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Transcriptional profiling of the nucleus of the solitary tract after alcohol dependence and stress

Emily Grantham, Gayatri Tiwari, Olga Ponomereva, Dayne Mayfield

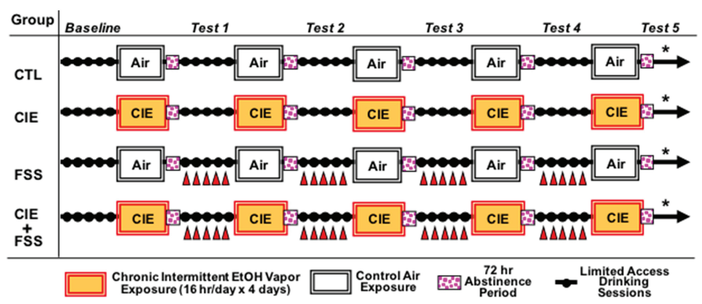
Waggoner Center for Alcohol and Addiction Research, The Unversity of Texas at Austin, Austin, TX 78712

Abstract

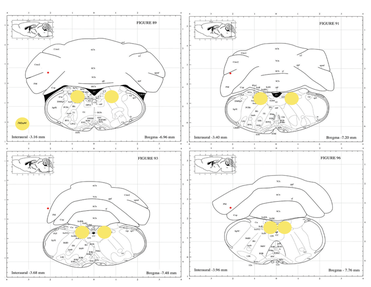
Chronic stress exposure contributes to the development of drug and alcohol addiction. Animal models show that chronic stress exacerbates escalations in alcohol consumption in alcohol-dependent animals. The nucleus of the solitary tract (NTS) is a critical brainstem region for integrating and relaying peripheral signals to regulate stress response. To examine the molecular adaptions within this brain region that may contribute to stress-induced alcohol drinking, we exposed animals to chronic repeated bouts of ethanol vapor, forced swim stress, or both and then transcriptionally profiled the NTS at three different timepoint after the last test (0-h, 72-h, and 186-h). Through these comparisons, we identified a role for interferon signaling, specifically Irf7, in stress-induced escalations of alcohol consumption. Differentially expressed genes at 0-h and 72-h timepoints were enriched for type I interferon signaling in animals exposed to EtOH + Stress. Irf7 was uniquely upregulated at the 72-h timepoint in the EtOH + Stress group, but not the others. Ingenuity pathway analysis identified Irf7 as a predicted upstream regulator of gene expression patterns in the EtOH + Stress group across all three timepoints. Overall, these results summarize the transcriptomic changes across time in the NTS that are critical to the development of stress-induced alcohol consumption and alcohol dependence.

Methods

Experimental desgin



NTS Coordinates



Animal Model: Adult male C57BL/6J (B6J) mice, 10 weeks of age, were purchased from Jackson Laboratories (Bar Harbor, ME) and individually housed under a 12-h light/dark cycle (lights on at 8:00 a.m.).

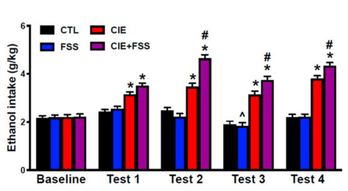
Procedure: Mice were evaluated in the CIE-FSS Drinking model. Briefly, mice were first trained to drink 15% (v/v) ethanol with access for 1 h/day starting 3 h after lights off. Once stable baseline intake was established (~3 weeks), mice were separated into four groups (equated for baseline level of alcohol intake): non-dependent alcohol consumption (CTL), FSS, CIE, and CIE + FSS. Mice in the CIE and CIE+FSS groups received CIE vapor exposure in inhalation chambers (16 h/day for 4 days), as described previously. After a 72-h abstinence period, drinking test sessions resumed for 5 consecutive days under the same limited access conditions as before. This pattern of weekly chronic intermittent ethanol (or air) exposure alternated with weekly limited access drinking sessions was repeated for 4 cycles (Test Cycles 1–4) (Left). Mice in the FSS and CIE + FSS groups experienced 10 min of forced swim stress (FSS) 4 h prior to each of the test drinking sessions, as detailed below. The remaining mice (CTL and CIE groups) remained in their home cage undisturbed. Mice were sacrificed after a fifth and final CIE (or air control) cycle, either immediately (0-h), 72 h, or 7 days after the last CIE (or air) exposure. Mice were decapitated, and whole brains were immediately snap-frozen.

RNA Sequencing and Bioinformatics Analysis: Isolated total RNA from mouse NTS (above, right) was submitted to the Genomic Sequencing and Analysis Facility at The University of Texas at Austin. Sequencing libraries were constructed using a 3′ Tag-based approach (TagSeq), targeting the 3′ end of RNA fragments from ~16 ng/μL of each RNA sample. This tag-based approach is a cost-efficient alternative to whole-transcriptome RNA sequencing, comparable with respect to accuracy and quantification of detected transcripts. Samples were sequenced on the HiSeq 2500 (Illumina) platform with a read depth of approximately 7.6 million reads (single-end 100 bp reads; 4.6 million high quality reads per sample after trimming). A total of 89 samples were included with an average of 1.8 uniquely mapped reads after mapping. TagSeq detected a total of 56,262 transcripts. On average, 38,000 transcripts per sample were detected, representing ~22,000 protein coding genes. Read quality was assessed using MultiQC (version 1.7). Reads were mapped to the mouse reference genome (Gencode GRCm38.p6 release M23) using a STAR (version STAR_2.5.4b) aligner. Read distribution of bam files over reference genomic features was evaluated with RSeQC (version 3.0.0). Raw counts were quantified using HTSeq (version 0.11.2).

Differential Gene Expression Analysis and Ingenuity Pathway Analysis: The R (version 3.5.1) package DESeq2 (version 1.22.2) was used to identify changes in expression across different levels with a likelihood ratio test (LRT) using the DESeq function. A nominal p-value less than or equal to 0.05 was selected to ascertain shared and nonshared changes in gene expression among the groups and timepoints with respect to affected biological categories and pathways. Differentially expressed genes were analyzed for enrichment of canonical gene ontologies and molecular pathways using the bioinformatic tool Enrichr. DEG lists were entered in to Ingenuity Pathway Analysis (version 1-19-02) with log2 fold-change, p-value, and baseMean as inputs. IPA identified canonical pathways and predicted upstream regulators based on gene expression patterns submitted with p-value <0.05.

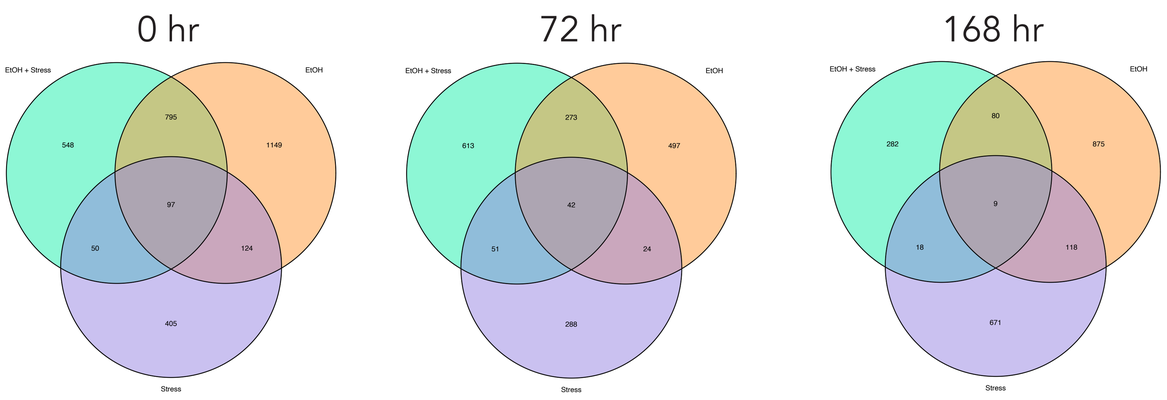
Results

Animals exposed to EtOH + Stress drink significantly more than EtOH or Stress alone



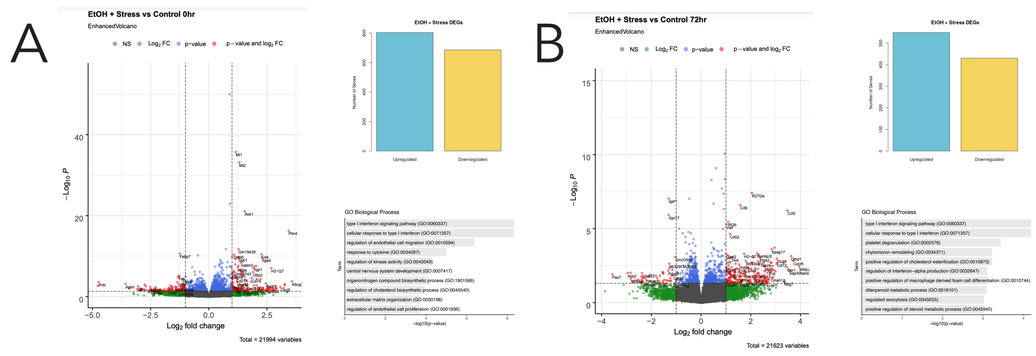
Over the course of the CIE + FSS procedure decribed above, animals voluntarily drank alcohol in a drinking-in-the-dark procedure. Animals exposed to CIE + FSS drank significantly more alcohol than CIE alone, suggesting stress-induced escalations in alcohol consumption.

Differential gene expression analysis idenified overlapping and unique genes in each group across time



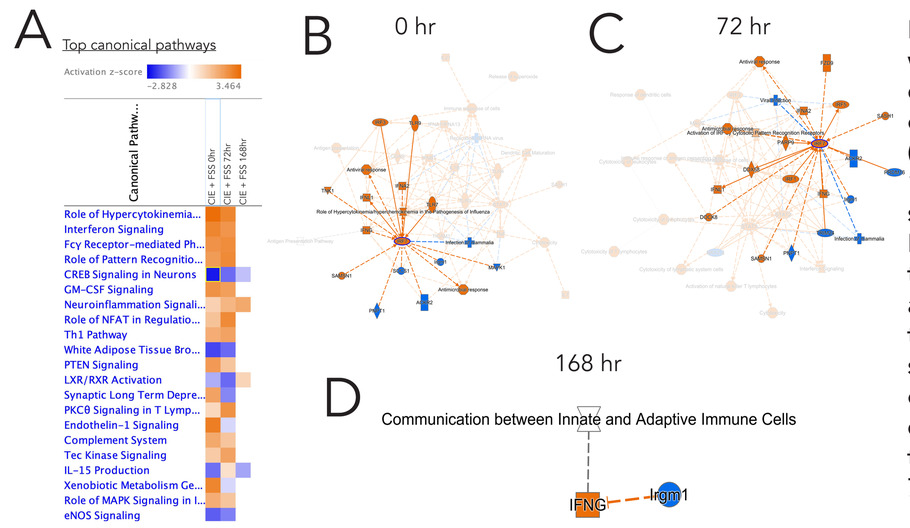
Venn diagrams show the number of DEGs identified in each treatment group at 0-h (left), 72-h (middle), and 168-h (right). The highest number of DEGs was observed at the 0-h timepoint. By 168-h there were very few DEGs that overlapped across all three treatment groups.

Differentially expressed genes in EtOH + Stress group enriched for interferon signaling and antigen presentation



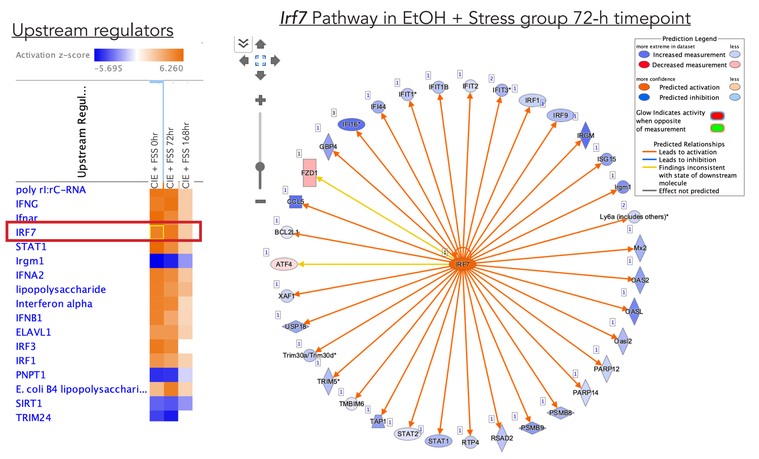
Volcano plots show differentially expressed genes in the EtOH + Stress group at 0-h (A), 72-h (B), and 168-h (C) timepoints. Bar plots show the number of DEGs up- vs down-regulated at each timepoint. Bar graphs show the top 10 Biological Processes enriched in each full set of DEGs at each timepoint. Type 1 interferon signaling was enriched at 0-h and 72-h. At 168-h, DEGs were enriched for antigen processing and presentation.

Ingenuity Pathway Analysis identifies role for interferon signaling in EtOH + Stress group across time



Ingenuity pathway analysis was used to identify top canonical pathways activated or inhibited in each dataset (CIE + FSS 0-h, 72-h and 168-h). Comparison analysis shows Hypercytokinemia and Interferon signaling as the top two Canonical pathways activated by CIE + FSS in the NTS (A). Graphical summaries connect a subset of the most significant entities to show major themes in DEGs at 0-h (B), 72-h (C), and 168-h (D).

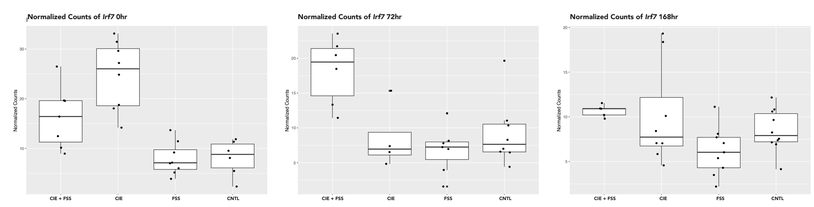
Upstream regulator analysis predicts Irf7 as a positive regulator of gene expression patterns observed in EtOH + Stress group



Upstream regulator analysis uses log2 fold-change and p-value information from differentially expressed gene lists to predict upstream regulators of gene expression patterns. (Left) Predicted upstream regulators of CIE + FSS gene expression patterns at 0-h, 72-h, and 168-h. (Right) Diagram showing pathway and activity patterns of *Irf7* and downstream genes at 72-h timepoint.

Irf7 is uniquely upregulated at 72 hr timepoint in EtOH + Stress group

Normalized read counts of Irf7 at 0-h (left), 72-hr (middle), and 168-h (right) in the EtOH + Stress group.



Summary and future directions

By profiling gene expression patterns in the NTS after EtOH + Stress exposure, we identified type I interferon signaling, and specifically Irf7, as important potential regulators of stress-induced alcohol consumption. Next steps in analysis will be to profile gene expression changes in the other treatment groups and identify differences to better understand molecular changes leading to stress-induced and dependence-induced drinking behavior. Future studies will manipulate interferon signaling in the NTS to determine a causal relationship between this pathway and alcohol consumption.

Integrative Neuroscience Initiative on Alcoholism INIA

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NIAAA National Institute on Alcohol Abuse and Alcoholism