NMDA receptor modulation in protracted ethanol withdrawal

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Introduction

The primary driver of drug craving and relapse results from the functional dysregulation of the glutamate system during withdrawal from chronic drug exposure. Psychostimulant use studies in animal models have demonstrated dynamic alterations in NMDA receptor function and expression that contribute to drug seeking behavior. These changes begin as early as 5 days following cessation of drug use are persistently expressed into long term withdrawal (>60d). Evidence of NMDA receptor dependent changes during short term withdrawal (24h) suggest that similar mechanisms may drive drug craving and relapse behaviors following chronic exposure to ethanol. To this end, NMDA receptor protein and surface expression during protracted withdrawal from chronic intermittent ethanol (CIE) exposure using western blot was investigated. The basolateral amygdala (BLA) was a primary focus of the study, as glutamatergic signaling in this region is robustly modulated by short term (24h) withdrawal from CIE (10d, 12hr/day) and regulates anxiety-like behavior expressed during withdrawal. Adolescent male rats were exposed to repeated cycles of CIE (12hr/day, 4d on/3d off, 3 cycles) and brain tissue was collected during protracted withdrawal. Optimization of experimental procedures is currently being conducted and analysis of total and membrane surface expression will be analyzed by surface biotinylation for NMDAR subunits (GluN1, GluN2, GluN3). Cell surface biotinylation will be used to isolate cell surface membrane proteins to detect and quantify expression of NMDAR subunits. We expect to identify an increase in membrane surface expression for NMDAR subunits, in agreement with functional data obtained in the laboratory.

Methods

Animals

- All procedures approved by DMU IACUC (2018-09) and approved by DMU Animal Care and Use Committee. Male Sprague Dawley rats utilized for study.

Ethanol Exposure

- Group housed animals (3/cage) within custom built plexiglass ethanol exposure chamber to deliver vaporized chronic intermittent ethanol (CIE) for 12h on/12h off cycle for 4 consecutive days followed by a 3-day intermittent withdrawal period. This cycle is repeated for 3 cycles and animals are returned to home cages to enter protracted withdrawal prior to experimental use.
- Blood ethanol concentrations (BEC) were analyzed by tail blood sampling and were conducted once during each cycle with levels between 150-250 mg/dL. Air levels of EtOH were measured daily for vaporization dosing verification.

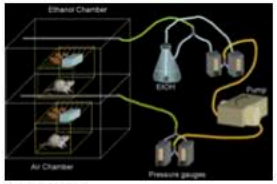


Figure 1. Diagram of vapor exposure equipment. Top chamber houses EtOH exposure animals. Bottom chamber houses air exposure control (CON) animals.

Cell Surface Labeling

- External cellular surface was labeled with biotin reagent to determine surface protein expression allowing for comparison of surface receptors to intracellular fractions.

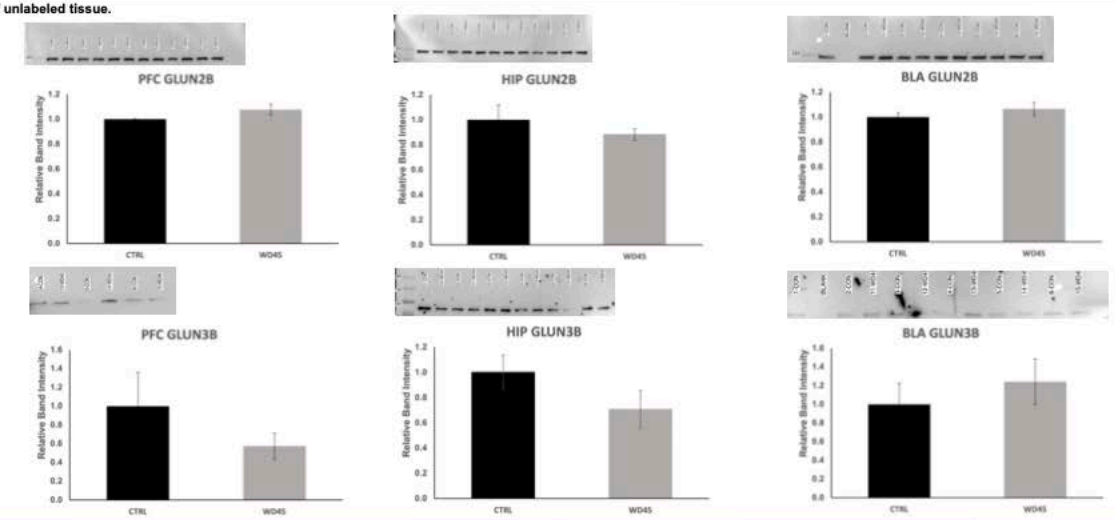
Western Blot Analysis

- Lysate processed for gel electrophoresis after treatment of tissue samples using 4-15% gradient gels and PVDF membrane transfer. Antibodies of interest for specific receptor subunits (GluN1, GluN2, GluN3) and trafficking partners (PACSIN1) were used. Imaging and analysis was conducted with ImageLab software.

Results

Western Blot analysis of unlabeled tissue.

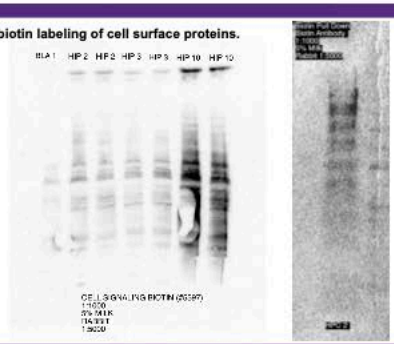
Figure 2. Western Blot analysis of GLUN2B and GLUN3B subunits conducted on total unlabeled lysate in prefrontal cortex (PFC), hippocampus (HIP), and basolateral amygdala (BLA) in control and CIE animals during withdrawal day (WD) 45. Preliminary data of total lysate will be used for comparison against biotinylated lysate to quantify surface expression of proteins.



Western Blot procedure to confirm biotin labeling of cell surface proteins.

Figure 3. Western Blot procedure confirmed biotin labeling procedure (left). Western Blot confirmation of Avidin-Biotin Pull-Down conducted with High Capacity NeutrAvidin Resin (Thermo Sci Cat # 29204) (right).

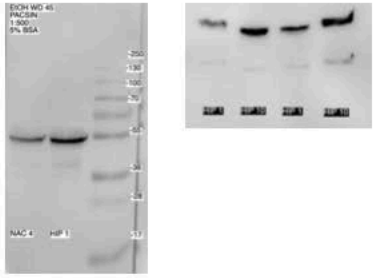
Anti-biotin antibody confirmed successful biotinylation procedure of sample protein to be used for further study of surface expressed receptors. Samples from control tissue (1-9) and withdraw day (WD) 55 (10-18).



Western Blot procedure to determine the role of PACSIN on NMDA receptor modulation.

Figure 4. Western Blot procedure demonstrated PACSIN expression within control lysate. Further studies will utilize PACSIN to determine its role in receptor trafficking of specific subunits (GLUN3A) (left).

Western Blot using GLUN3A antibody within HIP tissue. Samples from control (1-9) and WD 55 (10-18) (right).



Conclusion and Future Directions

Interactions between ethanol and NDMA-Rs have been shown to induce modulation of the glutamate system within the central nervous system. Identification of cellular surface expression of NMDA-Rs in a CIE animal model will help to identify regulation of specific subunits and their contribution to glutamatergic receptor dysfunction and their role in chronic alcohol withdraw.

Elucidating ratios of differential subunit expression will support previous functional data that has been identified by the Christian lab during protracted withdrawal. Receptor activity regulation and function can be concluded from identification of subunits which may lead to differential ion permeability and excitatory response within the basolateral amygdala (BLA).

Preliminary results as discussed within unlabeled tissue have determined differences in subunit expression within brain regions of interest (PFC, HIP, BLA) with an increased expression within the BLA.

Preliminary results of cell surface labeling with biotin reagent determine treatment was effective and will be used to detect and quantify expression of NMDA-Rs.

Future directions will determine sex-based differences in surface expression of NMDA-R and determination of molecular characterization of subunit trafficking to provide explanation for functional changes within synapses.

References

Christian, D. T., Stefanik, M. T., Bean, L. A., Loweth, J. A., Wunsch, A. M., Funke, J. R., Briggs, C. A., Lyons, J., Neal, D., Milovanovic, M., D'Souza, G. X., Stutzmann, G. E., Nicholson, D. A., Tseng, K. Y., & Wolf, M. E. (2021). GluN3-Containing NMDA Receptors in the Rat Nucleus Accumbens Core Contribute to Incubation of Cocaine Craving. *The Journal of Neuroscience*, *41*(39), 8262-8277.

Pérez-Otaño, I., Luján, R., Tavalin, S. J., Plomann, M., Modregger, J., Liu, X.-B., Jones, E. G., Heinemann, S. F., Lo, D. C., & Ehlers, M. D. (2006). Endocytosis and synaptic removal of NR3A-containing NMDA receptors by PACSIN1/syndapin1. *Nature neuroscience*, *9*(5), 611-621.

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