Ethanol Disinhibits Dorsolateral Striatal Medium Spiny Neurons Through Activation of A Presynaptic Delta Opioid Receptor

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The dorsolateral striatum mediates habit formation, which is expedited by exposure to alcohol. Across species, alcohol exposure disinhibits the DLS by dampening GABAergic transmission onto this structure's principal medium spiny projection neurons (MSNs), providing a potential mechanistic basis for habitual alcohol drinking. However, the molecular and circuit components underlying this disinhibition remain unknown. To examine this, we used a combination of whole-cell patch-clamp recordings and optogenetics to demonstrate that ethanol potently depresses both MSN- and fast-spiking interneuron (FSI)-MSN GABAergic synaptic transmission in the DLS. Concentrating on the powerfully inhibitory FSI-MSN synapse, we further show that acute exposure of ethanol (50 mM) to striatal slices activates delta opioid receptors that reside on FSI axon terminals and negatively couple to adenylyl cyclase to induce a long-term depression of GABA release onto both direct and indirect pathway MSNs. These findings elucidate a mechanism through which ethanol may globally disinhibit the DLS. *Neuropsychopharmacology* (2016) **41**, 1831–1840; doi:10.1038/npp.2015.353; published online 13 January 2016

INTRODUCTION

The dorsolateral subregion of the striatum (DLS) mediates habits, such as ethanol (EtOH) consumption in late-stage alcoholism, while the dorsomedial subregion (DMS) mediates goal-directed action strategies (Balleine *et al*, 2009). Animals with DMS lesions only express habit response strategies, while those with lesions to the DLS exclusively use a goal-directed action strategy (Yin *et al*, 2004, 2005). Thus, the relative output of one striatal subregion over the other dictates the expression of its cognate action strategy. Animals previously exposed to EtOH respond habitually for EtOH at earlier time points than non-addictive substances such as food pellets or sucrose solution (Dickinson *et al*, 2002; Corbit *et al*, 2012). In addition, EtOH-drinking rats form habits for sucrose solution more rapidly than EtOH-naïve animals (Corbit *et al*, 2012). A mechanistic understanding of how EtOH may interact with the DLS to achieve this is lacking.

The effects of acute and chronic EtOH exposure on dorsal striatal function provide clues as to how this drug mediates a possible global activation of the DLS. Blomeley *et al* (2011), as well as Wilcox *et al* (2014) demonstrated that acute EtOH

exposure to striatal slices depresses inhibitory synaptic transmission onto principal medium spiny neurons (MSNs) of the DLS. This acute EtOH effect may persist in chronic drinking models as chronic EtOH consumption in rodents and non-human primates depresses GABA release onto MSNs of the DLS/putamen (Cuzon Carlson *et al*, 2011; Wilcox *et al*, 2014). Despite the mounting evidence of EtOH-induced disinhibition of DLS MSNs, the microcircuit and molecular determinants of EtOH-induced MSN disinhibition remain unknown.

MSNs receive a majority of inhibitory inputs from neighboring MSNs and parvalbumin (PV)-containing fast-spiking interneurons (FSIs; Koos *et al*, 2004; Tepper *et al*, 2004). MSNs synapse upon the distal dendrites of other MSNs while FSIs form multiple synapses upon MSNs perisomatically to powerfully regulate MSN output (Koos *et al*, 2004). The FSI population increases in density laterally through the dorsal striatum (Schlösser *et al*, 1999; Luk and Sadikot, 2001), positioning these cells to influence DLS output to a greater extent than DMS output. FSIs and other striatal cell types, like MSNs, express a host of presynaptically localized $G_{i/o}$ -coupled receptors such as the cannabinoid type 1 receptor (CB1) and 5-HT_{1b}, among others (Mathur *et al*, 2011, 2013; Atwood *et al*, 2014a, b). When activated, these receptors dampen neurotransmitter release. As such, EtOH may be acting through one of these receptors to dampen GABA release onto MSNs. To examine this possible mechanism of action of EtOH on the depression of inhibitory synapses on DLS MSNs, we use whole-cell

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Received 9 September 2015; revised 14 November 2015; accepted 1 December 2015; accepted article preview online 11 December 2015

patch-clamp electrophysiology and optogenetics to isolate potential EtOH effects at either MSN-MSN or FSI-MSN synapses. We show that EtOH induces LTD at MSN and FSI inputs onto MSNs, and that the powerfully inhibitory FSI-MSN synapse is depressed through activation of a presynaptic delta opioid receptor (DOR). Further, we identify adenylyl cyclase/cAMP as the signaling pathway responsible for this novel form of EtOH-induced synaptic plasticity.

METHODS

All experiments were performed in accordance with the United States Public Health Service Guide for Care and Use of Laboratory and were approved by the Institutional Animal Care and Use Committee at the University of Maryland, Baltimore and the National Institute on Alcohol Abuse and Alcoholism. Mice were housed with littermates (2–5 per cage) under a 12-h light/dark cycle (lights on at 0700 hours, off at 1900 hours) with *ad libitum* access to food and water.

Viral-Mediated Channelrhodopsin (ChR2) Expression

To virally express ChR2 selectively in PV-containing FSIs or MSNs in the DLS, >2-month old *Pvalb-cre* or *RGS9-cre* (C57BL/6 background) transgenic mice, respectively, were stereotactically injected with an AAV vector under an EF1a promoter containing a DIO-ChR2-mCherry or eYFP construct flanked by loxP sites (AAV-flox-DIO-ChR2-mCherry/eYFP) at a volume of 400 nl and a rate of 20 nl/min into the DLS (AP +0.6 mm, ML $\pm$ 2.25 mm, DV – 2.4 mm from bregma), following our previously described methods (Mathur *et al*, 2013). To target FSIs for ChR2 expression and record from direct (MSN_D) or putative indirect (MSN_{ID}) MSNs, *Pvalb-cre* $\times$ *Drd1a-tdTomato* mice were injected with the same viral construct. Animals recovered for a minimum of 3 weeks before brains were sliced for electrophysiology experiments.

Brain Slice Preparation

At least 3 weeks post surgery, animals were anesthetized with isoflurane and brains were dissected out and submerged in 95% oxygen, 5% carbon dioxide (carbogen)-bubbled ice cold cutting solution (194 mM sucrose, 30 mM NaCl, 4.5 mM KCl, 1 mM MgCl₂, 26 mM NaHCO₃, 1.2 mM NaH₂PO₄, and 10 mM D-glucose). Brains were sliced coronally at 250 μ m with a vibratome (Leica VT 1200) and stored in carbogen-bubbled artificial cerebrospinal fluid (aCSF; 124 mM NaCl, 4.5 mM KCl, 2 mM CaCl₂, 1 mM MgCl₂, 26 mM NaHCO₃, 1.2 mM NaH₂PO₄, and 10 mM D-glucose). Sections were incubated at 32.4 °C for 30 min before they were removed and stored at room temperature until electrophysiological recordings were performed. Before recording, slices were hemisected and were constantly perfused with carbogen-bubbled aCSF (29–31 °C) via a gravity perfusion system throughout the experiment.

Whole-Cell Voltage-Clamp Recordings

Injection sites of ChR2-mCherry/eYFP into the DLS were visualized through the epifluorescent light path using a mercury bulb lamp (X-Cite series 120Q). Whole-cell

recordings were made from DLS MSNs receiving inputs from ChR2 infected cells as described previously (Mathur *et al*, 2013). Optically evoked IPSCs (oIPSC) were recorded using borosilicate glass pipettes with resistances in the 2–5 M Ω range. Pipettes were pulled on a Flaming Brown micropipette puller (Sutter Instruments) and filled with a CsCl-based internal solution (150 mM CsCl, 10 mM HEPES, 2 mM MgCl₂, 0.3 mM Na-GTP, 5 mM QX-314, 3 mM Mg-ATP, and 0.2 mM BAPTA) with osmolarity ranging from 305 to 310 mOsm at a pH of 7.3. oIPSCs were evoked with blue light (473 nm) using epifluorescent LED field illumination (Lumen Dynamics, XLED1). Blue light-evoked test pulses were delivered every 20 s with a pulse duration of 2–4 ms. Blue light intensity was adjusted to record oIPSCs with an average amplitude of 600 pA. Ultraviolet light (370 nm, pulse width of 4 ms) was used to photo-uncage bath-applied caged GABA (CNB-GABA, 50 μ M) to measure uncaged IPSCs (uIPSC). Electrically evoked IPSCs (eIPSC) were induced using a concentric bipolar stimulating electrode (World Precision Instruments) located locally in the DLS approximately 100 μ m from the recorded neuron. eIPSCs were recorded with AP5 (50 μ M) and NBQX (5 μ M) present in the bath. MSNs were voltage clamped at –60 mV using a MultiClamp 700B amplifier (Molecular Devices) and recordings were filtered at 2 kHz and digitized at 10 kHz. Clampex 10.4.1.4 software (Molecular Devices) was used for data acquisition. Cells were discarded from analysis if series resistance changed by more than 15% throughout the course of the experiment.

Statistical Analysis

Mean IPSC amplitudes were measured in Clampfit 10.4.1.4 and analyzed using GraphPad Prism 6.01. IPSC amplitudes were averaged per minute and expressed as a percent change from baseline measurements. Average baseline IPSC amplitudes and amplitudes in the final 5 min of recording were compared using a two-tailed paired *t* test. Changes in IPSC amplitudes between experiments were analyzed using a two-tailed unpaired *t* test.

Drugs

EtOH, SrCl₂ and GDP- β -S were purchased from Sigma-Aldrich. Cell-impermeant BAPTA tetracesium salt (molecular weight of 1004.03) and CNB-GABA were purchased from Invitrogen. All other drugs were purchased from Tocris Bioscience.

RESULTS

EtOH Depresses MSN- and FSI-MSN Synapses

The effect of EtOH on MSN- and FSI-MSN synaptic transmission was tested by measuring oIPSC amplitudes during and after 10 min acute exposure to 50 mM EtOH, a concentration of EtOH that correlates to a blood alcohol concentration (BAC) of 230 mg/dl in humans. This BAC is consistent with what late-stage alcoholics achieve (Brick and Erickson, 2009). EtOH depressed oIPSC amplitude of MSN inputs onto MSNs (MSN-MSN) to $75.69 \pm 7.03\%$ of baseline ($t = 4.06$, $df = 4$, $p = 0.02$, Figure 1a) in 5 out of 5 cells

recorded. FSI inputs onto MSNs were depressed to $70.79 \pm 5.733\%$ of baseline following EtOH application ($t = 5.1$, $df = 18$, $p < 0.001$, Figure 1b). EtOH-induced depression at this synapse was not due to decreased sensitivity of Chr2 to blue light during the 30 min experiment, as the amplitude of FSI-MSN oIPSCs was stable across 30 min of recording in the absence of EtOH (Figure 1b filled circles; $91.75 \pm 9.76\%$ of baseline, $t = 0.88$, $df = 9$, $p = 0.42$). Further, EtOH-induced depression of the FSI-MSN synapse was significantly different from the no drug control ($t = 2.9$, $df = 27$, $p = 0.01$). Unlike the MSN-MSN synapse, EtOH depressed FSI-MSN transmission in 14 out of the 19 cells recorded. EtOH-induced depression of both MSN- and FSI-MSN synapses persisted even 15 min following the end of EtOH application. Thus, we call this depression EtOH-LTD. Importantly, neither MSN-MSN nor FSI-MSN EtOH-LTD was due to a change in postsynaptic neuron series resistance (Figure 1c and d). We next determined if a range of EtOH concentrations that are achievable *in vivo* are capable of producing EtOH-LTD at the FSI-MSN synapse. Both 10 and 80 mM EtOH (the corresponding BAC values of which are 46 and 368 mg/dl, respectively) depressed the FSI-MSN synapse (Figure 1e). Similar to 50 mM, 10 mM EtOH depressed FSI transmission onto MSNs to $73.72 \pm 10.96\%$ of baseline ($t = 2.4$, $df = 8$, $p = 0.04$), while 80 mM EtOH depressed FSI-MSN transmission to $79.93 \pm 7.20\%$ of baseline ($t = 2.79$, $df = 8$, $p = 0.02$). To determine if the heterogeneity of the EtOH response at the FSI-MSN synapse is due to direct pathway MSN (MSN_D) or indirect pathway MSN (MSN_{ID}) DLS output specificity, we crossed *Pvalb-cre* mice with bacterial artificial chromosome-transgenic mice expressing tdTomato under the D₁-dopamine receptor promoter. We recorded the effect of EtOH at FSI- D₁-containing (tdTomato positive) MSNs_D or FSI-putative D₂-expressing MSNs_{ID} (tdTomato negative). EtOH depressed FSI transmission onto both MSNs_D and MSNs_{ID}; MSN_D oIPSC amplitude decreased to $79.83 \pm 8.76\%$ of baseline, $t = 2.30$, $df = 12$, $p = 0.04$; MSN_{ID} oIPSC amplitude was $74.74 \pm 6.69\%$ of baseline, $t = 3.78$, $df = 13$, $p = 0.002$ (Figure 1f), indicating EtOH-LTD is not pathway specific. The variation in the timing of onset of EtOH-LTD that occurs between FSI-MSN_D and FSI-MSN_{ID} could occur due to the percentage of cells in each group that respond to the EtOH application. Alternatively, this difference may be a function of the *Pvalb-cre* $\times$ *Drd1a-tdTomato* genotype. Because FSIs are the sole source of perisomatic synapses on MSNs (Koos *et al*, 2004; Tepper *et al*, 2004), they provide the most powerful inhibitory influence of all sources of GABA onto MSNs and, thus, are positioned to play a large role in EtOH effects on the disinhibition of the DLS. As such, we henceforth concentrated on elucidating the mechanism of EtOH-LTD at the FSI-MSN synapse.

FSI-MSN EtOH-LTD is Presynaptically Expressed

LTD of inhibitory synapses onto MSNs that is induced at a hyperpolarized or 'downstate' membrane voltage of -80 mV (iLTD) is present at FSI-MSN synapses and occurs through activation of presynaptically localized CB1 (Mathur *et al*, 2013). To test if EtOH-LTD at the FSI-MSN synapse is presynaptically expressed and, therefore, mutually occlusive with eCB-LTD, low frequency electrical stimulation (LFS, 1 Hz, 80 pulses) was locally delivered while voltage

clamping MSNs at -80 mV in the presence or absence of EtOH. LFS depressed the FSI-MSN synapse, consistent with our previous findings (Mathur *et al*, 2013; oIPSC amplitude $79.17 \pm 6.94\%$ of baseline, $t = 3.00$, $df = 5$, $p = 0.03$). Application of 50 mM EtOH following iLTD induction did not further depress the synapse (iLTD amplitude = $79.17 \pm 6.94\%$ of baseline compared with EtOH-LTD amplitude = $80.38 \pm 9.49\%$ of baseline, $t = 0.42$, $df = 5$, $p = 0.69$, Figure 2a). Additionally, induction of iLTD following the generation of EtOH-LTD did not further depress the synapse (EtOH-LTD amplitude = $89.02 \pm 5.73\%$ of baseline compared with iLTD amplitude = $83.70 \pm 6.82\%$, $t = 1.08$, $df = 5$, $p = 0.33$, Figure 2b).

To further assess whether FSI-MSN EtOH-LTD is expressed pre- or postsynaptically, we replaced CaCl₂ in the aCSF with 2 mM SrCl₂ to enable circuit-specific-asynchronous transmitter release. Changes in optically elicited Sr²⁺-enabled asynchronous IPSC (osIPSC) event frequency and amplitude were measured in the presence of EtOH (Choi and Lovinger, 1997). EtOH increased inter-event interval (IEI; 149.7 ± 13.06 ms in EtOH compared with control: 110.2 ± 7.47 ms, $t = 4.11$, $df = 8$, $p = 0.003$) but had no effect on event amplitude (EtOH: 23.93 ± 6.0 pA compared with control: 27.27 ± 7.7 pA, $t = 1.26$, $df = 7$, $p = 0.25$, Figure 2c), further suggesting EtOH-LTD is expressed presynaptically. We next tested if EtOH-LTD is caused by postsynaptic Ca²⁺-mediated processes that could result in either GABA_A endocytosis or the production of a retrograde transmitter (Della Rocca *et al*, 1999; Gerdeman *et al*, 2002) by loading the postsynaptic cell with cell-impermeable BAPTA (20 mM). BAPTA loading did not block EtOH-LTD ($83.16 \pm 6.3\%$ of baseline, $t = 2.67$, $df = 12$, $p = 0.02$, Figure 2d). To isolate potential changes on the postsynaptic side, we elicited IPSCs by photo-uncaging the caged-GABA compound CNB-GABA (50 μ M). Photo- uIPSC amplitudes during EtOH application did not depress in the presence of EtOH (uIPSC amplitude = $103.6 \pm 3.91\%$, $t = 0.93$, $df = 5$, $p = 0.40$), unlike eIPSC amplitudes in the same cell (eIPSC amplitude = $70.47 \pm 6.21\%$, $t = 4.76$, $df = 5$, $p = 0.01$, Figure 2e), further suggesting EtOH-LTD is presynaptically expressed. Electrically evoked IPSC amplitude depression following EtOH application was significantly different from uIPSC amplitude (uIPSC amplitude $103.6 \pm 3.91\%$ compared with eIPSC amplitude $70.47 \pm 6.21\%$, $t = 4.52$, $df = 10$, $p = 0.001$). Finally, to test if contributions from postsynaptic G-protein-coupled receptors underlie FSI-MSN EtOH-LTD, the G-protein receptor inhibitor GDP- β -S (500 μ M) was included in the internal pipette solution. EtOH application in the presence of GDP- β -S depressed the synapse (oIPSC amplitude = $71.20 \pm 6.67\%$ of baseline, $t = 4.32$, $df = 5$, $p = 0.01$, Figure 2f), indicating that contributions of postsynaptic G-protein-coupled receptors are not necessary for EtOH-LTD.

FSI-MSN EtOH-LTD is DOR Dependent

Our data show that EtOH-LTD is mutually occlusive with iLTD in the DLS, which is dependent on activation of the presynaptically localized, G_{i/o}-coupled CB1 (Mathur *et al*, 2013). To investigate if EtOH-LTD acts through this same CB1-dependent pathway, we pre-incubated slices in the CB1 receptor antagonist AM251 (5 μ M) for at least 35 min before exposing EtOH to the slice. EtOH application in the presence

Figure 1 Ethanol (EtOH) depresses medium spiny neuron (MSN) and fast-spiking interneuron (FSI) transmission onto MSNs. (a) EtOH application (10 min) depresses MSN–MSN optically evoked inhibitory postsynaptic current (olPSC) amplitude ($n = 5$ cells, $p < 0.05$). (b) EtOH depresses FSI inputs onto MSNs (open circles, $n = 19$ cells, $p < 0.05$). Changes in FSI–MSN strength are not owing to channelrhodopsin rundown, as a no drug control does not change synaptic strength (filled circles, $n = 10$, $p > 0.05$). (c) Top, raw data demonstrating that EtOH-induced depression of the MSN–MSN synapse is not due to changes in series resistance (R_s , bottom). (d) Top, raw data demonstrating that EtOH-induced depression of the FSI–MSN synapse is not caused by a change in R_s , bottom. (e) 10 mM (open circles) and 80 mM (filled circles) EtOH depress FSI–MSN synaptic strength (10 mM: $n = 9$, $p < 0.05$; 80 mM: $n = 9$, $p < 0.05$). (f) EtOH depresses FSI-direct pathway MSN (MSN_D) synapses (filled circles, $n = 13$ cells, $p < 0.05$) and FSI-indirect pathway MSN (MSN_{ID}) synapses (open circles, $n = 14$ cells, $p < 0.05$). All scale bars represent 300 pA (vertical) and 100 ms (horizontal). All example traces depict baseline (dark) and last 5 min of the experiment (light). Schematics represent optogenetic activation of MSNs or FSIs and whole-cell patch-clamp recordings from MSNs. All error bars indicate SEM.

Figure 2 FSI-MSN EtOH-induced long-term depression (EtOH-LTD) is presynaptically expressed. (a) EtOH-LTD is mutually occlusive with endocannabinoid-LTD. Delivering electrical low frequency stimulation while voltage clamping MSNs at -80 mV (LFS, arrow) results in a depression of the FSI-MSN synapse that is not further depressed by EtOH (last 15 min, $n = 6$ cells, $p > 0.05$). (b) EtOH application depresses FSI-MSN synapses and is not further depressed by electrical LFS (arrow, $n = 6$ cells, $p > 0.05$). (c) Left panel, optically evoked, Sr^{2+} enabled IPSC (oIPSC) inter-event interval (IEI) increases after EtOH application (filled circles) compared with baseline (open circles, $n = 9$ cells, $**p < 0.01$). oIPSC amplitude does not change following EtOH application (filled circles) compared with baseline amplitudes (open circles, $n = 8$ cells, $p < 0.05$). Right panel, representative traces of oIPSC events during baseline (open circle) and EtOH application (filled circle). Black circles indicate oIPSC events. (d) Including 20 mM BAPTA in the internal pipette solution does not eliminate FSI-MSN EtOH-LTD ($n = 13$ cells, $p < 0.05$). (e) EtOH depresses electrically evoked IPSCs (eIPSC, open circles, $n = 5$ cells, $p < 0.05$) but does not depress CNB-GABA photo-uncaged IPSCs (uIPSC, filled circles, $n = 6$ cells, $p > 0.05$). (f) Inclusion of GDP- β -S into the internal pipette solution does not eliminate EtOH-LTD ($n = 6$ cells, $p < 0.05$). Scale bars represent 300 pA (vertical) and 100 ms (horizontal). Example traces depict baseline (dark) and the last 5 min of the experiment (light). All error bars represent SEM.

of AM251 depressed both MSN and FSI transmission onto MSNs (MSN-MSN: oIPSC amplitude = $71.31 \pm 7.31\%$ of baseline, $t = 3.81$, $df = 4$, $p = 0.02$; FSI-MSN: oIPSC amplitude = $66.90 \pm 7.46\%$ of baseline, $t = 4.44$, $df = 3$, $p = 0.021$), indicating EtOH-LTD is CB1 independent.

Like CB1-dependent LTD, several other forms of LTD are known to depend on activation of a presynaptically localized

$G_{i/o}$ -coupled receptor (Atwood *et al*, 2014b). Given the known interactions between EtOH and the opioid system (Volpicelli *et al*, 1992; Drews and Zimmer, 2010), and that activation of these $G_{i/o}$ -coupled opioid receptors is capable of inducing striatal presynaptic LTD (Atwood *et al*, 2014a), we next assessed whether EtOH-LTD occurs in an opioid receptor-dependent fashion. MSN-MSN EtOH-LTD was not

eliminated in the presence of naloxone, a pan opioid receptor antagonist (oIPSC amplitude = $80.03 \pm 6.94\%$ of baseline, $t = 2.88$, $df = 6$, $p = 0.03$, Figure 3a). However, FSI-MSN EtOH-LTD was eliminated in the presence of naloxone ($5 \mu\text{M}$, oIPSC amplitude = $97.87 \pm 2.99\%$, $t = 0.71$, $df = 6$, $p = 0.50$, Figure 3b) and was significantly different from control EtOH-induced depression of oIPSC amplitude (EtOH-LTD oIPSC amplitude = $67.51 \pm 7.78\%$ compared with naloxone oIPSC amplitude $97.87 \pm 2.99\%$, $t = 3.28$, $df = 14$, $p = 0.01$), indicating EtOH-LTD at the MSN- and FSI-MSN synapses are mechanistically distinct.

To determine the specific opioid receptor involved in FSI-MSN EtOH-LTD, we then incubated slices in the DOR-specific antagonist naltrindole ($1 \mu\text{M}$) before applying EtOH. Naltrindole blocked EtOH-LTD at the FSI-MSN synapse (oIPSC amplitude = $97.65 \pm 7.32\%$ of baseline, $t = 0.32$, $df = 7$, $p = 0.76$, Figure 3c), and the EtOH effect in this condition differed from control EtOH-LTD (control EtOH-LTD oIPSC amplitude $66.67 \pm 6.54\%$, $t = 3.16$, $df = 14$, $p = 0.01$). To determine if this form of LTD is labile or static (Atwood *et al*, 2014b), we applied naltrindole ($1 \mu\text{M}$) to slices 15 min after EtOH washout (Figure 3d). EtOH-LTD did not return to baseline in the presence of naltrindole, suggesting this form of LTD is static (oIPSC following EtOH application = $75.95 \pm 3.07\%$ of baseline, $t = 6.23$, $df = 5$, $p = 0.02$; oIPSC in naltrindole = $63.58 \pm 9.91\%$ of baseline, $t = 3.58$, $df = 5$, $p = 0.02$). Because EtOH-LTD appears to be presynaptically expressed, and DOR, like CB1, may reside presynaptically, we hypothesized that DOR resides on the FSI terminal. Supporting this idea, application of the DOR agonist DPDPE (500 nM) depressed the FSI-MSN synapse (oIPSC amplitude = $81.98 \pm 3.49\%$ of baseline, $t = 5.16$, $df = 11$, $p = 0.0003$) but the μ opioid receptor agonist, DAMGO ($2 \mu\text{M}$) did not (oIPSC amplitude = $99.50 \pm 7.50\%$ of baseline, $t = 0.12$, $df = 5$, $p = 0.91$, Figure 3e). Using *PV-cre* $\times$ *Drd1a-tdTomato* mice we determined that DPDPE-induced LTD of oIPSC amplitude at the FSI-MSN synapse is not pathway specific; DPDPE depressed the FSI-MSN_D synapse to $66.46 \pm 8.36\%$ of baseline ($t = 4.01$, $df = 8$, $p = 0.004$) and depressed the FSI-MSN_{ID} synapse to $79.19 \pm 5.45\%$ of baseline ($t = 3.82$, $df = 6$, $p = 0.01$, Figure 3f). These data suggest that DOR is present on FSI terminals impinging upon both MSN_D and MSN_{ID}.

DOR-Mediated Depression of the FSI-MSN Synapse is Adenylyl Cyclase Dependent

DOR is a $G_{i/o}$ -coupled receptor that is known to decrease cAMP production when activated (Al-Hasani and Bruchas, 2011). Therefore, we tested the prediction that activating

adenylyl cyclase, the enzyme responsible for catalyzing the conversion of ATP to cAMP, will block DOR-induced LTD. Pre-incubating slices in $15 \mu\text{M}$ forskolin, an activator of adenylyl cyclase, for at least 20 min before DPDPE application indeed blocked DOR-induced LTD of oIPSC amplitude (oIPSC amplitude $97.04 \pm 5.10\%$ of baseline, $t = 0.58$, $df = 7$, $p = 0.58$, Figure 3g) as compared with control aCSF (oIPSC amplitude $67.04 \pm 10.65\%$ of baseline, $t = 3.10$, $df = 7$, $p = 0.02$). To test the downstream target of adenylyl cyclase, we incubated slices in the cell-permeable cAMP analog, 8-bromo-cAMP ($160 \mu\text{M}$) and measured oIPSC amplitude following DPDPE application. We found that DPDPE did not decrease oIPSC amplitude in the presence of 8-bromo-cAMP ($95.77 \pm 9.67\%$, $t = 0.44$, $df = 10$, $p = 0.67$, Figure 3h), as it did in control aCSF ($68.67 \pm 5.51\%$, $t = 5.68$, $df = 7$, $p = 0.001$), which suggests that DPDPE-induced LTD occurs through a cAMP-dependent signaling pathway. 8-bromo-cAMP blockade of DPDPE-induced LTD was significantly different than control LTD ($t = 2.20$, $df = 17$, $p = 0.04$).

DOR is Located Presynaptically on FSIs

DOR is known to co-localize with a subset of GABAergic neurons in mouse dorsal striatum (Scherrer *et al*, 2006), a finding that suggests co-localization onto a GABAergic interneuron population as opposed to the principal GABAergic MSN. These findings are consistent with our results that EtOH-LTD is presynaptically expressed and mediated through DOR activation. Thus, we tested the prediction that DOR is located on presynaptic FSI terminals by measuring changes in asynchronous oIPSC amplitude and event frequency in the presence of DPDPE. DPDPE application increased the IEI of oIPSC events (baseline IEI $95.09 \pm 2.81 \text{ ms}$; post-DPDPE IEI $130.1 \pm 7.60 \text{ ms}$, $t = 4.35$, $df = 15$, $p = 0.001$, Figure 4a). DPDPE application also depressed oIPSC amplitude (baseline amplitude $20.61 \pm 1.50 \text{ pA}$; post-DPDPE amplitude $16.47 \pm 1.90 \text{ pA}$, $t = 3.65$, $df = 14$, $p = 0.003$, Figure 4a). These data suggest that DPDPE lowers GABA release probability from FSIs. In addition, there is either a possible postsynaptic effect of DPDPE on GABA_A receptor availability or the reduction in probability of release reduces synchronous spontaneous event probability, which would in turn reduce event amplitude. To rule out contributions of postsynaptic DOR to FSI-MSN DPDPE-induced LTD, GDP- β -S ($500 \mu\text{M}$) was included in the internal pipette solution to disrupt G protein signaling in the postsynaptic cell. DPDPE application reliably depressed oIPSC amplitudes with GDP- β -S present (oIPSC amplitude = $70.08 \pm 10.75\%$ of baseline, $t = 2.78$, $df = 8$, $p = 0.02$, Figure 4b). Finally, to test if

Figure 3 EtOH-LTD is delta opioid receptor (DOR) dependent. (a) The pan opioid receptor antagonist, naloxone, does not eliminate EtOH-LTD at the MSN-MSN synapse ($n = 7$ cells, $p < 0.05$). (b) Naloxone eliminates EtOH-LTD at the FSI-MSN synapse (filled circles, $n = 7$ cells, $p > 0.05$), compared with control artificial cerebrospinal fluid (aCSF) (open circles, $n = 9$ cells, $p < 0.05$). (c) Blocking DOR with the antagonist naltrindole eliminates EtOH-LTD at the FSI-MSN synapse (filled circles, $n = 8$ cells, $p > 0.05$), compared with controls (open circles, $n = 8$ cells, $p < 0.05$). (d) EtOH-LTD is stable in the presence of naltrindole 15 min following EtOH washout ($n = 6$, $p < 0.05$). (e) The DOR agonist, DPDPE depresses the FSI-MSN synapse (open circles, $n = 12$ cells, $p < 0.05$), but the μ opioid receptor agonist, DAMGO, does not (filled circles, $n = 6$ cells, $p > 0.05$). (f) DPDPE depresses both FSI-MSN_D (filled circles, $n = 9$ cells, $p < 0.05$) and FSI-MSN_{ID} synapses (open circles, $n = 7$ cells, $p < 0.05$). (g) Incubating slices in the adenylyl cyclase activator forskolin eliminates DPDPE-induced LTD of the FSI-MSN synapse (filled circles, $n = 8$ cells, $p > 0.05$), compared with control aCSF (open circles, $n = 8$ cells, $p < 0.05$). (h) Incubating slices in the cAMP analog 8-bromo-cAMP also eliminates the DPDPE-induced LTD (filled circles, $n = 11$ cells, $p > 0.05$) compared with control aCSF (open circles, $n = 8$ cells, $p < 0.05$). All scale bars indicate 300 pA (vertical) and 100 ms (horizontal). Example traces represent baseline (dark) and the last 5 min of the experiment (light). Schematics depict optogenetic activation of MSNs or FSIs and whole-cell patch-clamp recordings from MSNs. All error bars indicate SEM.

Figure 4 Delta opioid receptors are located on FSI terminals. (a) Left panel, DPDPE increases IEL of oIPSCs and decreases the amplitude of oIPSC events (filled circles) compared with baseline (open circles, $n = 16$ cells, *** $p < 0.001$, ** $p < 0.01$). Right panel, representative traces of oIPSC events before DPDPE application (open circle) and after (filled circle). Black circles indicate detectable events. (b) The G-protein-coupled receptor blocker GDP- β -S does not eliminate DPDPE-induced LTD at the FSI-MSN synapse ($n = 9$ cells, $p < 0.05$). (c) Postsynaptic calcium chelation with BAPTA does not eliminate DPDPE-induced LTD at the FSI-MSN synapse ($n = 11$ cells, $p < 0.05$). Scale bars represent 300 pA (vertical) and 100 ms (horizontal). All example traces depict baseline (dark) and the last 5 min of the experiment (light). All error bars represent SEM. oIPSC, optically elicited Sr^{2+} -enabled asynchronous IPSC.

DPDPE-induced LTD results in the generation and liberation of a Ca^{2+} -induced retrograde signaling molecule that could signal presynaptically, we chelated postsynaptic Ca^{2+} using a high-BAPTA internal pipette solution (20 mM) and measured changes in oIPSC amplitude following DPDPE application. BAPTA (20 mM) in the postsynaptic cell did not block the DPDPE-induced LTD ($82.37 \pm 7.27\%$ of baseline, last 5 min, $t = 2.43$, $df = 10$, $p = 0.04$, Figure 4c), suggesting postsynaptic Ca^{2+} is not necessary for this LTD.

DISCUSSION

The data presented here describe a novel, EtOH-induced, DOR-dependent LTD of FSI-MSN synaptic transmission that, in conjunction with EtOH-LTD at MSN-MSN synapses, likely contributes to the increase in DLS output that occurs following EtOH exposure. We show that DOR-induced LTD at the FSI-MSN synapse occurs through the canonical adenylyl cyclase/cAMP pathway downstream of DOR activation (Figure 5). The discovery that DOR is positioned to regulate FSI-MSN synaptic transmission implicates the opioid system in the physiological expression of habit learning (Wassum *et al*, 2009) and other striatal-dependent behaviors. Finally, EtOH also reliably depresses the MSN-MSN synapse. Although additional work is necessary to unveil the mechanism underlying this effect, our data thus far suggest a presynaptic locus of expression of this form of LTD that functions independently of both CB1 and opioid receptors.

Figure 5 Proposed mechanism of EtOH-LTD. EtOH depresses FSI-MSN_D and -MSN_{ID} transmission by activating DOR on presynaptic FSI terminals. DOR activation decreases adenylyl cyclase (AC) activity, which in turn decreases cAMP levels. This form of DOR-mediated synaptic plasticity occludes CB1 receptor-mediated LTD (gray receptor, grayed out inhibition of AC). FSIs (circles, right) are more prevalent in the dorsolateral striatum (DLS) than the dorsomedial striatum. EtOH-LTD of FSI-MSN synapses is thus positioned to promote a global disinhibition of the dorsolateral striatum. EtOH, ethanol; FSI, fast-spiking interneuron; MSN, principal medium spiny neurons. A full color version of this figure is available at the *Neuropsychopharmacology* journal online.

We demonstrate that EtOH-LTD is mutually occlusive with a form of eCB-LTD at the FSI-MSN synapse but is not mediated through CB1 receptors (Figure 5). These findings are in line with previous work demonstrating that EtOH

blocks eCB-mediated disinhibition of the DLS in a CB1-independent manner (Adermark and Lovinger 2009; Clarke and Adermark, 2010). This full blockade of eCB-mediated DLS disinhibition by EtOH also suggests that the mechanism of action of EtOH-LTD at the MSN-MSN synapse is activation of a presynaptically localized $G_{i/o}$ -coupled receptor capable of occluding CB1 activation-induced depression of GABA release. Given that the present findings that EtOH-LTD occludes CB1-mediated LTD, this suggests that both MSN-MSN and FSI-MSN synapses are normally under manifold control by multiple presynaptically localized $G_{i/o}$ -coupled receptors. In the presence of EtOH, however, the DOR-mediated pathway overrides physiological opioid and eCB control of this synapse, possibly contributing to acute intoxication and/or lasting disinhibition of the DLS over time. Yet other presynaptic $G_{i/o}$ -coupled receptors, such as the kappa opioid receptor may also reside on the FSI terminal and the possible occlusion of their effects by EtOH will also have to be examined in future studies to gain a complete picture of the disruption of physiological control over FSI-MSN synapses by EtOH.

The mechanisms through which EtOH induces release of an endogenous DOR agonist and the cellular source of this release remain unknown. A potential candidate for the source is $MSNs_{ID}$; opioids are known to act as Ca^{2+} -dependent retrograde signaling molecules (Iremonger and Bains, 2009; Wamsteeker Cusulin *et al*, 2013) and $MSNs_{ID}$ contain pre-proenkephalin, the precursor for the most efficacious endogenous agonist of DOR (Gerfen *et al*, 1990; Surmeier *et al*, 1996). However, we found that EtOH depresses FSI transmission onto both $MSNs_D$ and $MSNs_{ID}$ and this depression is not eliminated with a postsynaptic Ca^{2+} chelator. Therefore, it is unlikely that EtOH induces release of a retrograde opioid signal from postsynaptic MSNs. It is possible that another source providing the endogenous DOR agonist is recruited by EtOH. One candidate is the external segment of the globus pallidus. This structure sends GABAergic projections to both MSNs and FSIs in the dorsal striatum (Bevan *et al*, 1998; Mallet *et al*, 2012; Mastro *et al*, 2014) and these projections express pre-proenkephalin (Mallet *et al*, 2012). Given that the variability in the induction of EtOH-LTD at the FSI-MSN synapse is not explained by differences in FSI- MSN_D and FSI- MSN_{ID} pathways, and that DPDPE application consistently induces FSI-MSN synaptic depression, the heterogeneous expression of FSI-MSN EtOH-LTD may be due to the variability in the EtOH-induced release of the endogenous DOR agonist.

While our results specifically describe the effects of EtOH on GABAergic transmission in the DLS, Wilcox *et al* (2014) report that acute EtOH exposure and chronic EtOH drinking induces a higher frequency of basal miniature IPSC (mIPSC) events in the DMS, and a lower frequency of mIPSC events in the DLS. This DMS to DLS response difference may be explained by the higher expression of FSIs in the DLS (Schlösser *et al*, 1999; Luk and Sadikot, 2001), as the majority of mIPSCs onto MSNs arise from FSIs (Koos *et al*, 2004). Taking the DOR-mediated depression of FSI-MSN synapses in the DLS following acute EtOH exposure together with the reported increase in GABA transmission onto DMS MSNs, EtOH appears to shape the global output of the striatum by modulating GABA synapses. Indeed, the importance of the

presently described mechanism in drinking is supported by the findings of Nielsen *et al* (2012) that show striatal DOR blockade decreases repeated EtOH intake in rats, while activating striatal DOR increases consumption. In light of this, additional studies are necessary to understand the effects of EtOH on DOR-mediated depression of the FSI-MSN synapse in the DLS following a chronic drinking paradigm.

FUNDING AND DISCLOSURE

For this work, MHP and BMR were supported by the National Institute on Alcohol Abuse and Alcoholism of the US National Institutes of Health (grant K22 AA021414 to BNM), the Division of Intramural Clinical and Biological Research of the NIAAA/NIH (DML) and the Integrative Neuroscience Initiative on Alcoholism of the NIAAA/NIH (DML). The authors declare no conflict of interest.

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