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

Dar'ya Pozhidayeva
Portland State University

Evan Firsick
Oregon Health & Science University

Kayla G. Townsley
Oregon Health & Science University

Dan Iancu
Oregon Health & Science University

Angela Ozburn
Oregon Health & Science University

See next page for additional authors

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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 tantastic intake. Here, we used clozapine-N-oxide (CNO) to selectively activate mutated excitatory Gq-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-M3Dq-mCherry and Cre-GFP in the NAc. We used a 6-week Drinking the Dark (DID) schedule where mice had 2hr access to 20% ethanol (DIOx). For 7 days, mice were injected with 1% DMSO in saline (vehicle, IP, 15 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 and drinking mice receiving either CNO or vehicle).

Conclusion: Based on these behavioral studies and analyses, we propose that chronically increasing NAc activity (via CNO/DREADD) 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.

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

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

Chronic NAc stimulation produces lasting reduction in binge-like drinking.

Experiment Design and Schedule: HDID-1 female mice were stereotaxically injected with AAV2-DIO-M3Dq-mCherry into the NAc and allowed to recover to 3 weeks before behavioral testing began.

Pareidol: Adult female HDID-1 mice were stereotaxically injected with AAV2/Cre-GFP and 0.5 uL AAV2/DIO-M3Dq-mCherry into the NAc (coordinates: +/-1.5 M/L, 7.5 A/2, 4.5 D/V). Animals were then 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) one-week schedule where mice received access to one bottle of 20% ethanol daily for 2 hours. There were 4 experimental groups: Ethanol DIOx with or without CNO and Water drinking groups with or without CNO (N = 12-15/group). Mice were from the 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 DIOg groups: weeks 1 and 6 were Vehicle injections and weeks 2, 3, 4, and 5 were 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 PurErase and RNA was isolated using Aurum Total RNA kit (Bio-Rad). RNA quality was assessed via BioAnalyzer and sequenced using library preparation and HiSeq 2000 Illumina by the Massively Parallel Sequencing Shared Resource at OHSU.

Analysis: Sequenced RNA was analyzed by aligning sample reads to the mus musculus genome using STAR, ultrashort 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 calculated 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).