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Selective alterations of NMDAR function and plasticity in D1 and D2 medium spiny neurons in the nucleus accumbens shell following chronic intermittent ethanol exposure

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Abstract

A major mouse model widely adopted in recent years to induce pronounced ethanol intake is the ethanol vapor model known as “CIE” or “Chronic Intermittent Ethanol.” One critical question concerning this model is whether the rapid induction of high blood ethanol levels for such short time periods is sufficient to induce alterations in *N*-methyl-D-aspartate receptor (NMDAR) function which may contribute to excessive ethanol intake. In this study, we determined whether such short term intermittent ethanol exposure modulates NMDAR function as well as other prominent electrophysiological properties and the expression of plasticity in both D1 (D1+) and D2 (D1–) dopamine receptor expressing medium spiny neurons (MSNs) in the nucleus accumbens (NAc) shell. To distinguish between the two subtypes of MSNs in the NAc we treated *Drd1a*-TdTomato transgenic mice with CIE vapor and electrophysiological recordings were conducted 24 h after the last vapor exposure. To investigate CIE induced alterations in plasticity, long-term depression (LTD) was induced by pairing low frequency stimulation (LFS) with post synaptic depolarization. In ethanol naïve mice, LFS induced synaptic depression (LTD) was apparent exclusively in D1+ MSNs. Whereas in slices prepared from CIE treated mice, LFS induced synaptic potentiation (LTP) in D1+ MSNs. Furthermore, following CIE exposure, LFS now produced LTD in D1– MSNs. We found that CIE exposure induced an increase in excitability in D1+ MSNs with no change in D1– MSNs. After CIE, we found a significant increase in spontaneous EPSCs (sEPSCs) frequency in D1+ but not D1– MSNs suggesting alterations in baseline α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA) mediated signaling. CIE induced changes in NMDAR function were measured using the NMDA/AMPA ratio and input-output curves of isolated NMDAR currents. We observed a significant increase in NMDAR function in D1+ MSNs and a decrease in D1– MSNs after ethanol vapor exposure. The reversal of NMDAR function may account for the CIE induced alterations in the expression of plasticity. The cell type specific alterations in excitatory signaling in the NAc shell may constitute

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an important neuroadaptation necessary for the expression of increased ethanol consumption induced by intermittent ethanol vapor exposure.

Keywords

Neuroadaptation; CIE; LTD; LTP

1. Introduction

The nucleus accumbens (NAc) is a critical component of the brain reward system and is composed primarily of medium spiny neurons (MSNs) of which there are two distinct subtypes, D1 and D2 dopamine receptor expressing MSNs. A prominent and well described form of plasticity expressed in the NAc is NMDAR-dependent synaptic long-term depression (“NMDAR-LTD”). This form of plasticity requires the internalization of GluA2-containing AMPA receptors (Brebner et al., 2005; Jeanes et al., 2014) and several reports have shown that drugs of abuse and ethanol can disrupt the expression of NMDAR-LTD in the NAc (Abrahao et al., 2013; Jeanes et al., 2014, 2011; Kasanetz et al., 2010; Mao et al., 2009; Martin et al., 2006; Shen and Kalivas, 2012; Thomas et al., 2001). Indeed, Brebner et al. (2005) demonstrated that the disruption of the internalization of GluA2-containing AMPA receptors can alter the expression of behavioral sensitization to psychomotor stimulants. Therefore, such drug or ethanol-induced synaptic changes in the NAc may be critical neuroadaptive responses underlying the induction and/or expression of drug-induced behaviors (Luscher and Malenka, 2011).

Chronic intermittent ethanol (CIE) exposure is a well-established model for inducing ethanol dependence and increasing volitional ethanol intake (Becker and Lopez, 2004; Griffin et al., 2009a, 2009b; Griffin, 2014; Lopez and Becker, 2005). In previous work from our lab, we focused upon identifying plasticity changes after a single cycle of CIE exposure in C57BL/6J mice. We observed a striking conversion in synaptic plasticity such that the NMDAR-LTD induction protocol which resulted in synaptic depression (LTD) in ethanol naïve mice produced synaptic potentiation (LTP) following a single cycle of ethanol vapor (Jeanes et al., 2011). At that time, we were unable to determine if ethanol differentially modulated plasticity between the D1 or D2 receptor-expressing MSN subtypes. In a second report, we used bacterial artificial chromosome (BAC) transgenic mice in which the expression of enhanced green fluorescent protein (eGFP) was controlled by the D1 receptor promoter in mice on a Swiss Webster background, thus allowing us to selectively record from D1+ or D1– MSNs (Matamales et al., 2009; Valjent et al., 2009). We observed that NMDAR-LTD was apparent only in D1+ MSNs with no change in synaptic strength in D1– MSNs following LFS conditioning. After CIE vapor exposure we found a reversal in the expression of plasticity in which LTD could be induced in D1– MSNs but not in D1+ MSNs (Jeanes et al., 2014). These findings strongly indicate that such differential neuroadaptations between MSN subtypes, which occur in response to CIE exposure, may underlie the development of excessive ethanol consumption.

However, this interpretation is limited by the fact that Swiss Webster mice do not voluntarily drink significant amounts of ethanol. Therefore, our first work using *Drd1a*-eGFP mice on the Swiss Webster background may not accurately reflect the neuroadaptations that contribute to the escalation of ethanol intake (Jeanes et al., 2014). In the present study, we used *Drd1a*-tdTomato mice on a C57BL/6 background, a strain that has a well-documented ethanol drinking phenotype. We aimed to characterize CIE induced effects on NMDAR function, synaptic plasticity, and intrinsic properties of MSNs in the NAc shell that occur in acute withdrawal, after a single 4 day cycle of intermittent ethanol exposure and thus may be critical for initiating neuroadaptive responses underlying enhanced ethanol intake in this widely adopted model.

2. Methods

2.1. Mice

Drd1a-tdTomato BAC transgenic male mice (MMRRC:030512-UNC) generated on a C57BL/6J background, at least 8 weeks of age, were used for *in vivo* ethanol vapor exposure and *in vitro* electrophysiological experiments. Mice were housed in pairs at 22 °C with a 12:12 light:dark cycle (lights on at 12 AM). Water and chow were available *ad libitum*. All of the following experimental procedures were approved by the University of Texas Institutional Animal Care and Use Committee.

2.2. Chronic intermittent ethanol exposure

Mice were exposed to ethanol vapor using the chronic intermittent ethanol (CIE) exposure model. Ethanol was volatilized by bubbling air through a flask containing 95% ethanol at a rate of 0.2–0.3 L/min. The resulting ethanol vapor was combined with a separate air stream to give a total flow rate of approximately 4 L/min, which was delivered to the mice housed in special mouse chamber units that contain an airtight top, a vapor inlet and an exhaust outlet (Allentown Inc., Allentown, NJ). Mice were exposed to ethanol vapor for 16 h followed by an 8 h withdrawal in which blood ethanol concentrations (BEC) return to zero (data not shown). This treatment is repeated for 4 consecutive days. Mice received intraperitoneal injections of a loading dose of ethanol (20% v/v, 1.5 g/kg) and pyrazole (1 mM, 68.1 mg/kg) in phosphate buffered saline (PBS) prior to vapor exposure to achieve a BEC of 150–200 mg/dl. Air control mice were handled the same but were injected with a solution of only pyrazole in PBS.

2.3. Blood ethanol concentrations

Tail blood samples were collected upon removal from the chambers each day and BECs were measured using a gas chromatograph (Bruker 430-GC) equipped with a flame ionization detector and Combi PAL autosampler (Bruker Corporation, Fremont, CA). Two, 10 µL samples of tail blood were collected and added to 10 mL vials containing 90 µL of saturated sodium chloride solution. Samples were warmed to 65 °C and the solid-phase micro extraction fiber (SPME; 75 µm CAR/PDMS, fused silica; Supelco, Bellefonte, PA) was used to absorb ethanol vapor from the samples. The stationary phase was a capillary column (30 m × 0.53 mm × 1 µm film thickness; Agilent Technologies, Santa Clara, CA) and helium, at a flow rate of 8.5 mL/min was used in the mobile phase. Resultant ethanol

peaks were analyzed using CompassCDS Workstation software (Bruker Corporation, Fremont, CA) and calibration was achieved using external ethanol standards.

2.4. Brain slice preparation

Parasagittal slices (240 μm thick) containing the NAc were prepared, 24 h after the last vapor exposure, using a Leica vibrating microtome (Leica Corp., Bannockburn, IL). Mice were anesthetized by inhalation of isoflurane and the brains were rapidly removed and placed in 4 °C oxygenated artificial cerebrospinal fluid (ACSF) containing the following (in mM): 210 sucrose, 26.2 NaHCO_3 , 1 NaH_2PO_4 , 2.5 KCl, 11 dextrose, bubbled with 95% O_2 /5% CO_2 . Slices were transferred to an ACSF solution for incubation containing the following (in mM): 120 NaCl, 25 NaHCO_3 , 1.23 NaH_2PO_4 , 3.3 KCl, 2.4 MgCl_2 , 1.8 CaCl_2 , 10 dextrose, were continuously bubbled with 95% O_2 /5% CO_2 ; pH 7.4, 32 °C, and were maintained in this solution for at least 60 min prior to recording.

2.5. Patch clamp electrophysiology

Whole cell voltage clamp recordings were made in the nucleus accumbens shell. Cells were identified using the MRK200 Modular Imaging system (Siskiyou Corporation, Grants Pass, OR) mounted on a vibration isolation table. Recordings were made in ACSF containing (in mM): 120 NaCl, 25 NaHCO_3 , 1.23 NaH_2PO_4 , 3.3 KCl, 0.9 MgCl_2 , 2.0 CaCl_2 , and 10 dextrose, bubbled with 95% O_2 /5% CO_2 . ACSF was continuously perfused at a rate of 2.0 mL/min and maintained at a temperature of 32 °C. Picrotoxin (50 μM) was included in the recording ACSF to block GABA_A receptor-mediated synaptic currents. Recording electrodes (thin-wall glass, WPI Instruments, Sarasota, FL) were made using a Brown-Flaming model P-88 electrode puller (Sutter Instruments, San Rafael, CA) to yield resistances between 3 and 6 M Ω . For current clamp experiments, electrodes were filled with (in mM): 135 KMeSO_4 , 12 NaCl, 0.5 EGTA, 10 HEPES, 2 Mg-ATP, 0.3 Tris-GTP, 260–270 mOsm (pH 7.3). For voltage clamp experiments, electrodes were filled with (in mM): 120 CsMeSO_4 , 15 CsCl, 8 NaCl, 10 HEPES, 0.2 EGTA, 10 TEA-Cl, 4 Mg-ATP, 0.3 Na-GTP, 0.1 spermine, and 5 QX-314-Cl. Input resistance and access resistance were monitored throughout the experiments. Cells in which input and access resistance varied more than 20% were not included for analysis.

2.6. Data acquisition and analysis

Glutamatergic afferents were stimulated with a stainless steel bipolar stimulating electrode (FHC, Inc., Bowdoin, ME) placed dorsal and anterior to the recording electrode, about 150–300 μm from the cell body. Excitatory postsynaptic currents (EPSCs) were acquired using an Axopatch 200B amplifier (Axon Instruments, Foster City, CA), filtered at 1 kHz, and digitized at 10–20 kHz with a Digidata 1440A interface board using pClamp 10.2 (Axon Instruments). In experiments for long term plasticity, EPSCs were evoked by local stimulation for at least 10 min at 0.1 Hz, to ensure stable recordings. To induce LTD, conditioning stimuli of 500 pulses at 1 Hz were paired with continuous postsynaptic depolarization at –50 mV. After pairing, EPSCs were monitored for 30 min at a rate of 0.1 Hz. Data were combined in 2 min bins and the magnitude of LTD was calculated by averaging normalized EPSC values from 20 to 30 min after the pairing protocol and comparing that value to the average normalized EPSCs during the 10 min baseline. Data

from each neuron within a treatment group was combined and represented as percent baseline values. Spontaneous EPSCs (sEPSCs) were recorded for 187 s separated into 20 consecutive sweeps. Events greater than 5 pA were included for analysis using Clampfit v10. Paired-pulse ratios were acquired by applying two stimuli of equal intensity, separated by an interstimulus interval (ISI) of 50 ms and calculated as a ratio of EPSC 2/EPSC 1. NMDA/AMPA ratios were calculated as the ratio of the EPSC recorded at +40 mV, 50 ms after afferent stimulation (NMDA) and the peak EPSC recorded at –80 mV (AMPA). NMDAR input-output curves were generated by determining the maximum subthreshold current response. The minimum stimulation intensity was defined as the minimum stimulation needed for a 5–10 pA response. NMDAR currents were recorded at +40 mV and were pharmacologically isolated using 50 μ M picrotoxin, 10 μ M DNQX, and 1 μ M CGP 52432. Current clamp experiments were conducted in normal ACSF (glutamate receptor antagonist were not included). The number of spikes were counted during each fixed current injection. A current-voltage curve was generated and the input resistance was calculated as the slope of the linear regression line. Action potential (AP) parameters were measured using the first AP fired during current injections. Fast afterhyperpolarization (fAHP) was measured at 4 ms after AP threshold. Summary data are presented as mean $\pm$ SEM. Statistical significance from baseline for within each treatment group is defined as $p < 0.05$ using a two-tailed Student's *t* test (assuming equal variance). Group comparisons were made using a two-way ANOVA and Bonferroni post hoc test. Statistical significance was defined as $p < 0.05$. The unit of determination was defined a slice.

3. Results

3.1. Differential modulation of plasticity in D1+ and D1– MSNs after CIE

In our previous work on *Drd1a*-eGFP on the Swiss Webster background we found that the expression of NMDAR-dependent LTD was restricted to D1+ MSNs in the NAc shell (Jeanes et al., 2014). In the present study using *Drd1a*-tdTomato mice on a C57BL/6J background we observed a similar pattern of expression in the air treated control mice where NMDAR-dependent LTD is expressed in D1+ MSNs (Fig. 1A, B) but not in D1– MSN (Fig. 1A, B). In CIE treated mice we observed a reversal in the expression of plasticity between the two MSN subtypes. In D1+ MSNs, 24 h after the last vapor exposure, the induction protocol that resulted in LTD in the air treated mice instead resulted in LTP (Fig. 1D, E). In addition, following CIE exposure we now observed LTD in D1– MSNs (Fig. 1D, E).

3.2. Increase in excitability in D1+ MSNs after CIE

Chronic ethanol exposure has also been shown to modulate the intrinsic properties of MSNs in the NAc (Hopf et al., 2010; Marty and Spigelman, 2012) however it is unknown if ethanol differentially modulates the intrinsic properties of D1 and D2 MSNs. To assess how CIE alters the excitability and intrinsic properties of D1+ and D1– MSNs of the NAc shell we conducted whole cell patch clamp recordings in current clamp mode. In ethanol naïve mice, D1– MSNs fired more action potentials than D1+ MSNs in response to fixed current injections ($p < 0.05$ at 300–400 pA). We found a significant increase in action potential firing in D1+ MSNs of CIE treated mice compared to D1+ MSNs from air control mice (Fig.

2A). There was no change in excitability after ethanol vapor exposure in D1– MSNs (Fig. 2B).

3.3. Effects of CIE on electrophysiological properties of D1+ and D1– MSNs

Changes in the passive membrane properties of MSNs in the NAc shell after CIE are shown in Table 1. We found a 12% decrease in the input resistance in D1– MSNs ($p = 0.051$) with no change in D1+ MSNs after CIE. We observed no change in the resting membrane potential in either D1+ or D1– MSNs after CIE. Further analysis of the action potential parameters revealed a significant increase in the fast after hyperpolarization (fAHP) ($p = 0.04$ versus air) as well as a significant decrease in the action potential half width ($p = 0.02$ versus air) in D1+ MSNs with no significant change in D1– MSNs. Action potential threshold and amplitude remained unchanged after CIE in both D1+ and D1– MSNs.

3.4. CIE induced increase in sEPSC frequency in D1+ MSNs

Changes in the intrinsic properties of MSNs in the NAc shell may not completely account for the reversal in the expression of plasticity since there was no change in excitability in D1– MSNs. To further explore CIE induced changes in excitatory signaling that may contribute to the alterations in plasticity we measured spontaneous EPSCs (sEPSCs) to determine if there was any change in AMPAR signaling. CIE exposure induced a significant increase in sEPSC frequency in D1+ MSNs (Fig. 3C) with no change in amplitude (Fig. 3B). No change in sEPSCs frequency (Fig. 3H) or amplitude (Fig. 3G) was detected in D1– MSNs. A change in frequency can often be detected as an increase in the probability of presynaptic neurotransmitter release. Paired pulse ratio (PPR) is inversely related to the probability of neurotransmitter release. Using the PPR with an interstimulus interval of 50 ms, we did not however detect any significant changes in PPR in either D1+ (Fig. 3E) or D1– MSNs (Fig. 3J).

3.5. Effects of CIE on NMDAR function in D1+ and D1– MSNs

Given that we are investigating a NMDAR-dependent form of plasticity, CIE induced alterations in NMDAR function may be responsible for the changes in the expression of plasticity that we have observed. To measure changes in NMDAR function we used the NMDA/AMPA ratio. We observed a larger NMDA/AMPA ratio in D1– MSNs compared to D1+ MSNs of air control mice (Fig. 4C, D). After CIE, there was a significant increase in NMDA/AMPA ratio in D1+ MSNs (Fig. 4C) and a trend towards a significant decrease in D1– MSNs compared to air controls (Fig. 4D). To further investigate CIE induced alterations in NMDAR function, NMDAR currents were measured at +40 mV and pharmacologically isolated to generate an input-output (I/O) relationship. There was significant increase in the I/O curve in D1+ MSNs (Fig. 4E) while D1– MSNs showed a significant decrease after ethanol vapor exposure (Fig. 4F).

4. Discussion

4.1. Changes in intrinsic measures

In the present study we observed that CIE treatment can alter the excitability and intrinsic properties of MSNs in the NAc shell in a cell type specific manner. In ethanol naïve mice,

D1– MSNs fired more action potentials in response to fixed current injections than D1+ MSNs (Fig. 2) suggesting that D1– MSNs are intrinsically more excitable. This result is similar to what has been previously reported in the dorsal striatum (Cepeda et al., 2008) as well as in the NAc core (Grueter et al., 2010). Exposure to CIE resulted in an increase in excitability in D1+ MSNs with no change in D1– MSNs and we coincidentally observed a significant increase in the fast afterhyperpolarization (fAHP) in D1+ MSNs. Evidence indicates that a variety of ethanol-sensitive K^+ channels may regulate NAc MSN firing. Large conductance voltage and Ca^{2+} -activated potassium (BK) channels are involved in action potential repolarization and are known to be modulated by ethanol (Dopico et al., 2014; Martin, 2010; Mulholland et al., 2009; Treistman and Martin, 2009). Furthermore, it has been previously shown that blocking BK channels in the NAc shell results in an increase in neuronal excitability (Ishikawa et al., 2009). Other voltage dependent channels such as the A-type K^+ channels and small conductance voltage and Ca^{2+} activated K^+ (SK) channels may also be involved in regulating neuronal excitability. In rats that received chronic oral intubation of ethanol, a similar increase in fAHP and decrease in action potential half width was observed due to an increase in the A-type K^+ channel function (Marty and Spigelman, 2012). In addition, withdrawal from ethanol self-administration has been shown to result in a decrease in SK channel function and an increase excitability of MSNs in the NAc core (Hopf et al., 2010).

The increase in excitability in D1+ MSNs after CIE exposure may be the result of homeostatic plasticity (Kourrich et al., 2015). For instance, strong suppression of excitatory transmission with the non-specific antagonist, kynurenic acid, increased excitability in NAc shell MSNs (Ishikawa et al., 2009). Likewise, shell MSNs may be adjusting membrane excitability to compensate for a reduction in excitatory synaptic transmission during ethanol vapor exposure. The increase that we observed in sEPSC frequency in D1+ MSNs of CIE treated mice may also be a compensatory change resulting from dampened excitatory signaling during ethanol vapor exposure. Similar increases in glutamate release have been observed after chronic ethanol exposure (Dahchour and De Witte, 1999; Dahchour et al., 2003; Griffin et al., 2014; Roberto et al., 2004; Rossetti and Carboni, 1995). The increase in sEPSC frequency was not accompanied by a change in PPR. This discrepancy may be due to differences in regulation of spontaneous and evoked neurotransmitter release (Kavalali, 2015; Ramirez and Kavalali, 2011).

4.2. Changes in synaptic measures

The ethanol-induced modulation of NMDAR-dependent plasticity differs between C57BL/6 and Swiss Webster mouse strains. This form of plasticity has been shown to require the internalization of postsynaptic AMPARs (Brebner et al., 2005; Jeanes et al., 2014). The difference in ethanol-induced modulation of this form of AMPA receptor plasticity may indicate a critical difference in ethanol related phenotypes between these strains. Consistent with our previous work, we found that D1+ MSNs express NMDAR-dependent LTD and D1– MSNs show no apparent change in synaptic strength in response to LFS synaptic conditioning in ethanol-naïve mice. However, a significant difference between the two strains is revealed after ethanol vapor exposure. In the Swiss Webster mice, CIE induced a reversal in the expression of plasticity; the induction protocol resulted in LTD in D1– MSNs

but no change in synaptic strength in D1+ MSNs (Jeanes et al., 2014). Here we report a similar result where D1– MSNs express LTD after CIE but D1+ MSNs express LTP. This latter finding is consistent with our previous work using wild-type (WT) C57BL/6 mice following CIE in which we observed a net synaptic potentiation in slices from all CIE-treated mice (Jeanes et al., 2011). Presumably, the net synaptic potentiation we observed in WT C57BL/6 mice following CIE was likely due to the relative degree of potentiation in D1+ MSNs versus a weaker degree of LTD in D1– MSNs. It is tempting to speculate that the appearance of LTP following LFS conditioning in C57BL/6 mice, which markedly respond to CIE by increasing volitional ethanol intake, may indicate that postsynaptic AMPA receptor cycling may constitute an important neuroadaptive response underlying this ethanol phenotype. Likewise, Swiss Webster mice, which show no net synaptic change in D1+ MSNs following CIE, do not volitionally drink significant amounts ethanol even under baseline conditions. Therefore, strain-dependent differences in AMPAR plasticity may even underlie baseline differences in ethanol intake.

Ethanol is a significant modulator of NMDAR function. Our lab has shown that acute ethanol exposure can inhibit NMDARs in the NAc (Maldve et al., 2002; Zhang et al., 2005, 2006). Other groups have shown that withdrawal from chronic ethanol exposure results in the upregulation of NMDAR function (Buck and Harris, 1991; Dodd et al., 2000; Hendricson et al., 2007; Siggins et al., 2003; Stuber et al., 2010). However, whether CIE can induce an upregulation of NMDAR function was unknown in shell NAc MSNs. In D1+ MSNs, we observed an increase in NMDAR function after CIE. This net increase in D1+ NMDAR function could also underlie the transition to synaptic potentiation due to LFS conditioning. Interestingly, we observed a decrease in NMDAR function in D1– MSNs after CIE which likewise may contribute to the expression of LTD by allowing for the expression of other forms of plasticity. In the NAc core, a form of endocannabinoid (eCB) dependent LTD was described that involved the activation of the vanilloid receptor, TRPV1, and was expressed only in D2 MSNs (Grueter et al., 2010). We have previously reported that TRPV1 dependent LTD can also be expressed in the NAc shell and requires increased eCB signaling (Renteria et al., 2014). Ethanol is a modulator of eCB signaling (Pava and Woodward, 2012) and acute withdrawal from CIE treatment may result in an upregulation of eCB signaling, allowing for LFS conditioning to result in eCB dependent LTD in D1– MSNs. Further studies need to be conducted to determine the mechanisms involved in the CIE induced alterations of plasticity in D1+ and D1– MSNs of the NAc shell.

The CIE protocol used in this study has documented effects on increasing volitional ethanol intake by other labs as well as ourselves, with each subsequent cycle of vapor exposure leading to more robust increases in ethanol drinking (Becker and Lopez, 2004; Griffin et al., 2009a, 2009b; Griffin, 2014; Lopez and Becker, 2005; Jeanes et al., 2011). In this study, mice are limited to one 4 day cycle of ethanol vapor exposure, therefore the changes in synaptic signaling and plasticity that we observed may reflect the initial neuroadaptations in NAc synaptic function that may later contribute to increased ethanol drinking. These complex changes in NMDAR function in D1+ and D1– MSNs respectively would be expected to markedly change in NAc output and thereby may alter volitional ethanol intake. Unfortunately we do not know the time course of the CIE induced changes in NMDAR function in relation to ethanol drinking behavior. Previous work from our lab has shown that

the differential disruption in plasticity in D1+ and D1– MSNs persist for up to one week after ethanol vapor exposure (Jeanes et al., 2014). Therefore, it is feasible that these observed changes in NMDAR function which may contribute to changes in the expression of plasticity can also contribute to increased ethanol drinking. Differential activity of D1 and D2 MSNs has been demonstrated to be important for the formation (Kai et al., 2015) and expression (Hikida et al., 2010) of psychostimulant behavioral sensitization. Specifically, NMDARs expressed in D1 MSNs are necessary for amphetamine sensitization (Beutler et al., 2011). It remains unclear how differential activity of D1+ and D1– MSNs contribute to ethanol related behaviors. Activity of D1 receptors are necessary for the acquisition of ethanol conditioned place preference (CPP) (Young et al., 2014) and it has been shown that NMDAR activation in the NAc is necessary for the expression of ethanol CPP (Gremel and Cunningham, 2009) but the distinct contribution of NMDAR function in D1 and D2 MSNs for the expression of ethanol CPP or ethanol drinking is unknown. Much work remains to be done to determine how differential activity of D1 and D2 MSNs modulate ethanol related behaviors.

5. Conclusions

The work in this study further supports the importance of understanding how NMDAR-dependent plasticity in the NAc can be altered by chronic drug or alcohol exposure. Although there may be unique neuroadaptations that are encoded with chronic drug or alcohol exposure there seems to be an interaction with the mechanisms of induction and/or expression of NMDAR-dependent plasticity in the NAc. Altered plasticity in the NAc may provide valuable information in detecting more specific changes in excitatory signaling and possibly identifying targets for the development of novel pharmacotherapies to treat addiction.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.neuropharm.2016.03.004>.

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Fig. 1.

CIE induced alterations in the expression of plasticity in D1+ and D1- MSNs. (A.) The pairing protocol which consisted of 1 Hz stimulation paired with depolarization to -50 mV resulted in LTD in D1+ MSNs but not in D1- MSNs of air control mice. (B.) Bar graphs representing the percentage change $\pm$ S.E.M. for average EPSC amplitude between baseline (min 0–10) and post-pairing (min 40–50) in D1+ MSNs (47.54 ± 11.14 , $n = 6$, 5 animals, $* = p < 0.05$ versus baseline) and D1- MSNs (99.76 ± 7.38 , $n = 5$, 4 animals) of air control mice. (C.) Sample of average EPSCs during baseline and post-pairing (40–50 min) of D1+ and D1- MSNs of air treated mice. (D.) Twenty four hours after ethanol vapor exposure, the pairing protocol resulted in LTP in D1+ MSNs and LTD in D1- MSNs. (E.) Bar graphs representing the percentage change $\pm$ S.E.M. for average EPSC amplitude between baseline (min 0–10) and post-pairing (min 40–50) 24 h after CIE in D1+ MSNs (130 ± 8.75 , $n = 6$, 5 animals, $* = p < 0.05$ versus baseline) and D1- MSNs (55.35 ± 13.95 , $n = 4$, 3 animals, $** = p < 0.01$ versus baseline). (F.) Sample of average EPSCs during baseline and post-pairing (40–50 min) of D1+ and D1- MSNs of CIE treated mice. Scale bars represent 50 pA (vertical) and 10 ms (horizontal).

Fig. 2.

CIE induced increase in excitability in D1+ MSNs. (A.) D1+ MSNs exhibit increased firing in response to depolarizing current injections after CIE exposure (Air, $n = 10$, 7 animals; CIE, $n = 14$, 8 animals, $* = p < 0.05$ versus air control). (B.) Excitability of D1- MSNs was unaffected by CIE treatment (Air, $n = 7$, 6 animals; CIE, $n = 13$, 7 animals). Data shown as mean $\pm$ SEM. D1+ Air vs. D1- Air ($p < 0.01$ at 300 pA and 350 pA, $p < 0.05$ at 400 pA).

Fig. 3.

Increase in sEPSC frequency in D1+ MSNs after CIE. (A.) Sample sEPSC traces of D1+ MSNs from air control and CIE treated groups. (B.) No change in sEPSC amplitude in D1+ MSNs after CIE treatment (Air, 12.7 ± 1.09 , n = 12, 8 animals; CIE, 14.24 ± 0.78 , n = 29, 14 animals). (C.) A significant increase in frequency was observed in CIE treated mice (5.20 ± 0.59 , n = 29, 14 animals) compared to air controls (3.66 ± 0.75 , n = 12, 8 animals * = $p < 0.05$ versus air controls). (D.) Sample traces of PPR of D1+ MSNs from air and CIE treated mice. (E.) There was no change in the PPR in D1+ after CIE treatment (Air, 1.68 ± 0.23 , n = 6, 4 animals; CIE, 1.56 ± 0.13 , n = 14, 8 animals). (F) Sample sEPSC traces of air control and CIE treated groups in D1- MSNs. (G.) No change in amplitude in D1- MSNs after CIE treatment (14.9 ± 1.38 , n = 16, 12 animals) compared to air controls (13.63 ± 1.20 , n = 11, 7 animals). (H.) No change in frequency in D1- MSNs after CIE treatment (3.79 ± 1.20 , n = 16, 12 animals) compared to air controls (3.67 ± 0.59 , n = 11, 7 animals). (I.) Sample traces of PPR of D1- MSNs from air and EtOH treated mice. (J.) There was no change in the PPR in D1- after CIE treatment (Air, 1.41 ± 0.26 , n = 4, 4 animals; CIE, 1.65 ± 0.19 , n = 8, 6 animals). Scale bars represent 50 pA (vertical) and 25 ms (horizontal).

Fig. 4.

Cell type specific reversal of NMDAR function after CIE. (A. & B.) Sample current traces of NMDA (top trace, +40 mV, 50 ms after stimulus) and AMPA (bottom trace, -80 mV) from D1+ MSNs of both air and CIE treated mice. (C.) Significant increase in NMDA/AMPA ratio in D1+ MSNs after CIE (Air, 0.22 ± 0.05 , $n = 6$, 6 animals; EtOH, 0.33 ± 0.02 , $n = 10$, 7 animals). * = $p < 0.05$ versus air control. (D.) A decrease in NMDA/AMPA ratio in D1- MSNs after CIE but was not significant (Air, 0.39 ± 0.08 , $n = 5$, 4 animals; EtOH, 0.23 ± 0.05 , $n = 8$, 5 animals; $p = 0.09$). (E.) D1+ MSNs showed a significant increase in the I/O curve after CIE compared to air controls (Two-way ANOVA, stimulation intensity, $F_{(5,70)} = 11.44$, $p < 0.0001$; * = $p < 0.05$ versus air control). (F.) D1- MSNs showed a significant decrease in the I/O curve after CIE (Two-way ANOVA, stimulation intensity, $F_{(5,50)} = 14.22$, $p < 0.0001$; * = $p < 0.05$ versus air control). Scale bars represent 50 pA (vertical) and 10 ms (horizontal).

Table 1

CIE induced alterations in electrophysiological properties in D1+ MSNs.

	D1 + MSNs		D1 – MSNs	
	Air, n = 11	CIE, n = 11	Air, n = 8	CIE, n = 11
Input resistance (MU)	117.79 ± 3.78	119.21 ± 6.88	139.72 ± 4.97	123.16 ± 5.87
RMP (mV)	-74.54 ± 0.78	-74.89 ± 0.47	-72.58 ± 1.02	-73.55 ± 0.85
fAHP (mV)	-17.6 ± 0.96	-20.55 ± 0.99 *	-16.71 ± 1.47	-19.34 ± 1.35
AP half width (ms)	1.18 ± 0.04	1.07 ± 0.03 *	1.17 ± 0.06	1.1 ± 0.05
AP amplitude (mV)	58.24 ± 1.5	61.16 ± 1.48	55.46 ± 1.00	58.73 ± 2.05
Threshold (mV)	-22.98 ± 1.13	-20.71 ± 4.62	-28.3 ± 0.78	-26.59 ± 1.07

Values are shown as the mean ± SEM.

*
= p < 0.05 versus air control.