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Effects of Chronic Stimulation of Nucleus Accumbens on Binge Drinking and Transcriptome

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Presenter Information

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Abstract

The nucleus accumbens (NAc) is an important brain region in alcohol use disorders. Previously, we found increasing NAc activity decreases binge drinking of alcohol, without altering fluid or tastant intake. Here, we used clozapine-N-oxide (CNO) to selectively activate mutagenized excitatory muscarinic G protein-coupled receptors (hM3Dq) in the Nac. We found that the effects of chronically increasing NAc activity produced lasting reductions in binge-like drinking, based on these behavioral results we proposed that transcriptional changes potentially underlie these changes.

Methods: High Drinking In the Dark (HDID-1) mice were stereotaxically injected with AAV2-DIO-hM3Dq-mCherry and Cre-GFP into the NAc. We used a 6 week Drinking the Dark (DID) schedule where mice had 2hr access to 20% ethanol (EtOH). For 7 days, mice were treated with 1% DMSO in saline (vehicle, IP, 30 minutes prior to DID) to determine baseline intake. Mice were then treated with CNO (1 mg/kg) prior to daily drinking for 28 days. Then, mice were administered vehicle for another 7 days to determine whether the effects of chronic CNO treatment were lasting. There were 3 control groups (ethanol-drinking mice not receiving CNO, and water-drinking mice receiving either CNO or vehicle).

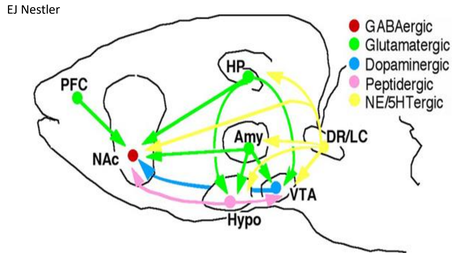
Conclusion: Based on these behavioral studies and analyses, we propose that chronically increasing NAc activity (via CNO/DREADDs) can induce molecular and cellular plasticity. By using these analyses, we are working to identify changes in gene expression related to harmful binge-like drinking and CNO/DREADD-induced reductions in binge-drinking. We plan to identify key hubs for pharmacological manipulation of binge-like drinking.

Research Question

What are the effects of changing NAc activity on binge-drinking and gene expression networks?
Can we reverse alcohol-induced changes in gene expression using DREADDs?

Using DREADDs to Manipulate Activity in the Nucleus Accumbens

EJ Nestler

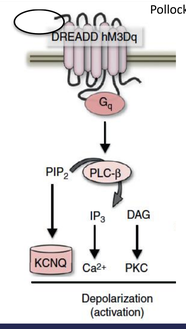


The Nucleus Accumbens (NAc) is a site of convergent inputs and an important regulator of mood.

Pollock and Satterlee, 2013

Designer Receptors Exclusively Activated by Designer Drugs: DREADDs.

The hM3Dq DREADDs increases Gq signaling, Ca+2 levels and neuronal activity when CNO is administered.



Chronic NAc stimulation produces lasting reduction in binge-like drinking

Experimental Design and Schedule:
HDID-1 female mice were stereotaxically injected with AAV2-DIO-hM3Dq-mCherry into the NAc and allowed to recover to 3 weeks before behavioral testing began.

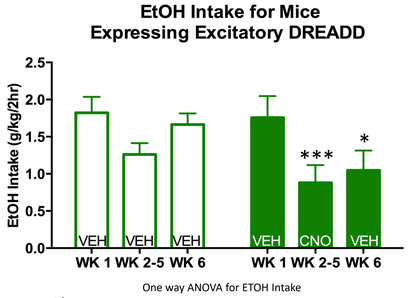
BASELINE DRINKING MEASUREMENT
Week 1
Veh (IP) 30 min prior to DID
2hr 20% EtOH or water access (3hr after lights off)

→

TREATMENT
Week 2-5
CNO 1mg/kg (IP) or Veh 30 min prior to DID
2hr 20% EtOH or water access (3hr after lights off)

→

WASHOUT PERIOD
Week 6
Veh (IP) 30 min prior to DID
2hr 20% EtOH or water access (3hr after lights off)



One way ANOVA for ETOH Intake

n=11-12/group
Control Group (VEH/VEH/VEH): n/s
Experimental Group (VEH/CNO/VEH): $F(2,35)=8.28$, $p < 0.001$, Dunnett's post-hoc test revealed significant differences for Veh1 (baseline) vs CNO (*** p<0.001) and Veh1 (baseline) vs Veh2 (washout; * p<0.05)

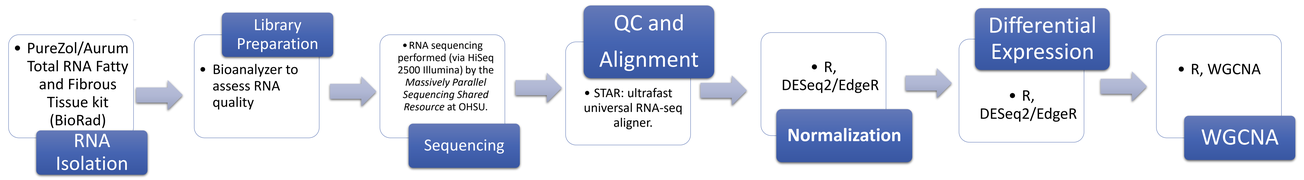
Methods

Paradigm: Adult female HDID-1 mice were stereotaxically injected with 0.5uL AAV2/5 Cre-GFP and 0.5 uL AAV2-DIO-hM3Dq-mCherry into the NAc (coordinates: +/-1.5 M/L, +1.34 A/P, -4.5 D/V) bilaterally and allowed to recover for three weeks prior to testing. Binge-like ethanol intake was tested using a variation of the Drinking in the Dark (DID) six-week schedule where mice received access to one bottle of 20% ethanol daily for 2 hours. There were 4 experimental groups: Ethanol DID with or without CNO and Water drinking groups with or without CNO (n = 11-12/group). Mice were from same selection generation. Mice received daily injections (IP) of either 1 mg/kg clozapine-n-oxide (CNO) or vehicle (1% DMSO in saline) approximately 30 minutes before a 2-hour DID session. For CNO groups: weeks 1 and 6 were vehicle injections and weeks 2-5 are CNO injections (1 mg/kg). Vehicle groups were injected with 1% DMSO in saline daily (IP) for 6 weeks. Mice were euthanized via cervical dislocation one day after the last session.

Sample Preparation and Processing: Tissue was mechanically homogenized in PureZol and RNA was isolated using Aurum Total RNA Fatty and Fibrous Tissue kit (Bio-Rad). RNA quality was assessed via BioAnalyzer and sequenced using library preparation and HiSeq 2500 Illumina by the Massively Parallel Sequencing Shared Resource at OHSU.

Analysis: Sequenced RNA was analyzed by aligning sample reads to *Mus musculus* genome using STAR: ultrafast universal RNA-seq aligner. After alignment, read counts were filtered and normalized to produce a count matrix. To perform Differential Expression analysis (DE), DESeq2 (in R) was used to process the matrix and determine significantly up or down regulated genes based on p-value threshold. Next Weighted Gene Co-expression Analysis (WGCNA) was used to identify modules (co-regulated genes) and hubs (genes correlating strongly with a significant number of related expressed modules). The WGCNA package (in R) was used to build a network on the basis of gene-gene correlations. Gene-gene correlations were collected in an adjacency matrix from the count matrix. The adjacency matrix was further processed (using soft threshold) and then clustered to detect gene modules (groups of genes that are expressed).

Workflow: How do we analyze changes in gene expression?

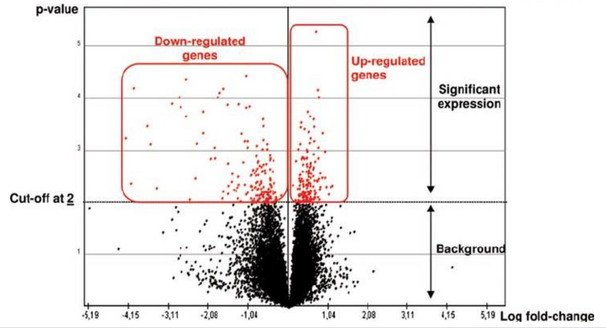


1. Quantifying Gene Expression Differences Between Groups

Differential expression analysis (DE) is used to analyze gene expression levels between treatment groups.

- Sequenced RNA from NAc tissue of mice from both CNO and VEH (Water and EtOH) treatment groups is aligned to the *Mus musculus* genome, and normalized.
- Regions of the sequenced genome in one sample cover regions of *Mus musculus*. Different samples between groups may have variability in alignment coverage.
- Based on this coverage between samples, we can see how genes are being expressed (Up-regulated/Down-regulated).
- How does CNO change gene expression? (What are the significant genes? What are their functions?)

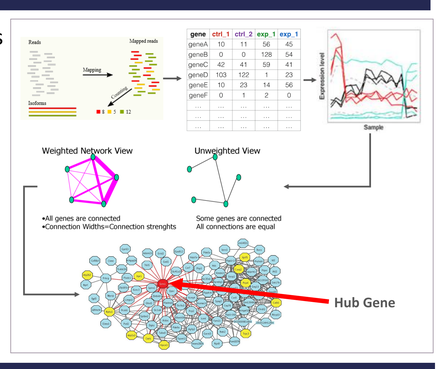
Volcano plot: common figure used to illustrate gene expression levels. Satih 2009



2. How Do Differentially Expressed Genes Correlate?

Weighted Gene Co-expression Analysis (WGCNA) is used to describe correlation patterns between genes. It can identify sets of tightly co-regulated genes (modules) based on network analysis. Understanding connectivity between genes can be used to single out important genes (hubs).

- In gene co-expression networks, RNA-Seq data is transformed into a count matrix. This matrix is used to compare gene expression between samples. Each gene (and its counts) correspond to a node; two nodes are connected if their expression values are highly correlated.
- The network is built on the basis of gene-gene correlations, which are collected in an adjacency matrix.
- The adjacency matrix is further processed and then clustered to detect gene modules (groups of genes that are coexpressed and often participate in the same biological processes).
- Genes that have many strong correlations are denoted as hubs.
- Our assumption is that they are important and potential targets for manipulation and/or therapy.
- Network properties in combination with differential expression can offer insights into molecular mechanisms.



Conclusions

- We found that chronically increasing NAc activity (via CNO/DREADDs) can induces lasting reductions in binge-drinking and propose that molecular plasticity underlies this effect.
- By using these analyses, we are identifying changes in gene expression related to harmful binge-like drinking and CNO/DREADD-induced reductions in binge-drinking.
- We plan to identify key hubs (WGCNA) for pharmacological manipulation of binge-like drinking.

Acknowledgements

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