Increased NMDA Receptor Function during Protracted Withdrawal from Chronic Intermittent Ethanol Exposure

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Introduction

Functional dysregulation of the glutamatergic receptor system during withdrawal from chronic drug exposure is a primary driver of drug craving and relapse. Animal models of psychostimulant use have demonstrated dynamic alterations in both AMPA and NMDA receptor function and expression that contribute to drug seeking behavior. Several studies have identified an increased contribution of atypical GluN3-containing triheteromeric NMDARs. Short term withdrawal (24hr) from chronic exposure to ethanol also induces functional dysregulation of AMPA and NMDA receptors suggesting that similar mechanisms may be regulating drug craving and relapse behaviors across drugs of abuse. Far less is known about changes in glutamatergic receptor function during protracted withdrawal from chronic ethanol exposure. We utilized an ethanol vapor model to exposure rats who then entered protracted withdrawal. We then utilized whole-cell patch clamp electrophysiology to measure evoked NMDA receptor mediated synaptic responses in both control (air exposed) and in previously exposed withdrawal animals with the primary aim of 1) characterizing basal NMDAR transmission during withdrawal and CON conditions; 2) elucidating subunit specific contributions to overall NMDAR-mediated responses using pharmacological agents; and 3) investigate sex-dependent alterations in NMDAR mediated transmission across experimental conditions.

Methods

Animals: All procedures were approved by the DMU IACUC (2018-09). Studies utilized male Sprague Dawley rats (~100g) 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.

Electrophysiology: Whole cell patch clamp recordings of basolateral amygdala (BLA) pyramidal neurons were conducted in coronal brain slices (400uM) in room temperature aCSF. Picrotoxin (0.1 mM) and 6-Cyano-7-nitroquinoxaline-2,3-dione disodium (CNQX, 0.02 mM) were used to block GABAergic and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor mediated synaptic transmission, respectively. Patch pipettes (6-8MΩ) were filled with internal solution containing (in mM): 140 CsCl, 10 HEPES, 2 $MgCl_2$, 5 NaATP, 0.6NaGTP, 2 QX-314.

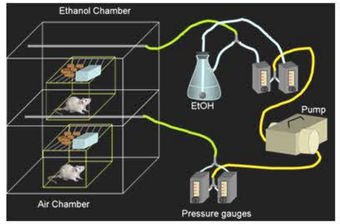

Figure 1. Diagram of vapor exposure equipment. Top chamber houses EtOH exposure animals. Bottom chamber houses Air exposure control (CON) animals.

Previous Results – Cocaine SA

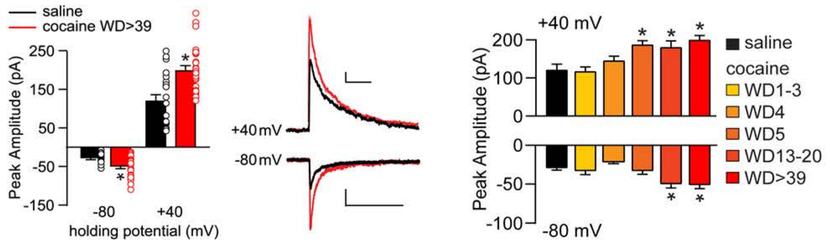

Figure 2. Time dependent regulation of NMDAR-mediated synaptic transmission in NAc core medium spiny neurons (MSN) during withdrawal from extended-access cocaine self-administration. Whole-cell patch-clamp recordings of NMDAR-mediated EPSCs were performed in NAc core MSNs of saline rats (23 cells/15 rats) on or after withdrawal day (WD) 16 and from cocaine rats (29 cells/22 rats) between WD39 and WD68. IV plots of NMDAR mediated synaptic transmission of cocaine cells at progressive time intervals of withdrawal: WD1-3 (15 cells/7 rats), WD4 (16 cells/8 rats), WD5 (11 cells/7 rats), and WD13-20 (15 cells/12 rats). Suggests multiple mechanisms contribute to alterations across holding potentials. Increased NMDAR function at -80mV likely has outsized impact on cellular function. Christian et al, Under Revision, JNeurosci * = p>0.05. Scale bars, +40mV: 50pA x 250ms; -80mV: 20pA x 250ms.

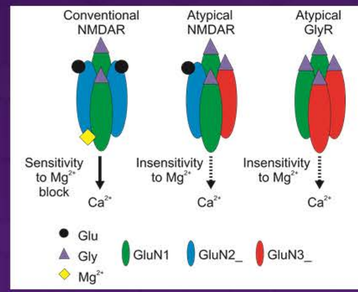

Figure 3. Diagram of NMDAR subunit composition. Left) Conventional NMDA receptor subunit composition. B) Atypical subunit composition. The inclusion of GluN3 subunits conveys distinct functional properties, including removal of Mg2+ block which could account for increased NMDAR function at -80mV. C) Alternate subunit composition that results in glycine receptors. Modified from Kehoe et al., Neural Plasticity 2013;

Results – Ethanol Vapor Exposure

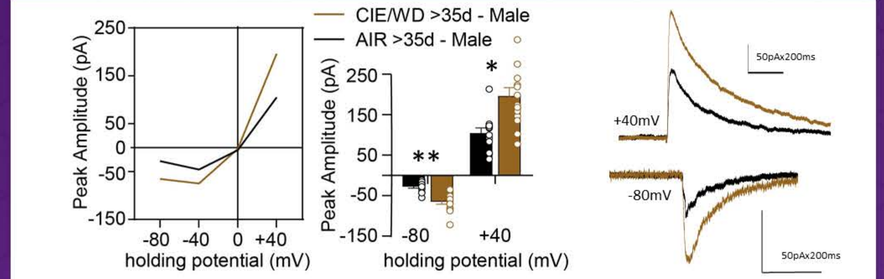

Figure 4. Increased NMDAR function during protracted withdrawal following 3 cycles of chronic intermittent ethanol vapor exposure. Left) IV curve plot demonstrating increased NMDAR mediated responses at -80mV and +40mV. Middle) Bar graph of individual data points showing increased NMDAR responses during protracted withdrawal at -80 and + 40mV. Similar patter of results as those found during protracted withdrawal from cocaine. Right) representative traces of responses at each holding potential. * = p>0.05; ** p>0.01. Student t-test.

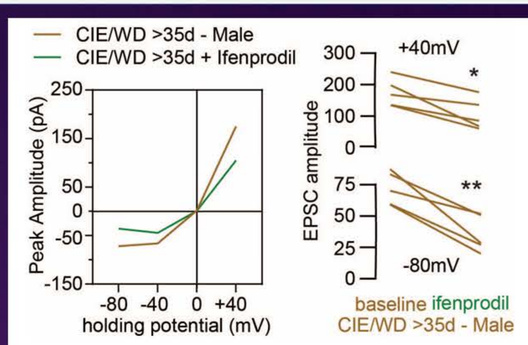

Figure 5. Increased NMDAR function is mediated by GluN2B-containing receptors. Left) IV Plot of evoked responses at various membrane holding potentials. Drug application was conducted onto single cells "within cell" in male rats during protracted withdrawal from CIE exposure. Right) Within-cell data showing effect of ifenprodil (5μM) application on response amplitudes at various membrane potential. Effect at -80mV suggest atypical NMDARs are present during protracted withdrawal from CIE. * = p>0.05, ** = p>0.01; Paired t-tests

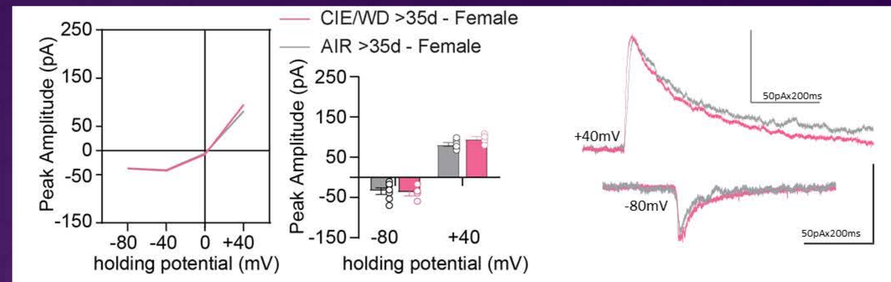

Figure 6. NMDAR function during protracted withdrawal following 3 cycles of chronic intermittent ethanol vapor exposure is unchanged in Female rats. Left) IV curve plot demonstrating increased NMDAR mediated responses at -80mV and +40mV. Middle) Bar graph of individual data points showing no significant change in NMDAR responses during protracted withdrawal at -80 and + 40mV. Right) Representative traces of responses at each holding potential. Data suggests sex-specific regulation of NMDARs during protracted withdrawal from CIE exposure.

Conclusions/Future Directions

- Functional profile of male synaptic transmission matches that shown in NAc neurons during protracted withdrawal following cocaine self administration.
- Preliminary data suggest sex-specific effect on NMDAR mediated synaptic transmission in BLA pyramidal neurons (i.e. no differences in female rats).
- Pharmacological studies utilizing TK30 (pan-GluN3) and TK80 (GluN3b) to characterize the functional contribution of GluN3 subunits are currently being conducted.
- Future studies will be geared to understanding the time course along which functional alterations shown in male rats develops. Studies in Females will also investigate if functional alterations are demonstrated in a time and/or exposure dependent manner and expand sample sizes.
- Future studies will seek to identify gene expression, protein level, and surface expression of NMDAR subunits in the BLA across withdrawal time points and animal sex.

References

- Kehoe, L.A., Y. Bernardinelli, and D. Muller, *GluN3A: an NMDA receptor subunit with exquisite properties and functions.* Neural Plast, 2013. **2013**: p. 145387
- Christian, D.T., et al., *GluN3-containing NMDA receptors in nucleus accumbens core are required for incubation of cocaine craving.* Soc Neurosci Abstr, 2017. Under Revision JNeurosci.
- This work was supported by the Iowa Osteopathic Research Fund.