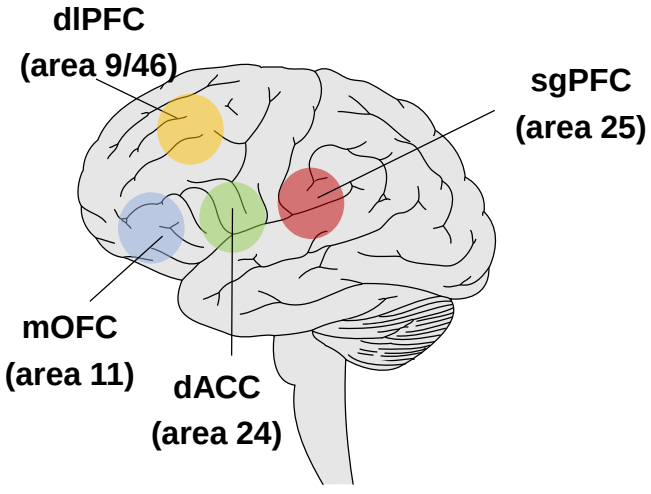
<u>In PTSD:</u>

- Decreased GM
- Decreased Volume



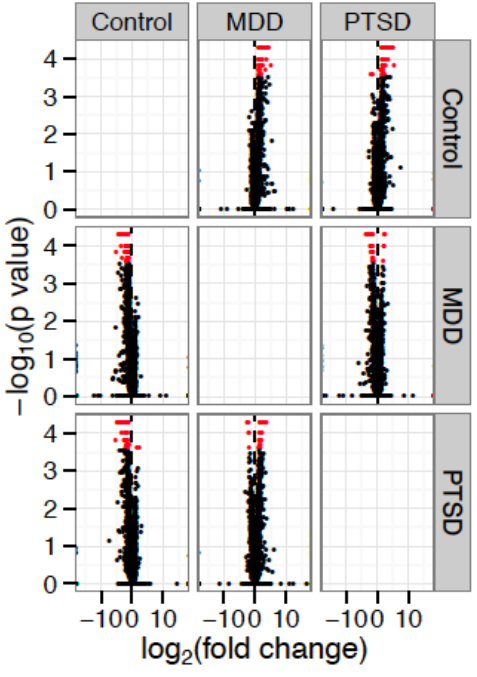
Adapted from Nees, et al. 2017

Identifying the molecular mechanisms of PTSD

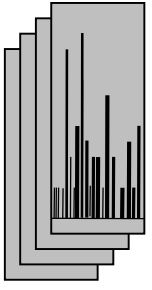


CON, MDD, PTSD
n=50

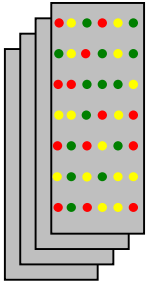
RNA-seq



Mass Spectrometry
Proteomics



Bisulfite Chip
CpG Methylation



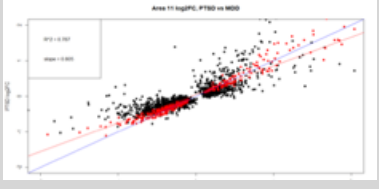
Girgenti et. al. 2018

Gene Expression Data
Illumina HiSeq, 75bp, paired end

Normalization
- Outlier Removal
- Covariate Regression
- Dimensional Scaling (PCA)

Differential Gene Expression (DGE)
- FPKM of each transcript

Global Transcriptome Comparison



Network Analysis
- Batch Correction for study
- Robust weighted gene co-expression network analysis (WGCNA)
- Module-disease association
- Module-disease-region association
- Module characterization (pathway analysis, cell-type specificity, key driver)
- Convergence of regional transcript changes

GWAS/Psychiatric Disorder Overlap
- SNP based co-heritability
- Shared molecular convergence

Differential Expression Modeling

We fit this statistical model to the data using DESeq2 within each brain region:

raw count for gene i, sample j

The mean is taken as "normalized counts" scaled by a normalization factor

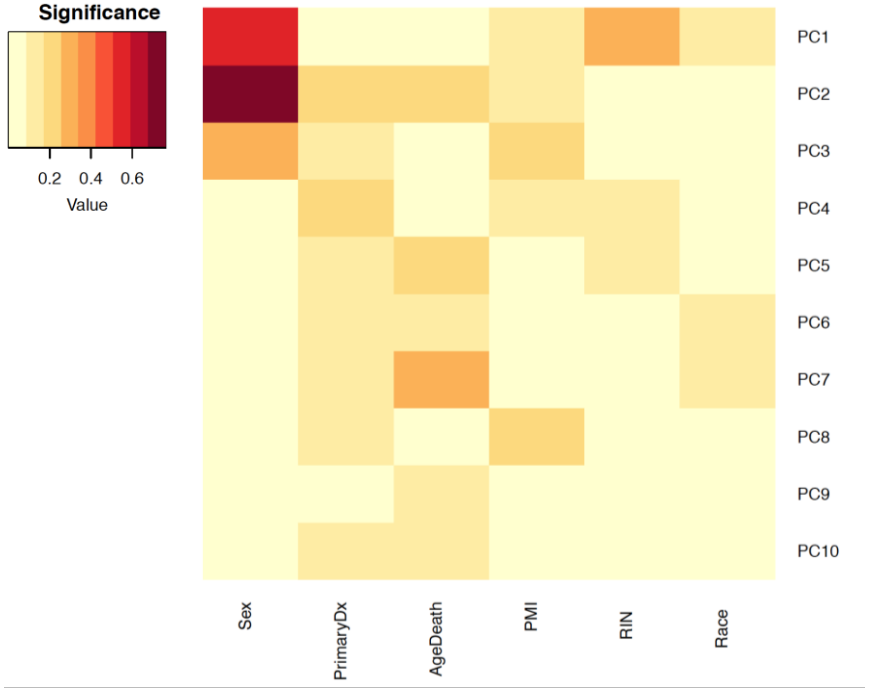
one dispersion per gene

$$K_{ij} \sim \mathrm{NB}(s_{ij} q_{ij}, \alpha_i)$$

normalized counts for gene i, sample j

log2 fold change between conditions

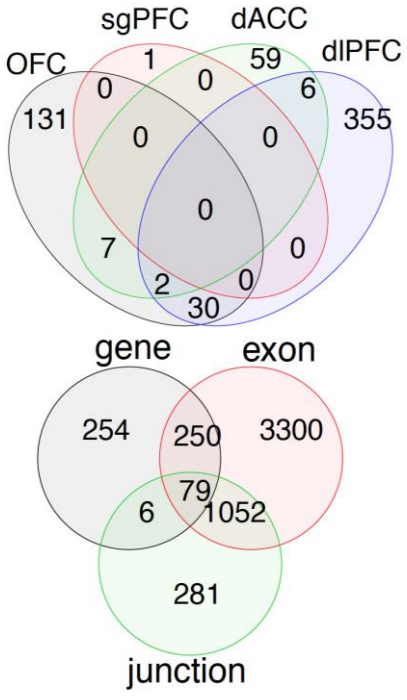
$$\log 2 q_{ij} = \Sigma_r x_{jr} \beta_{ir}$$

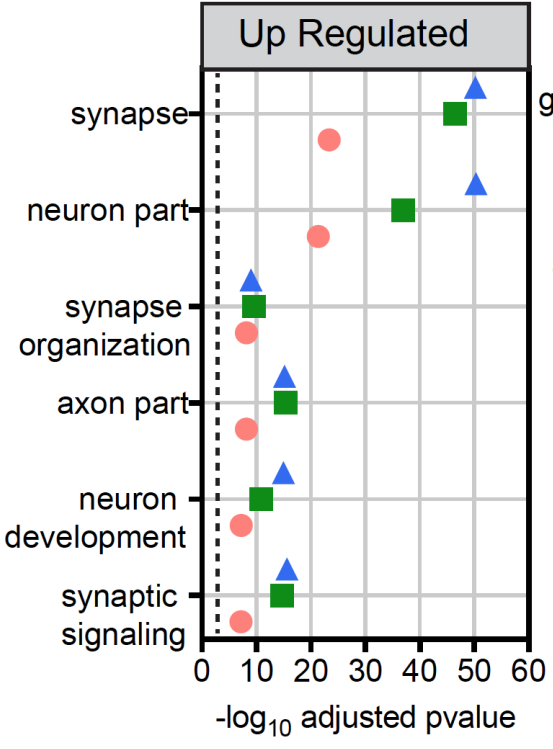


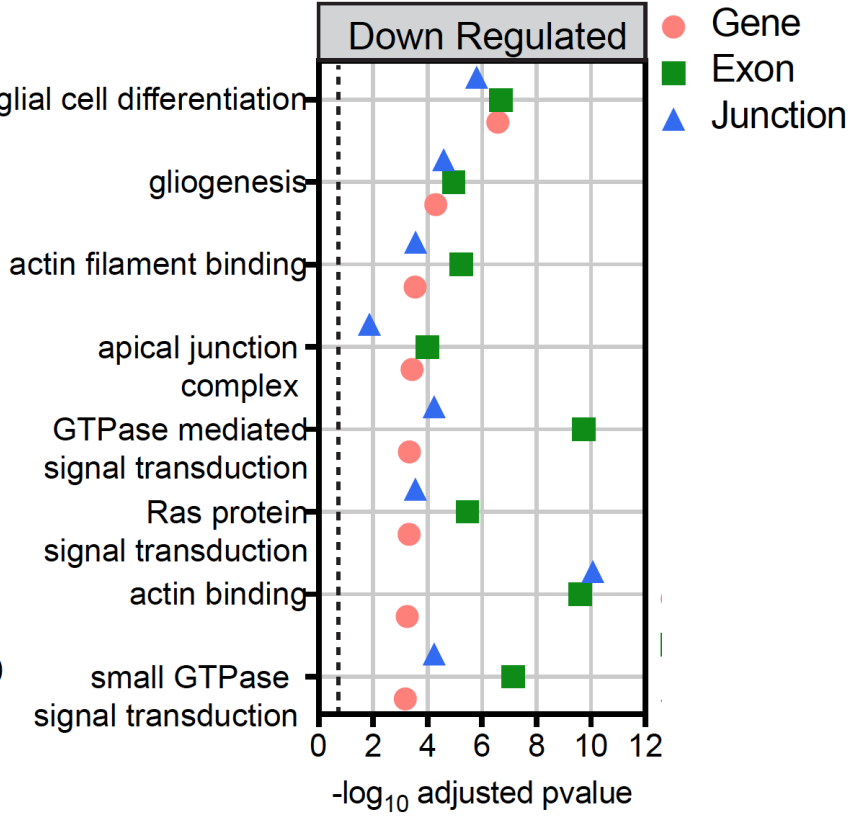
Covariates~ Dx, age, sex, RIN, PMI, and race

SVA for batch effects

Gene Set Enrichment of PTSD Cortical DEGs







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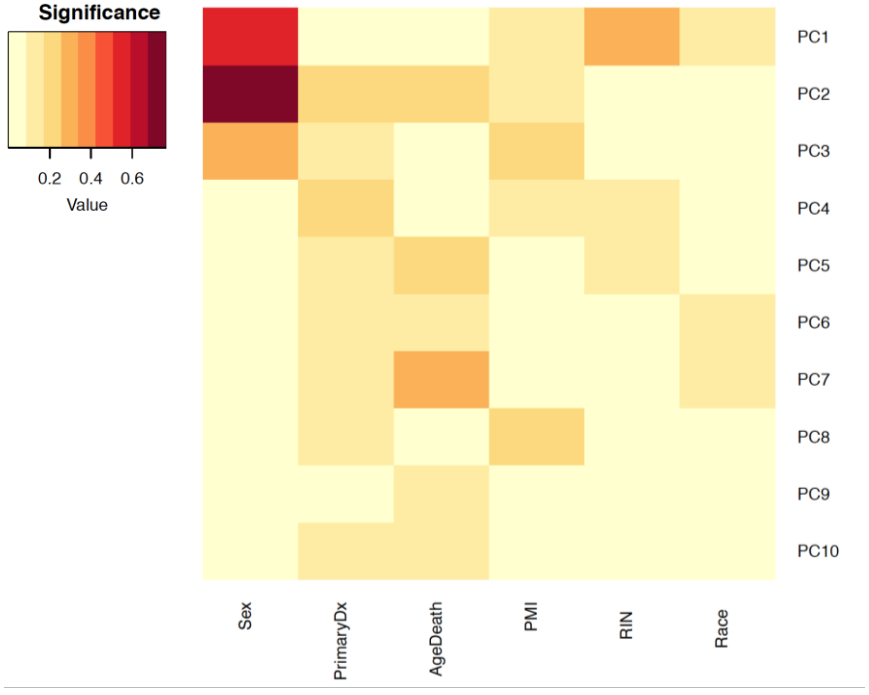
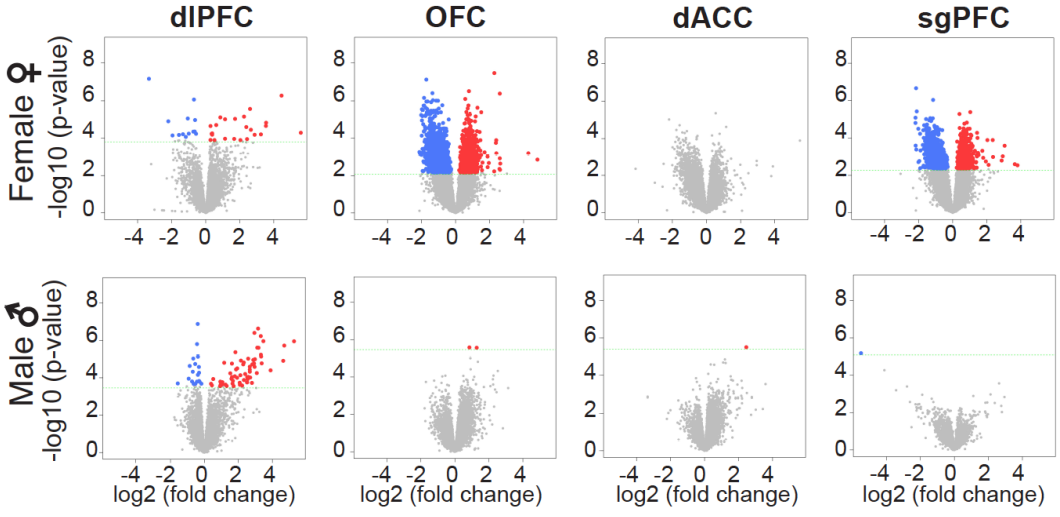
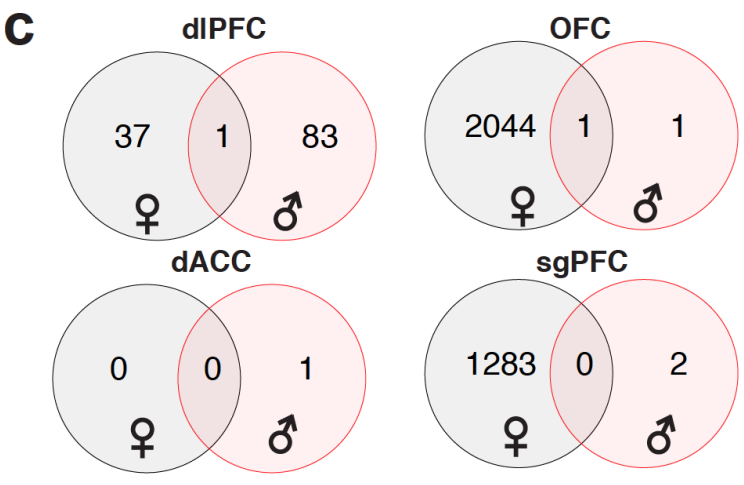


Covariates~ Dx, age, sex, RIN, PMI, and race

SVA for batch effects

Sex-specific PTSD DEGs





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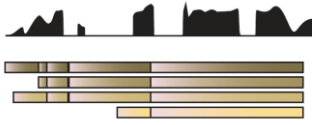
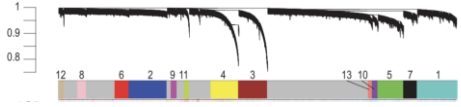
Gene Network Specificity

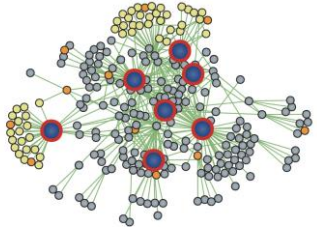


Gene Expression

Network Analysis

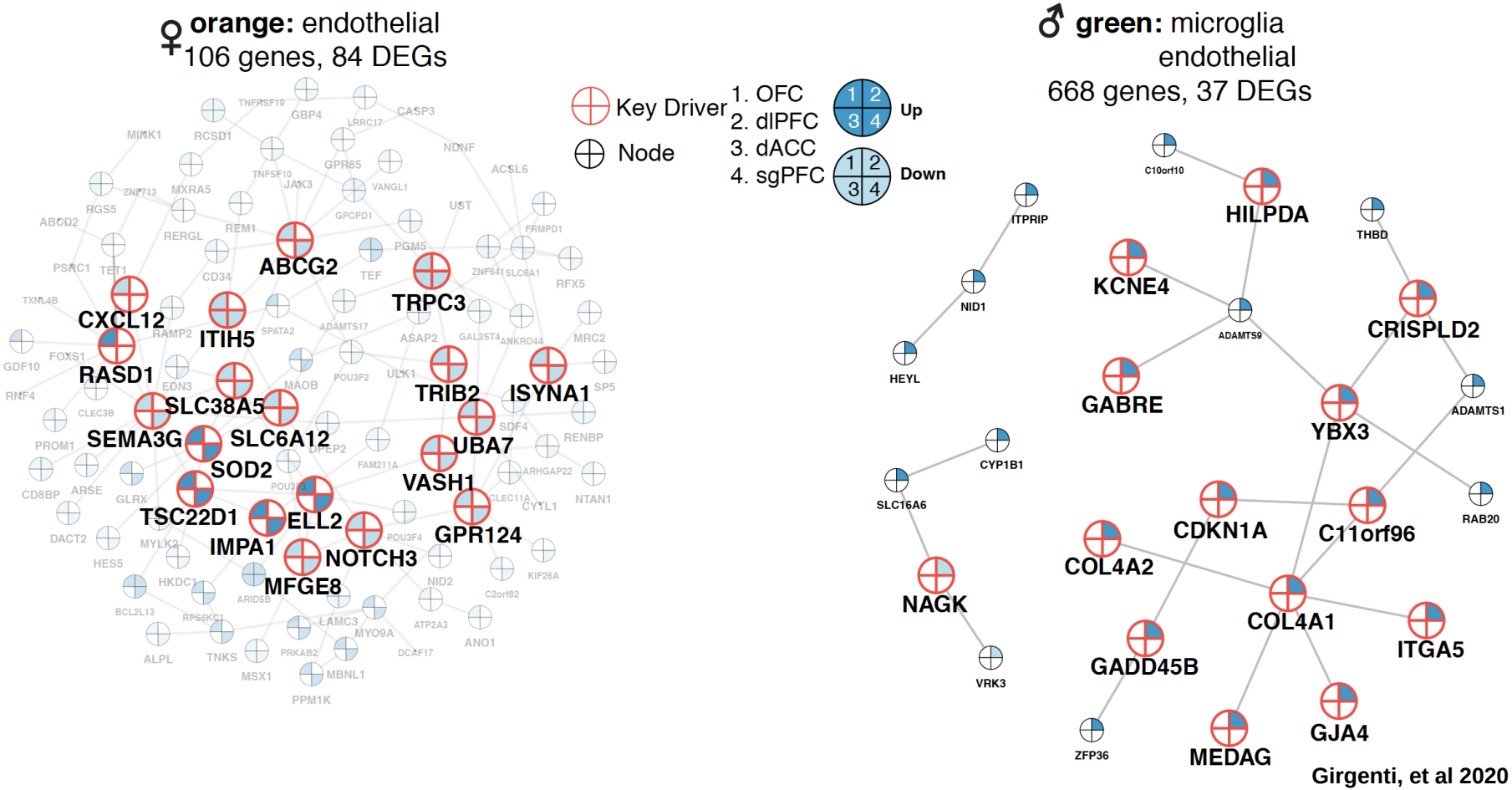


Integrate/Contrast



Genes do not act independently- form networks with common functions

Top Sex-specific Co-expression Modules



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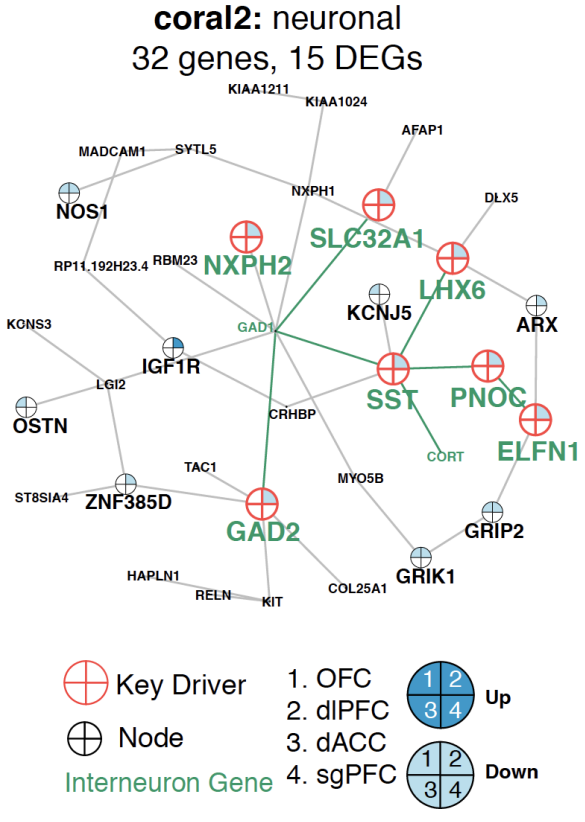
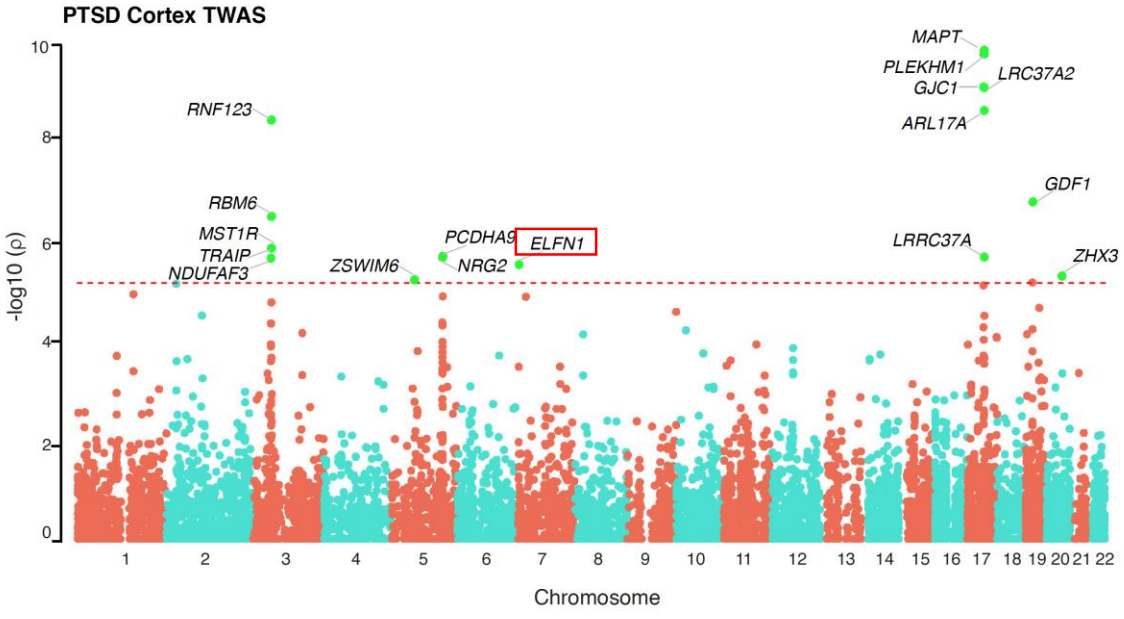
coral2 is Enriched with Interneuron DEGs





Much of this GABA network is recapitulated in a combine-sex module

Largest PTSD GWAS (MVP)
GTEx eQTL weights (54 non-diseased tissue types)
- Cortex panel
- CNS panel
- non-CNS panel

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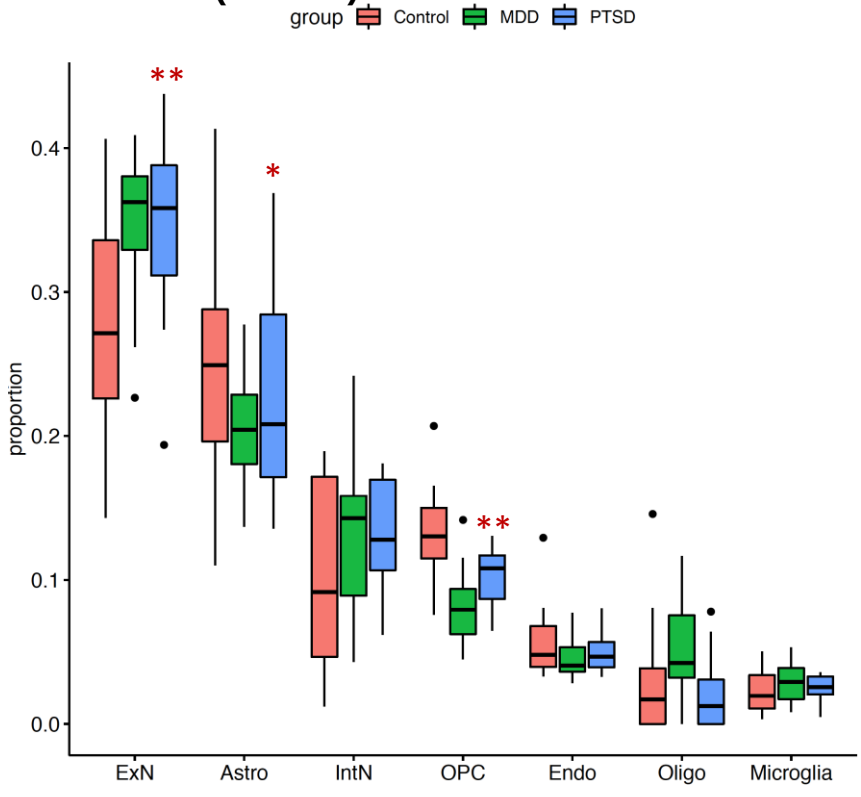
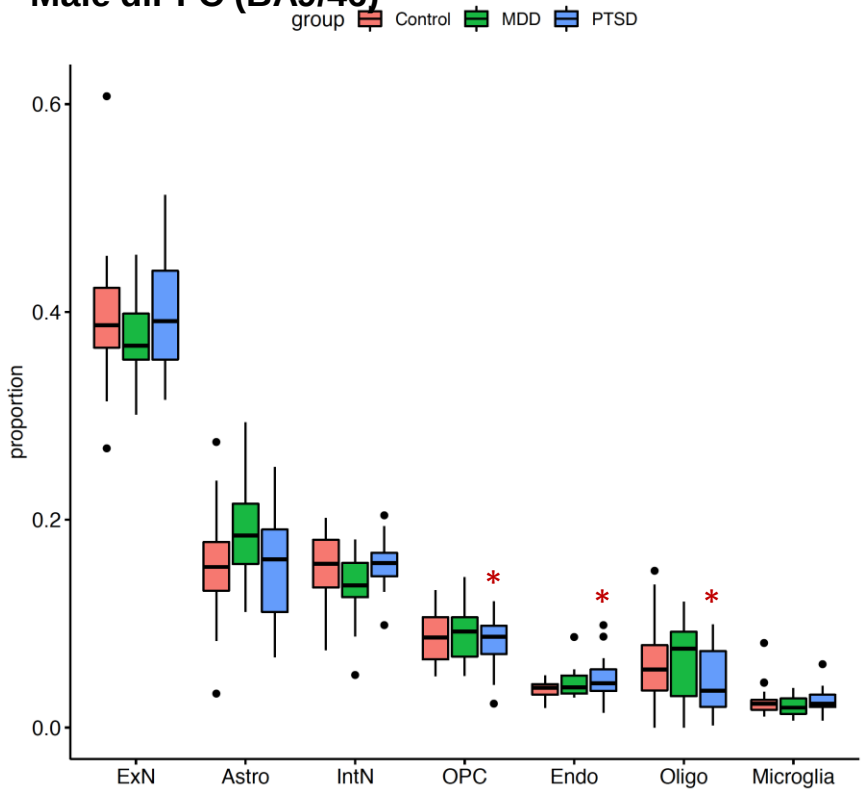
Sex-specific cell type proportion changes

Female dlPFC (BA9/46)



Male dlPFC (BA9/46)



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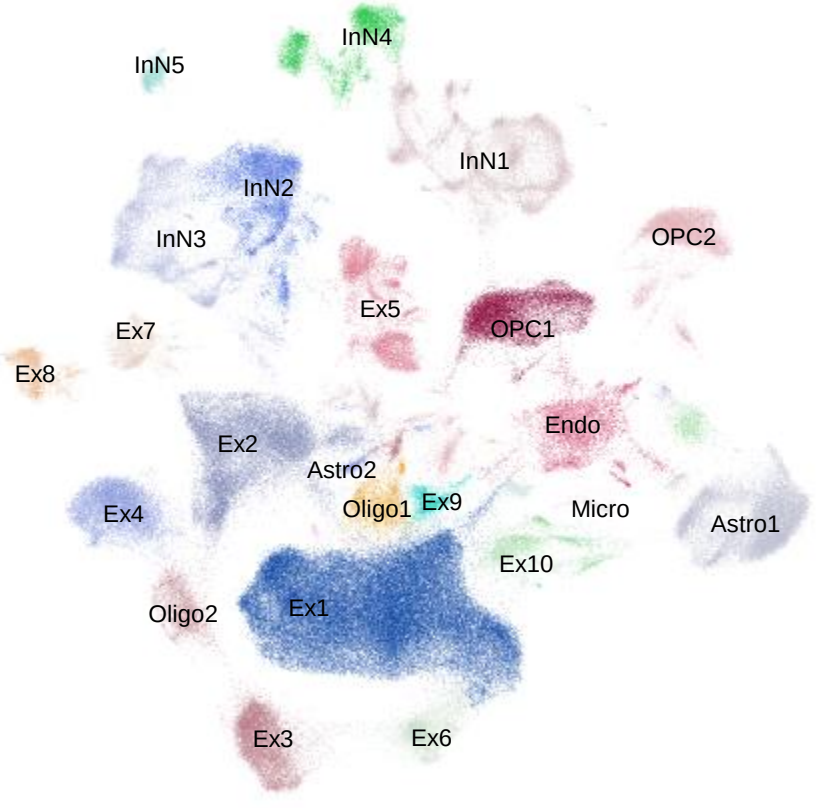
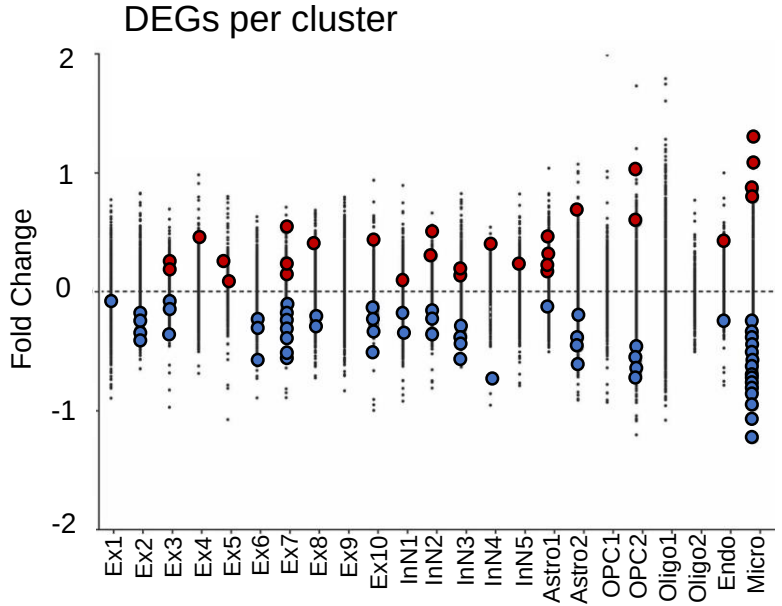
Single Cell type-specific Transcriptomics

Female dlPFC (BA 9/46)



Identified 23 clustered cell types



127 DEGs

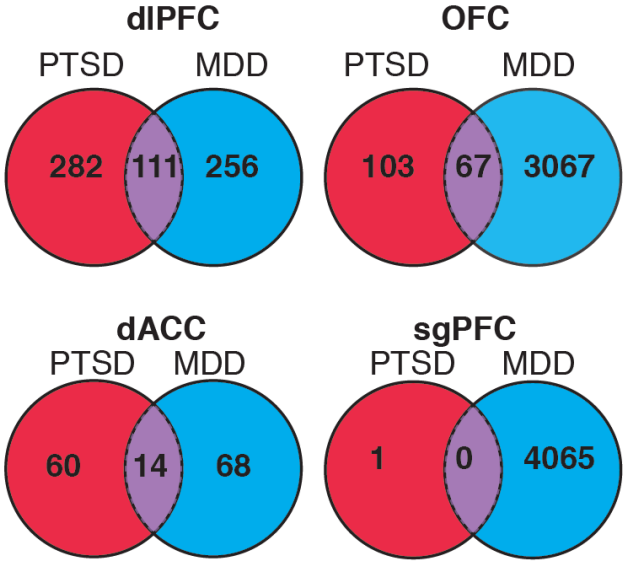
49 Up
78 Down

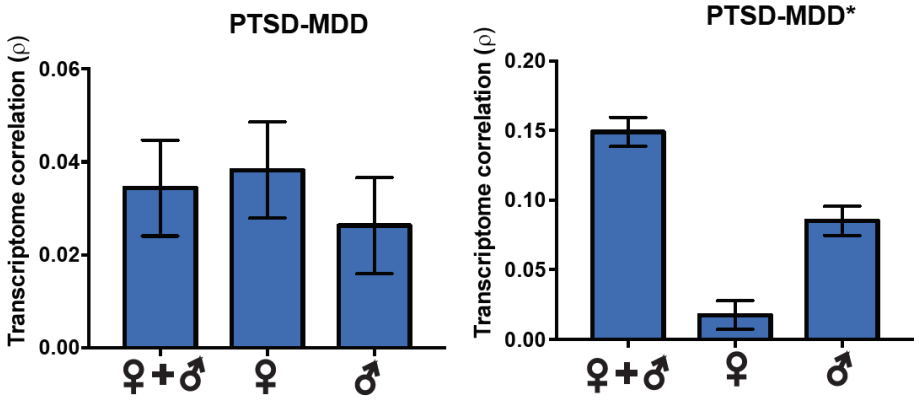
17 Ex DEGs
24 InN DEGs
57 Micro DEGs

Non-PTSD Psychiatric Control Group- MDD

	Control	MDD	PTSD
	n=46	n=45	n=52
Sex (% Male)	56.5	60.0	50.0
Race (% Caucasian)	65.2	77.8	81.0
Age at Death	48.5 ±12.4	45.4 ±13.1	42.8 ±11.5
PMI	21.8 ± 6.6	18.7 ± 6.1	20.5 ± 6.3
RIN: OFC	7.7 ± 0.97	7.8 ± 0.95	7.9 ± 1
RIN: sgPFC	8.1 ± 0.85	8.1 ± 0.77	7.9 ± 0.99
RIN: dACC	7.4 ± 0.98	7.5 ± 0.97	7.3 ± 1.06
RIN: dlPFC	7.4 ± 1.19	7.6 ± 1.1	7.7 ± 0.92
Tobacco (% ATOD)	21.7	68.0	65.0
Alcohol (% ATOD)	0.0	28.9	35.0
Opioids (% ATOD)	8.7	22.2	15.4
Antidepressant (% ATOD)	0.0	56.5	60.0
Manner of death (% Suicide/ %Natural)	0.0	22.2/ 77.8	12.0/ 88.0
Drug Related Death (%)	0.0	42.2	52.0

Diverging Molecular Mechanisms between PTSD and MDD





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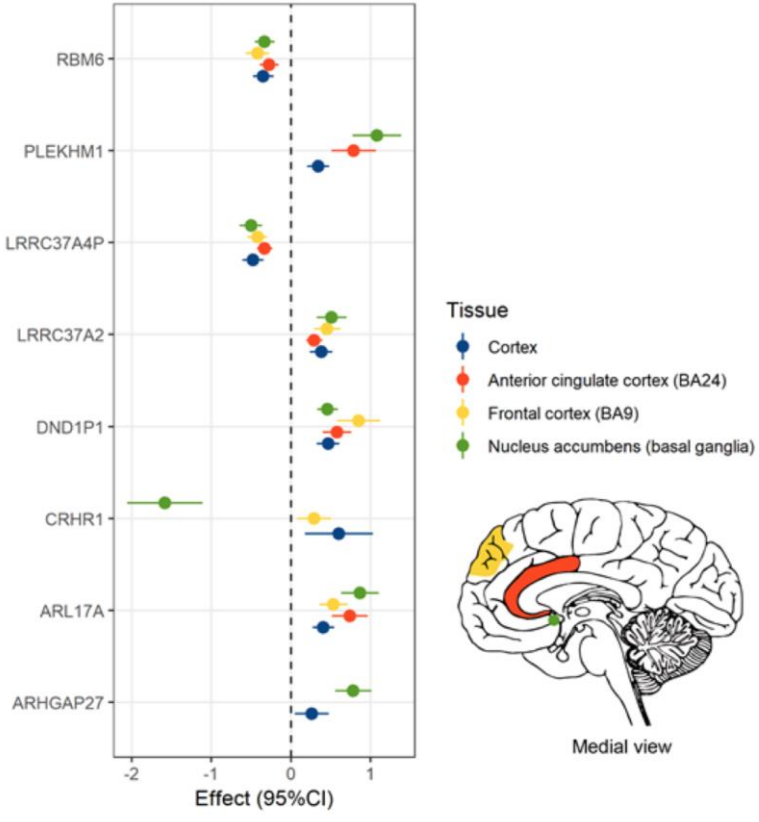
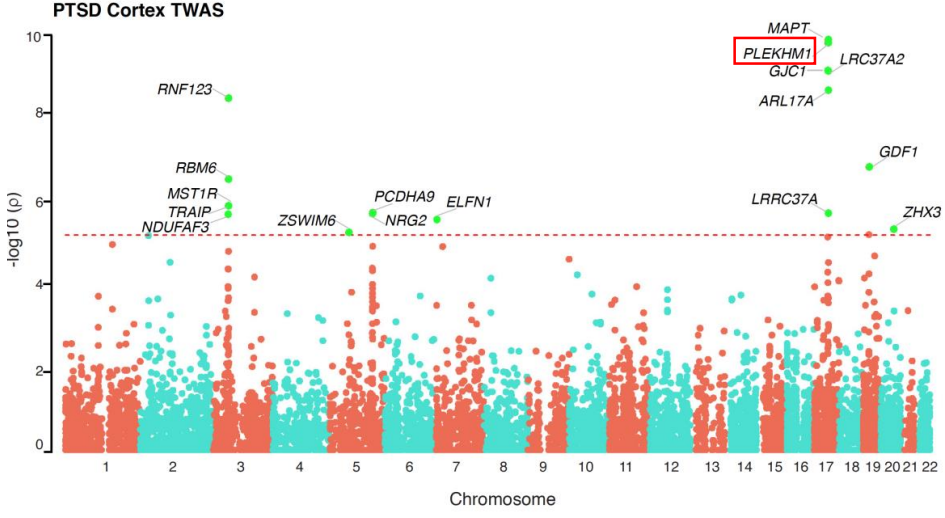
Drug Repositioning Analyses





SIGNIFICANCE

1.) There is highly connected, down-regulated set of interneuron transcripts in PTSD PFC.

2.) Interneuron gene *ELFN1* confers significant genetic liability and a likely functional role in PTSD pathophysiology specifically in females.

3.) Despite high co-morbidity between PTSD and MDD there is little molecular pathological changes that overlap between them.

4.) PTSD single cell work pipeline is developed and there is an emerging biology centered on sex- and cell- type-specific changes.

5.) Reverse transcriptomics and drug repositioning based on postmortem transcriptomic data is a promising direction for development of novel therapeutics

ACKNOWLEDGEMENTS

John Krystal, M.D.
Ronald Duman, Ph.D.

Mario Skarica, M.D.
Jiawei Wang
Dingjue Ji, Ph.D.
Jing Zhang, Ph.D.
Sydney Muchnik
Hongyu Li
Rose Terwilliger

Yale
Hongyu Zhao, Ph.D.
Nenad Sestan, M.D., Ph.D.

VACT- West Haven
Joel Gelernter, M.D.
Ismene Petrakis, M.D.
Brenda Martini

Dartmouth
Matthew Friedman, M.D.
Paul Holtzheimer, M.D.

UCSD
Murray Stein, M.D., MPH

Duke University
Doug Williamson, Ph.D.
Dianne Cruz

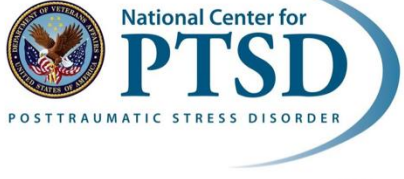
UPMC
David Lewis, M.D.

Funding Sources:
VA Career Development Award (VISN1)

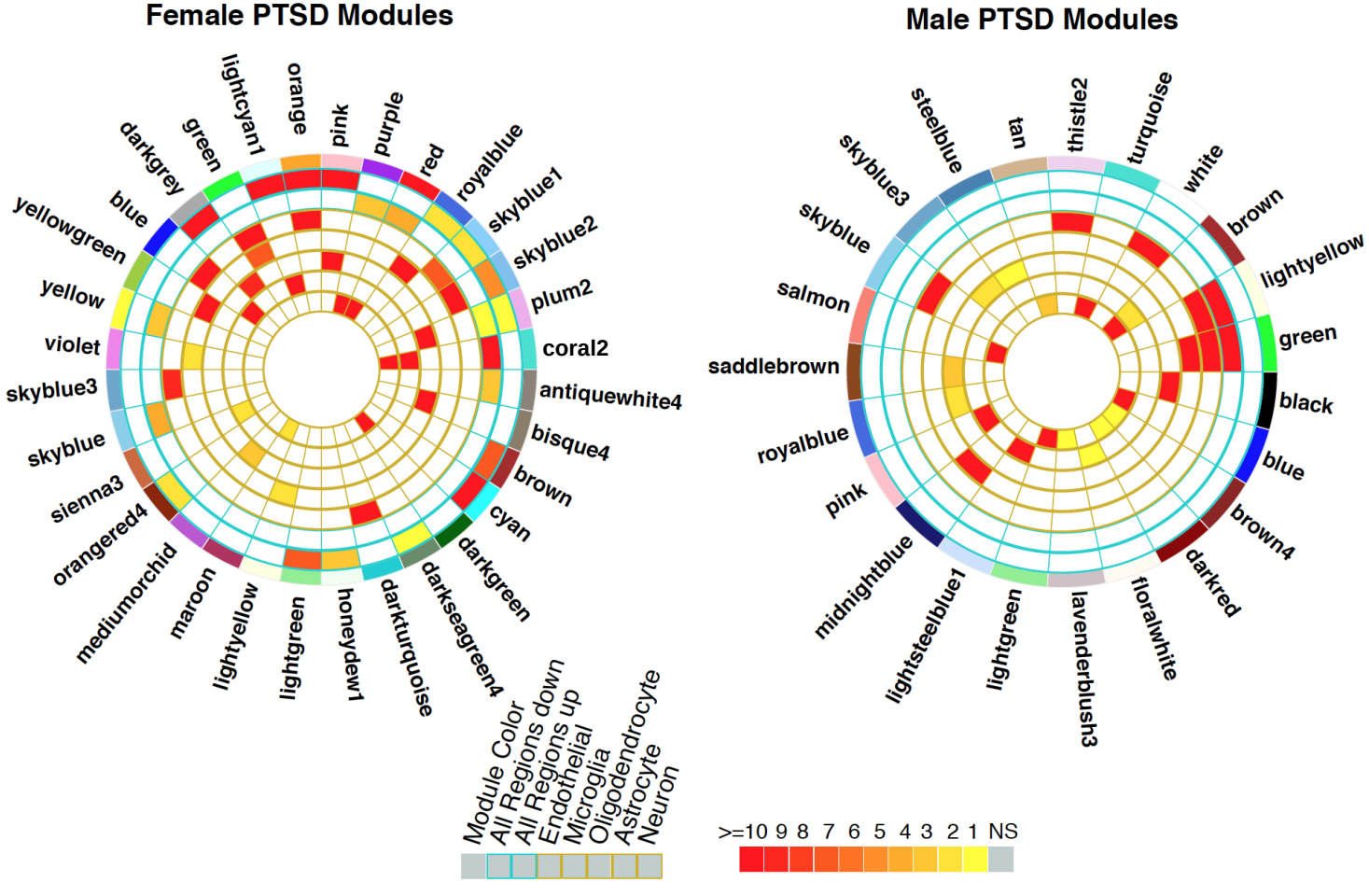
NARSAD Young Investigator Award

Yale/NIDA Neuroproteomics Pilot Grant

State of Connecticut Department of Mental Health & Addiction Services



Sex-specific Co-expression Modules



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