Changes In Metabotropic Glutamate Receptor Trafficking During Protracted Ethanol Withdrawal

Sarah Mainya, Jennifer Giles, Daniel T. Christian
Des Moines University, Physiology and Pharmacology Department, West Des Moines, IA 50266

DES MOINES UNIVERSITY
MEDICINE & HEALTH SCIENCES

Methods

Animals: All procedures were approved by the DMU IACUC (2018-09). Studies utilized male (~100g) and female (~75g) Sprague Dawley rats for all studies. All studies were approved by the Des Moines University Animal Care and Use Committee.

Ethanol Exposure: Animals were group housed (2-3/cage) inside custom built plexiglass ethanol exposure chambers. The chronic intermittent ethanol (CIE) exposure paradigm consisted of vaporized ethanol for 12h on/ 12hr off for 4 consecutive days followed by a 3-day intermittent withdrawal period. This 4d on/3d off pattern was repeated for 3 cycles. Following the last exposure animals were placed in their home cages and allowed to enter protracted withdrawal prior to experimental use. Blood ethanol concentrations were analyzed from tail blood samples collected once per exposure cycle (3x/animal). BEC levels for all animals included in the presented data are 196 mg/dl. Air levels of EtOH were measured daily.

Biotinylation, BS3, Western Blots: All treatments followed previously published methods. Following the dissection of the BLA, membrane surface protein labels NHS-SS-BIOTIN (APExBIO) or BS3 (Covachem) were added to the tissue. These membrane impermeant reagents bind to proteins on the cell membrane surface. This allowed for the separation of proteins in surface (membrane bound) and internal (unbound) fractions. Unbound fractions were not analyzed for Biotinylation studies. Tissue was then processed for western blot analysis. Tissue was separated by weight using 4-12% gradient gels and transferred using either wet or semidry transfer methods. Relative protein expression was quantified following membrane incubation in primary and secondary antibodies. List as requested.

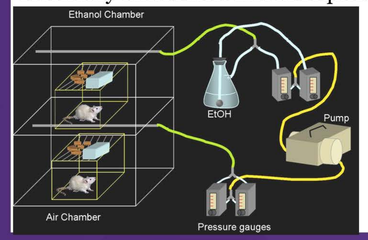
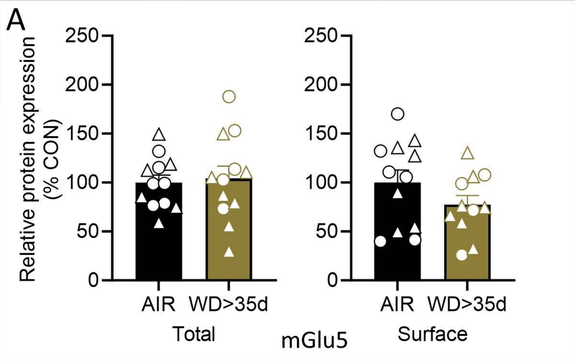
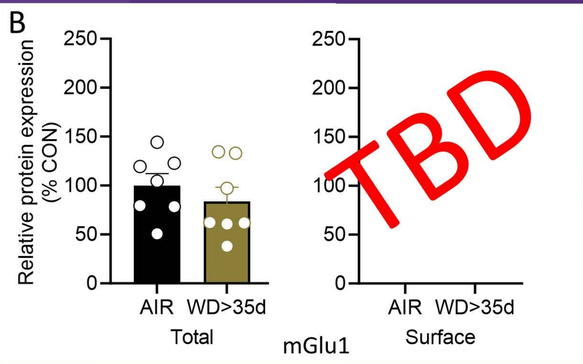


Figure 1. Diagram of vapor exposure equipment. Top chamber houses EtOH exposure animals. Bottom chamber houses Air exposure control (CON) animals.

Introduction

As a result of chronic ethanol exposure, the brain undergoes neuroadaptive changes in the glutamatergic receptor system. Along with functional alterations, protein expression and regulation are thought to occur, and to contribute to overall glutamatergic system dysregulation. These functional/expression alterations are associated with long term propensity to relapse to drug use. In this study, we quantified the expression of group I metabotropic glutamate receptors (mGlu1 and mGlu5) during withdrawal from chronic intermittent ethanol exposure. mGlu expression and function are known to be altered during protracted withdrawal from other drugs of abuse. In addition, we also quantified known trafficking/anchoring partners, including Homer1bc and Homer2 proteins. We seek to identify any alterations in protein expression/localization that could contribute to increased relapse vulnerability in our exposure model. We utilized western blot analysis on whole tissue lysate as well as a biotin labeling of surface (membrane bound) proteins in withdrawal and control rats.





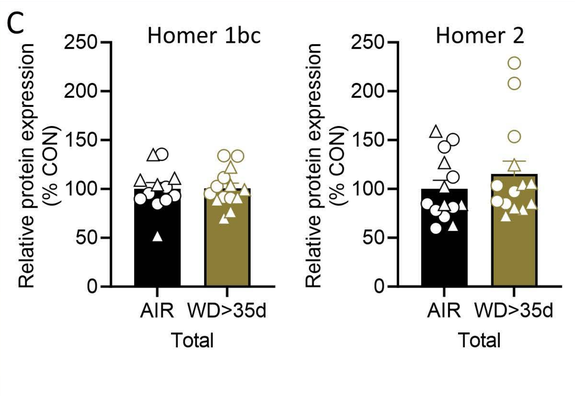


Figure 2. Surface and Total protein expression of mGlu and Homer proteins during protracted ethanol withdrawal are unaltered compared to Air exposed control tissue.

A) mGlu5 protein expression did not differ across total ($t(22)=0.27$, ns) or biotinylated surface expressed fractions ($t(21)=1.4$, ns).

B) mGlu1 total protein expression was not different between exposure groups ($t(12)=0.85$, ns). Surface expression has not yet been quantified/collected.

C) Left: Homer 1bc protein (total) was not differentially expressed in protracted ethanol withdrawal compared to control tissue ($t(24)=0.09$, ns). Right: Homer2 total protein levels did not vary between ethanol exposure and control tissues ($t(26)=0.98$,ns).

Across mGlu5, mGlu1 and Homer proteins, we see no effect of long-term withdrawal on protein expression in total or surface tissue fractions. This suggests that mGlu trafficking/expression at this time period during protracted withdrawal is 1) resistant to alteration, 2) does not contribute to ongoing functional differences, or that 3) protein/localization alterations occur earlier in withdrawal.

mGlu and Homer proteins for physical bonds *in vivo*, which these assays do not measure. It is possible that these interactions are disrupted, which could contribute to altered functional outputs.

We have not measured the functional contributions of mGlu receptors under these experimental conditions. It is possible that the functional output of these receptors is altered despite no apparent change in physical location.

Future studies will address protein–protein interactions of mGlu, Homer, and other ionotropic glutamatergic receptor proteins across earlier withdrawal time points.

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