Chronic Intermittent Ethanol Exposure Induces Spatial Changes in Lipid Raft Localization of G-Proteins and Their Interacting Proteins in Rat Prefrontal Cortex

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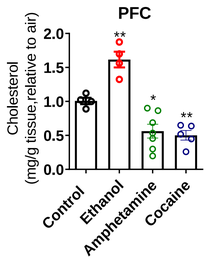
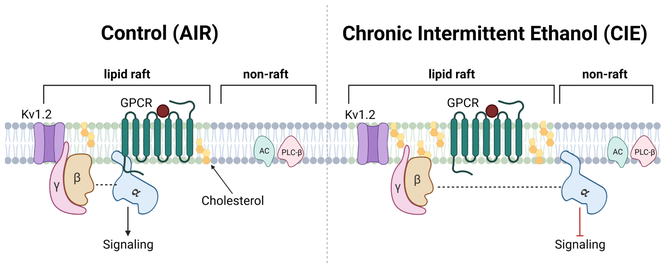
INTRODUCTION

- Chronic intermittent ethanol (CIE) exposure produces alterations in many membrane proteins such as GPCRs (e.g., mGluR2) and voltage-gated ion channels (e.g., Kv1.2) in various brain regions, but the molecular mechanism is unclear
- These membrane proteins are functionally regulated by heterotrimeric G-proteins, which are composed of a Gα and a dimeric Gβγ subunit
- Protein conformation and the assembly of signaling complexes in the plasma membrane is influenced by cholesterol content, which divides the membrane into lipid raft and non-raft microdomains
- Many membrane proteins contain cholesterol-binding motifs and may be regulated by membrane cholesterol
- Alterations to cholesterol content may lead to protein mislocalization within lipid rafts, resulting in impaired protein function
- Our lab has shown that drugs of abuse differentially alter membrane cholesterol content in the prefrontal cortex (PFC)



HYPOTHESIS

CIE exposure induces spatial changes in the membrane localization of G-proteins and their coupling partners within lipid raft and non-raft microdomains, which represents a potential underlying mechanism of CIE-related protein dysfunction



METHOD

Ethanol Vapor Exposure:

- Male Sprague Dawley rats were exposed to either ethanol vapor (CIE) or room air (AIR) during the light cycle (12 hr/day) for 7 consecutive days
- Following 24 hr withdrawal from the last ethanol exposure, all animals were euthanized, and PFC tissue or basolateral amygdala (BLA) slices were prepared

***Ex Vivo* Whole-Cell Patch-Clamp Electrophysiology:**

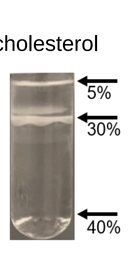
- Whole-cell patch clamp recordings were used to measure mGluR2 presynaptic function at *stria terminalis* inputs to BLA neurons

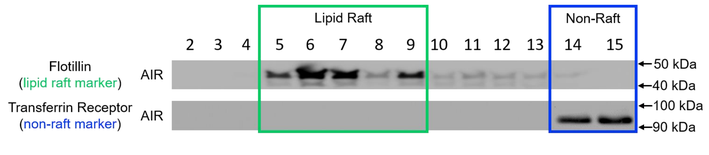
Cholesterol Loading of N2A Cells:

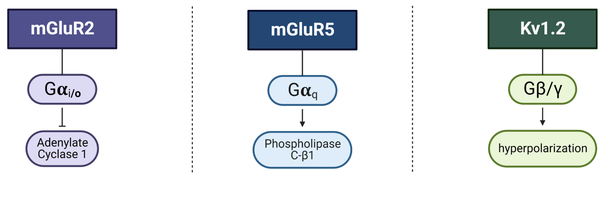
- Mouse neuroblastoma 2a cells were loaded with 5 mM cholesterol for 30 min and immediately lysed with lysis buffer

Sucrose Density Gradient Ultracentrifugation:

- Samples were homogenized and separated by discontinuous sucrose density (5%/30%/40%) ultracentrifugation into 15 fractions
- Western blotting was performed to determine the localization of G-protein subunits and their interacting proteins within lipid raft and non-lipid raft microdomains

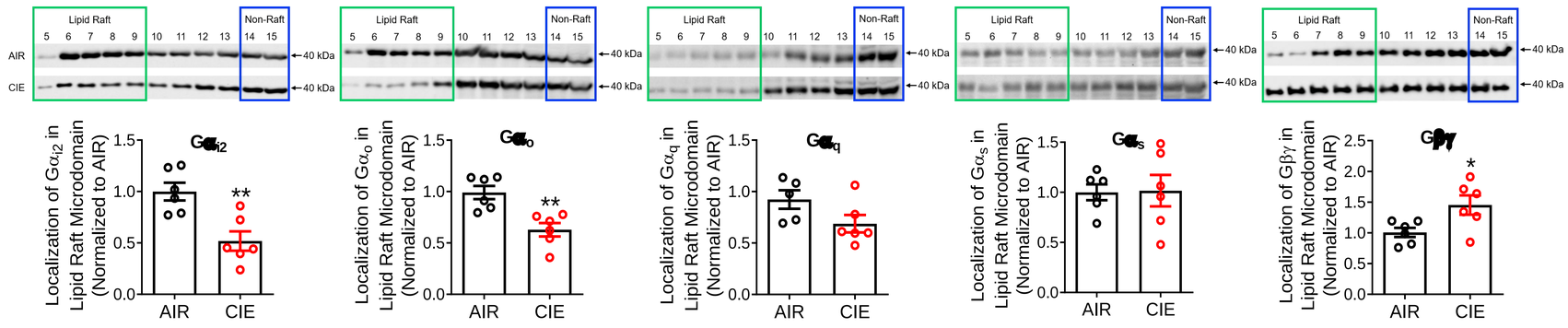




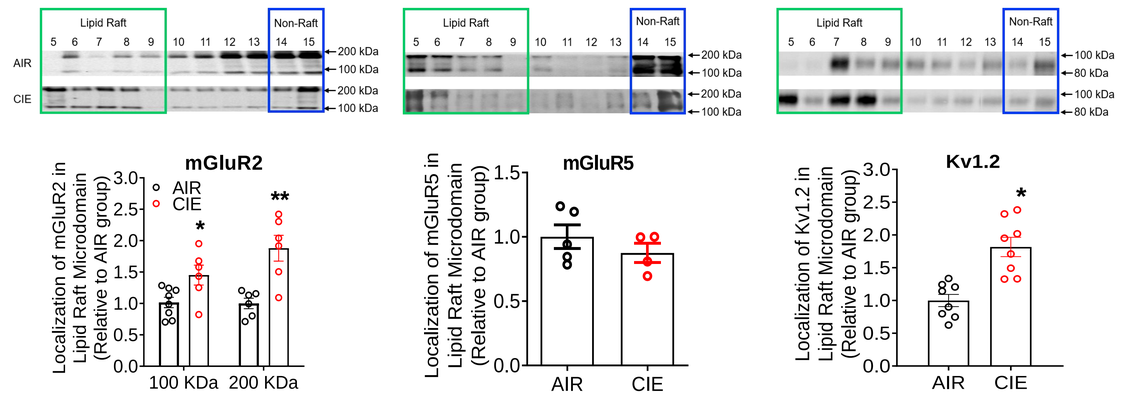


RESULTS

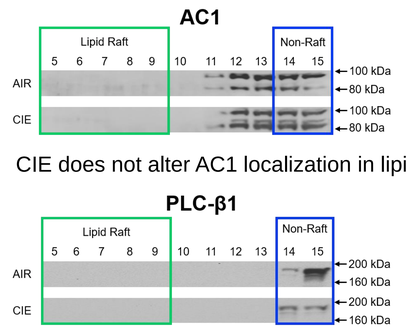
1. CIE Decreases $G\alpha_{i2}$ and $G\alpha_o$ Subunit and Increases Gβγ Localization within Lipid Raft Microdomains



2. CIE Increases mGluR2 and Kv1.2 Localization within Lipid Raft Microdomains



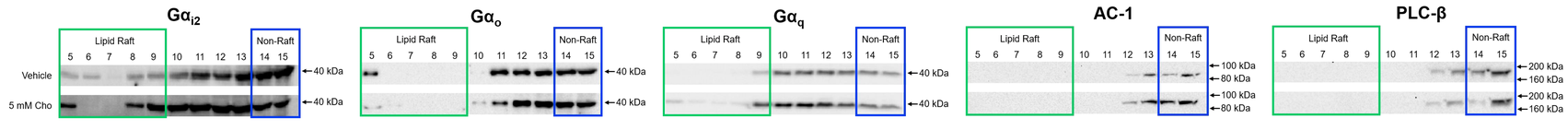
3. CIE Does Not Alter AC1 or PLC-β1



CIE does not alter AC1 localization in lipid rafts

CIE does not alter PLC-β1 localization in lipid rafts

4. Cholesterol Loading of N2A Cells Mimics the Effects of CIE on G-Protein and Interacting Protein Localization



Cholesterol ↓ $G\alpha_{i2}$ in lipid rafts

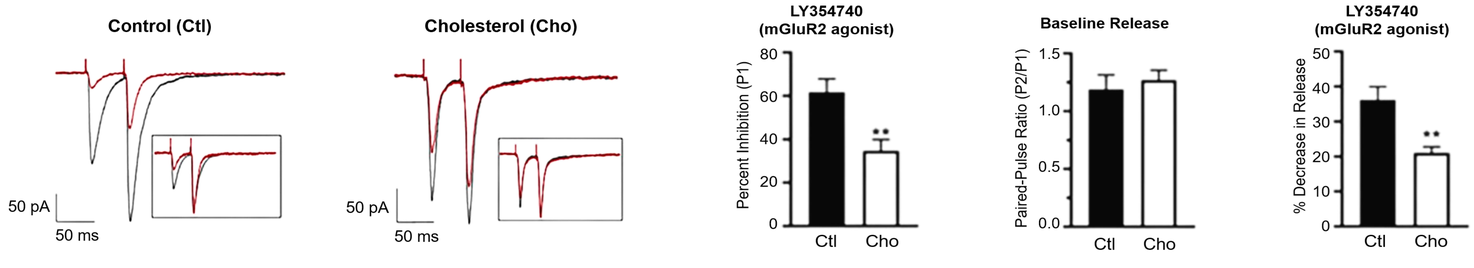
Cholesterol ↓ $G\alpha_o$ in lipid rafts

Cholesterol does not alter $G\alpha_q$ in lipid rafts

Cholesterol does not alter AC1 in lipid rafts

Cholesterol does not alter PLC-β1 in lipid rafts

5. Cholesterol Loading *ex vivo* Disrupts mGluR2 Inhibition of Glutamate Release



Paired-pulse response from control and cholesterol-loaded (0.5 mg/mL, 1h, 25° C) BLA neurons. Baseline (black traces) and effects of LY354740, an mGluR2 agonist, (red traces) on EPSCs are shown. Inset: responses scaled to the second amplitude to show effects of LY354740 on paired pulse ratio. Cholesterol loading has no effect on basal paired pulse ratios but decreases both the percent inhibition of the first response and the percent decrease in release by LY354740.

CONCLUSIONS

- CIE differentially alters the spatial localization of G-proteins and their coupling partners
- $G\alpha_{i/o}$ and Gβγ, along with their interacting proteins, mGluR2 and Kv1.2, are sensitive to the effects of CIE on lipid raft compartmentalization
- CIE in rats and cholesterol loading in N2A cells produce similar effects on G-protein localization in lipid rafts, suggesting a direct causal relationship between increased cholesterol and membrane protein localization
- Cholesterol loading in brain slices disrupts GPCR (mGluR2) function, demonstrating the functional consequences of altered mGluR2 localization in lipid rafts
- The present study highlights a potential role of lipid homeostasis in GPCR dysregulation and may open a new avenue for targeting cholesterol metabolism as a treatment for alcohol use disorder

ACKNOWLEDGEMENTS

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