Changes in AMPA Receptor Trafficking Observed In Protracted Ethanol Withdrawal

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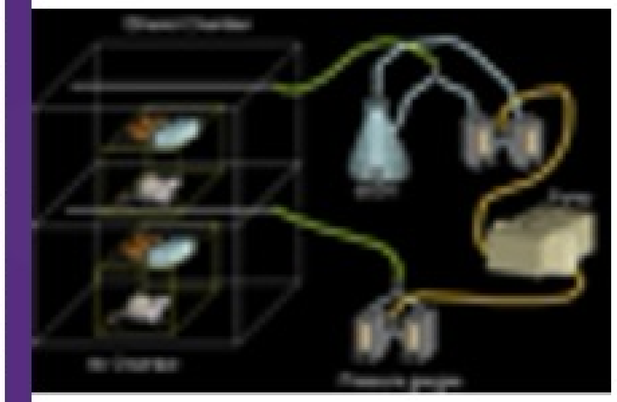
Introduction

Dysregulation of glutamatergic transmission within the brain is thought to underlie the development and expression of drug craving/relapse. Increased trafficking of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs) has been observed in animal models characterizing short-term ethanol withdrawal (~24h) Investigating these alterations after longer term withdrawal periods (30-45 days) may provide new insight into how this dysregulation alters propensity to relapse/drug craving. Thus, we are investigating AMPAR expression following chronic intermittent ethanol (CIE) exposure and an extended withdrawal period in male and female rats. We focused on the basolateral amygdala (BLA), as glutamatergic signaling in this region is robustly modulated by short term (24h) withdrawal and regulates anxiety-like behaviors expressed during withdrawal. 5–9-week-old male and female rats undergo a CIE exposure protocol and are allowed to enter protracted withdrawal. The BLA was dissected and treated with a cell impermeant tag bis(sulfosuccinimidyl)suberate (BS3). Western blots were run to identify pools of BS3-tagged surface proteins and untagged intracellular proteins using antibodies towards GluA1, GluA2, and GluA3 AMPAR subunits. Preliminary data suggest our BS3 treatment was successful, and we can identify proteins isolated to each compartment. On-going studies are expected to indicate an increase in AMPAR trafficking of GluA1 subunits in CIE/Withdrawal animals in comparison to control tissue. We also hypothesize that CIE/Withdrawal female animals will show smaller increases in AMPAR surface expression in comparison to both female control and male withdrawal animals.

Methods

Animal Model and Exposure Paradigm

- 5–9-week-old male and female rats
- Rats were allowed to acclimate for 7 days and placed into either exposure or control chambers (see below)
- Rats were exposed for 12 hr. periods, and this was repeated for 4 days followed by a 3-day withdrawal period. 7-day cycle was repeated a minimum of 3 times
- After completing these exposure cycles, an extended withdrawal period of >40 days was started.



- Schematic of exposure chambers

Tissue Collection and Crosslinking Treatment

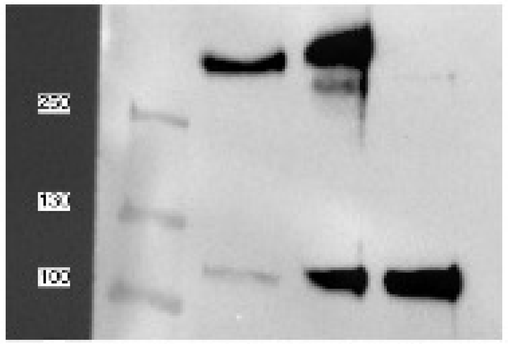
- Rats were taken from their chambers and given an intraperitoneal chloral hydrate injection for anesthetization
- Rats were decapitated, brains were extracted and sliced and placed in artificial cerebral spinal fluid (aCSF)
- Relevant brain regions were dissected and then the BS3 crosslinking treatment was performed[1]

SDS PAGE and Western Blot Analysis

- BCA protein assay was performed to calculate protein concentration of samples
- Equal amounts of sample were added to each 50 µL well in a 4-15% gradient polyacrylamide gel
- Proteins were separated by size then transferred to a PVDF membrane
- The membranes were then treated with AMPAR subunit specific antibodies for visualization

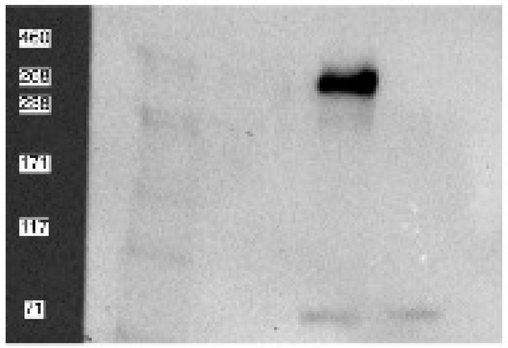
Results

GluA1 Subunit



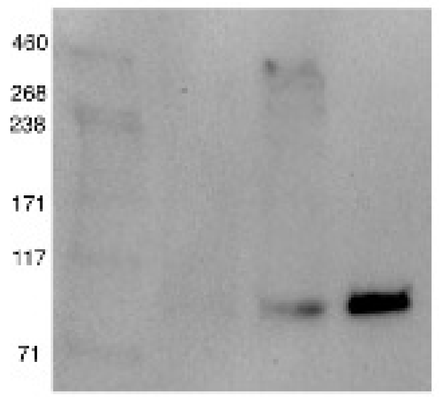
- Lanes from left to right: BS3 treated Amygdala, BS3 treated Hippocampus, non-treated BLA. Primary GluA1 antibody Abcam 109450

GluA2 Subunit



- Lanes from left to right: BS3 treated Amygdala, BS3 treated Hippocampus, non-treated BLA. Primary GluA2 antibody Neuromab 75-002

GluA3 Subunit



- Lanes from left to right: BS3 treated Amygdala, BS3 treated Hippocampus, non-treated BLA. Primary GluA3 antibody Cell Signaling 3437

Conclusion

- Preliminary data suggest that the crosslinking treatment was effective.
- Initial characterization supports and increase in GluA1 surface trafficking, but no change in localization for GluA2 and GluA3 subunits.

Future Directions

- Repeat western blot analysis with CIE and non–treated control tissue in order to visualize any potential differences in AMPAR trafficking and expression.
- Samples will be analyzed across all three antibodies. Female tissue will be collected to analyze sex differences in AMPAR expression and trafficking across male and female rats.

References

1. Boudreau, Amy C et al. "A protein cross-linking assay for measuring cell surface expression of glutamate receptor subunits in the rodent brain after in vivo treatments." *Current protocols in neuroscience* vol. Chapter 5 (2012): Unit 5.30.1-19. doi:10.1002/0471142301.ns0530s59
2. Christian, Daniel T et al. "Chronic intermittent ethanol and withdrawal differentially modulate basolateral amygdala AMPA-type glutamate receptor function and trafficking." *Neuropharmacology* vol. 62,7 (2012): 2430-9. doi:10.1016/j.neuropharm.2012.02.017
3. Morales, Melissa et al. "Chronic Intermittent Ethanol Exposure Modulation of Glutamatergic Neurotransmission in Rat Lateral/Basolateral Amygdala is Duration-, Input-, and Sex-Dependent." *Neuroscience* vol. 371 (2018): 277-287. doi:10.1016/j.neuroscience.2017.12.005

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