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CELL TYPE-SPECIFIC SYNAPTIC ENCODING OF ETHANOL EXPOSURE IN THE NUCLEUS ACCUMBENS SHELL

Z. M. JEANES,[a] T. R. BUSKE[a] AND R. A. MORRISETT[a,b,c,d]*

[a] *The Division of Pharmacology and Toxicology, The College of Pharmacy, The University of Texas at Austin, Austin, TX 78712-1074, United States*

[b] *The Waggoner Center for Alcohol and Addiction Research, The University of Texas at Austin, Austin, TX 78712-0125, United States*

[c] *The Institute for Molecular and Cellular Biology, The University of Texas at Austin, Austin, TX 78712-0125, United States*

[d] *The Institute for Neuroscience, The University of Texas at Austin, Austin, TX 78712-0125, United States*

Abstract—Synaptic alterations in the nucleus accumbens (NAc) are crucial for the aberrant reward-associated learning that forms the foundation of drug dependence. Altered glutamatergic synaptic plasticity, in particular, is thought to be a vital component of the neurobiological underpinnings of addictive behavior. The development of bacterial artificial chromosome-eGFP (enhanced green fluorescent protein) transgenic mice that express eGFP driven by endogenous D1 dopamine receptor (D1R) promoters has now allowed investigation of the cell type-specific synaptic modifications in the NAc in response to drugs of abuse. In this study, we used whole-cell *ex vivo* slice electrophysiology in *Drd1*-eGFP mice to investigate cell type-specific alterations in NAc synaptic plasticity following ethanol exposure. Electrophysiological recordings were made from eGFP-expressing medium spiny neurons (D1+ MSNs) and non-eGFP-expressing (putative D2 receptor-expressing) (D1− MSNs) from the shell subregion of the NAc. We observed low frequency-induced long-term depression (1 Hz-LTD) of α-amino-3-hydroxy-5-methyl-isoxazole-4-propionic acid (AMPA)-mediated excitatory postsynaptic currents (EPSCs) solely in D1+ MSNs. However, 24 h following four consecutive days of *in vivo* chronic intermittent ethanol (CIE) vapor exposure, 1-Hz LTD was conversely observed only in D1− MSNs, and now absent in D1+ MSNs. Complete recovery of the baseline plasticity phenotype in both cell types required a full 2 weeks of withdrawal from CIE vapor exposure. Thus, we observed a cell type specificity of synaptic plasticity in the NAc shell, as well as, a gradual recovery of the pre-ethanol exposure plasticity state following extended withdrawal. These changes highlight the adaptability of NAc shell MSNs to the effects of ethanol exposure and may represent critical neuroadaptations underlying the development of ethanol dependence.

Key words: synaptic plasticity, drug dependence, mesocorticolimbic, long-term depression, metaplasticity, neuroadaptation.

INTRODUCTION

The nucleus accumbens (NAc) is an important brain region of convergence for the widespread projections involved in reward processing and guidance of goal-directed behaviors (Ikemoto, 2007). In the NAc, γ-aminobutyric acid (GABA)-ergic medium spiny neurons (MSNs) receive dopaminergic innervations from the ventral tegmental area and glutamatergic inputs from the prefrontal cortex, hippocampus, and amygdala. Limbic region afferents to the NAc interface with motor control circuitry to regulate goal-directed behavior (Mogenson et al., 1980; Nicola et al., 2000; Wise, 2004). Persistent adaptive changes within the NAc in response to drugs of abuse are posited to underlie drug dependence (Koob and Le Moal, 2001; Kauer and Malenka, 2007). These adaptations involve neuronal signaling and synaptic mechanisms similar to those implicated in neural models of learning and memory, in particular long-term synaptic plasticity (Nestler, 2001; Hyman et al., 2006). Specifically, long-term depression (LTD) of α-amino-3-hydroxy-5-methyl-isoxazole-4-propionic acid (AMPA)-mediated excitatory postsynaptic currents (EPSCs) normally present in drug-naïve animals is absent in MSNs from animals sensitized to the behavioral effects of psychostimulants (Thomas et al., 2001; Brebner et al., 2005). In addition, LTD in the NAc core remains occluded in rats that had self-administered cocaine 21 days earlier (Martin et al., 2006). Thus, it is increasingly apparent that drug-induced modifications of synaptic plasticity may share a common mechanism that underlies drug dependence.

*Correspondence to: R. A. Morrisett, The University of Texas at Austin, PHAR-Pharmacology, 1 University Station, A1915, Austin, TX 78712-0125, United States. Tel: +1-512-471-1911; fax: +1-512-475-6088.

E-mail address: ramorris@mail.utexas.edu (R. A. Morrisett).

Abbreviations: ANOVA, analysis of variance; ACSF, artificial cerebrospinal fluid; CIE, chronic intermittent ethanol; CPP, conditioned place preference; D1+ MSNs, eGFP-expressing medium spiny neurons; D1− MSNs, non-eGFP expressing (putative D2 receptor-expressing); D1R, D1 dopamine receptor; D2R, dopamine D2 receptor; drd1a, dopamine D1a receptor; eGFP, enhanced green fluorescent protein; EGTA, ethylene glycol tetraacetic acid; EPSCs, excitatory postsynaptic currents; GENSAT, Gene Expression Nervous System Atlas; HEPES, 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid; 1 Hz-LTD, low frequency-induced long-term depression; LFS, low frequency stimulation; LTD, long-term depression; MSNs, medium spiny neurons; NAc, nucleus accumbens; NMDAR, *N*-methyl-D-aspartate receptor.

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NAc MSNs are commonly divided into two major categories based on their expression of releasable peptides, dopamine receptor subtype expression, and their axonal projection targets (Gerfen and Young, 1988; Gerfen et al., 1990; Le Moine et al., 1990). Dopamine D1 receptor (D1R)-expressing and dopamine D2 receptor (D2R)-expressing MSNs characterize the striatonigral (direct) and striatopallidal (indirect) pathways, respectively (Alexander et al., 1986; Groenewegen et al., 1999). To facilitate investigation into the functional differences between D1R- and D2R-expressing MSNs, bacterial artificial chromosome transgenic mice in which expression of enhanced green fluorescent protein (eGFP) is controlled by D1R or D2R promoters have been created (Gong et al., 2003). Studies capitalizing on this advancement provide insights into the specific synaptic characteristics of both MSN subtypes in the dorsal striatum and NAc core (Cepeda et al., 2008; Grueter et al., 2010). One such report described how activation of eGFP-expressing medium spiny neurons (D1+ MSNs) in the NAc enhances cocaine sensitization and conditioned place preference (CPP), whereas activating D2+ MSNs diminishes these behaviors (Lobo et al., 2010). Additionally, aberrant expression of *N*-methyl-D-aspartate receptor (NMDAR) LTD in the NAc may facilitate the transition of rats to an ''addicted'' behavioral state (Kasanetz et al., 2010).

While there is extensive literature detailing how alterations in NAc signaling are important in behavioral responses to psychostimulants, reports investigating NAc synaptic plasticity and ethanol are few. In fact, our lab published the first report demonstrating a disruption in NAc shell NMDAR-dependent LTD following *in vivo* ethanol exposure. We observed that chronic intermittent ethanol (CIE) exposure reverses the polarity of synaptic plasticity from depression to potentiation (Jeanes et al., 2011). In addition, modulation of ethanol-related behaviors can occur in a dopamine receptor-specific fashion within the NAc. One study demonstrated that siRNA-mediated downregulation of D1 receptors in the NAc decreases ethanol intake and preference, behavioral sensitization, and acquisition of ethanol-induced CPP (Bahi and Dreyer, 2012); while another study showed that blocking D1 receptors in the NAc dose-dependently attenuates reinstated ethanol seeking in rats (Chaudhri et al., 2009). Taken together, these observations formed the impetus for our investigation of the cell type specificity of NAc shell LTD and its potential conversion to synaptic potentiation subsequent to CIE vapor exposure.

In the current study, we used *Drd1*-eGFP transgenic mice expressing eGFP in direct pathway D1+ MSNs to determine synaptic properties in both D1+ and non-eGFP-expressing (putative D2 receptor-expressing) (D1− MSNs). We investigated the cell type-specific expression of low frequency-induced LTD (1 Hz-LTD) in the NAc shell of ethanol-naïve and CIE-exposed mice at several withdrawal time points. Collectively, we aimed to determine whether significant alterations in synaptic plasticity occurred in cell type-specific manner either before or after CIE exposure. Additionally, we sought to detail how quickly disruptions in NAc 1-Hz LTD following CIE exposure recovered to pre-CIE levels after extended withdrawal. These findings could provide a crucial insight into the cell type-specific synaptic adaptations within the NAc shell thought to contribute to the development of ethanol dependence.

EXPERIMENTAL PROCEDURES

Subjects

Dopamine D1a receptor (*drd1a*) promoter-dependent eGFP BAC transgenic mice, generated by the GENSAT (Gene Expression Nervous System Atlas) project, were purchased from the Mutant Mouse Regional Resource Center and outcrossed onto the Swiss Webster background to create hemizygous progeny. Mice were housed under a 12-h light/dark cycle (lights on at 0700 h) and cared for by the University of Texas at Austin Animal Resource Center. Food and water were available *ad libitum*, and all of the following experimental procedures were approved by the University of Texas Institutional Animal Care and Use Committee.

Brain slice preparation

Parasagittal slices (210–250 μm thick) containing the NAc were prepared from the brains of 4–8-week-old male mice from either the C57BL/6J mouse line (The Jackson Laboratory, Bar Harbor, ME, United States) or our in-house mouse line derived from hemizygous *Drd1*-eGFP transgenic mice backcrossed onto the Swiss Webster background (Harlan Laboratories, Indianapolis, IN, United States). Mice were lightly anesthetized with isofluorane, and the brains were rapidly removed and placed in ice-cold (4 °C) oxygenated artificial cerebrospinal fluid (ACSF) containing the following (in mM): 87 NaCl, 25 $NaHCO_3$, 1.25 NaH_2PO_4, 2.5 KCl, 7 $MgCl_2$, 0.5 $CaCl_2$, 25 dextrose, 75 sucrose, bubbled with 95% O_2/5% CO_2. Sagittal slices were cut and then transferred to an incubation ACSF for a minimum of 60 min prior to recording that contained 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, bubbled with 95% O_2/5% CO_2; pH 7.4, 32 °C. Unless otherwise noted, all drugs and chemicals were obtained from Sigma–Aldrich (St. Louis, MO, United States).

Patch clamp electrophysiology

We conducted all recordings at 31–33 °C in ACSF containing (in mM): 120 NaCl, 25 $NaHCO_3$, 1.23 NaH_2PO_4, 3.3 KCl, 0.9 $MgCl_2$, 2 $CaCl_2$, 10 dextrose, bubbled with 95% O_2/5% CO_2. The $GABA_A$ receptor antagonist, picrotoxin (50 μM), was added to the external recording solution throughout all recordings to inhibit $GABA_A$ receptor-mediated synaptic currents and improve the reliability of synaptic plasticity in the dorsal and ventral striatum by favoring postsynaptic depolarization during conditioning stimuli (Berretta et al., 2008). Whole-cell voltage and current clamp recordings were obtained from NAc shell eGFP-expressing MSNs (referred to as D1+ MSNs) and non-eGFP-expressing