Molecular and Cellular Neuroscience 85 (2017) 93–104

Contents lists available at ScienceDirect

Molecular and Cellular Neuroscience

journal homepage: www.elsevier.com/locate/ymcne

Chronic ethanol exposure increases inhibition of optically targeted phasic dopamine release in the nucleus accumbens core and medial shell ex vivo

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ARTICLE INFO

Keywords:
Dopamine
Nucleus accumbens
Ethanol
Optogenetics
Voltammetry
Kappa opioid receptors

ABSTRACT

Dopamine signaling encodes reward learning and motivated behavior through modulation of synaptic signaling in the nucleus accumbens, and aberrations in these processes are thought to underlie obsessive behaviors associated with alcohol abuse. The nucleus accumbens is divided into core and shell sub-regions with overlapping but also divergent contributions to behavior. Here we optogenetically targeted dopamine projections to the accumbens allowing us to isolate stimulation of dopamine terminals ex vivo. We applied 5 pulse (phasic) light stimulations to probe intrinsic differences in dopamine release parameters across regions. Also, we exposed animals to 4 weeks of chronic intermittent ethanol vapor and measured phasic release. We found that initial release probability, uptake rate and autoreceptor inhibition were greater in the accumbens core compared to the shell, yet the shell showed greater phasic release ratios. Following chronic ethanol, uptake rates were increased in the core but not the shell, suggesting region-specific neuronal adaptations. Conversely, kappa opioid receptor function was upregulated in both regions to a similar extent, suggesting a local mechanism of kappa opioid receptor regulation that is generalized across the nucleus accumbens. These data suggest that dopamine axons in the nucleus accumbens core and shell display differences in intrinsic release parameters, and that ethanol-induced adaptations to dopamine neuron terminal fields may not be homogeneous. Also, chronic ethanol exposure induces an upregulation in kappa opioid receptor function, providing a mechanism for potential over-inhibition of accumbens dopamine signaling which may negatively impact downstream synaptic function and ultimately bias choice towards previously reinforced alcohol use behaviors.

1. Introduction

Alcohol use disorders (AUD) and drug addiction share a common feature—persistent seeking and use despite delayed negative consequences on both physical and social health. These aberrant actions suggest abnormalities in the neural circuitry governing motivated behavior (Hyman et al., 2006; Stuber et al., 2010b), and have prompted research into elucidating ethanol induced neuroadaptations that may underlie the switch from casual to obsessive attention towards alcohol. An area of particular interest is the nucleus accumbens (NAc), which integrates diverse cognitive and limbic inputs onto motor plan-initiating output signals of the basal ganglia, implicating this region in motivation and behavioral selection. For instance, repeated drug exposure causes long term synaptic plasticity within the NAc that results in sensitized locomotor responses (Creed et al., 2015; Pascoli et al., 2011) and incubation of craving during withdrawal (Conrad et al., 2008), prompting increased attention on identifying the synaptic manifestations of chronic ethanol exposure in this region (Jeanes et al., 2014; Abrahao et al., 2013). Dopamine (DA) signaling in the NAc is thought to encode reward learning via modulation of synaptic signaling and gating aspects of synaptic plasticity (Shen et al., 2008; Surmeier et al., 2011; Tritsch and Sabatini, 2012; Creed and Lüscher, 2013), processes that are believed to ultimately bias decision making towards previously reinforced behaviors. Indeed, aberrations in DA signaling have been linked to obsessive/compulsive behaviors (Sesia et al., 2013) and behavioral rigidity (Beeler et al., 2014).

Kappa opioid (κ-opioid) receptors are expressed within the NAc where they inhibit DA signaling (Spanagel et al., 1992) upon activation by endogenous dynorphin, providing a negative feedback mechanism to regulate local DA levels (Steiner and Gerfen, 1996). Further, the dynorphin/κ-opioid system appears to be upregulated following chronic ethanol exposure (Sirohi et al., 2012). Specifically, the κ-opioid

Abbreviations: aCSF, artificial cerebral spinal fluid; AUD, alcohol use disorder; BEC, blood ethanol concentration; ChR2, channelrhodopsin-2; CIE, chronic intermittent ethanol; DA, dopamine; FSCV, fast scan cyclic voltammetry; NAc, nucleus accumbens; nor-BNI, nor-binaltorphimine; PBS, phosphate buffered saline; TH, tyrosine hydroxylase; Vmax, maximal rate of uptake; VTA, ventral tegmental area; κ-Opioid, kappa opioid receptor

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http://dx.doi.org/10.1016/j.mcn.2017.09.007
Received 2 June 2017; Received in revised form 8 September 2017; Accepted 18 September 2017
Available online 20 September 2017

receptor antagonist nor-binaltorphimine (nor-BNI) reduces the escalation of intake observed in alcohol dependent animals while having no effect on non-dependent animals (Walker and Koob, 2008), suggesting that recruitment of κ-opioid receptor activity contributes to dependence and may provide a potential mechanism for ethanol-induced adaptations in DA transmission and the subsequent modulation of synaptic function (Shippenberg et al., 2007).

The NAc is divided into two sub-regions, the core and the shell, which receive unique assortments of afferent inputs and differentially contribute to reward aspects of behavior (Kelley, 2004). The core is involved in reinforcement learning and adaptive instrumental behavior, while the shell is connected with viscero-endocrine effector systems involved in reward processing and motivational states (Kelley, 1999). For example, by virtue of its afferent innervation from the ventral hippocampus (Britt et al., 2012) and efferent projections to the lateral hypothalamus (Kelley, 2004) the shell is considered to be a component of the extended amygdala, a collection of structures heavily implicated in exaggerated stress and anxiety states during alcohol withdrawal (Koob, 2013; Lovinger and Kash, 2015). Both regions receive DA innervation from the ventral tegmental area (VTA); however, DA signaling does not appear to be homogenous across the NAc (Aragona et al., 2009). Acute drug and alcohol administration selectively increase DA release in the shell compared to the core (Di Chiara et al., 2004; Howard et al., 2008), and chronic drug exposure results in differential dopaminergic adaptations in the core and shell (Saddoris, 2016; Saddoris et al., 2016). This is coupled with differences in phasic release parameters of DA terminal fields across regions (Jones et al., 1996; Zhang et al., 2009), supporting region-specific heterogeneity in DA signaling.

One level of DA signal regulation occurs at the terminals, where expression of a variety of release-regulating heteroreceptors in the terminal membrane (Zhang and Sulzer, 2012; Sulzer et al., 2016) allows local environmental influence of terminal physiology and results in diverse micro-domains within terminal fields (Wightman et al., 2007; Pickel, 2000; Zhang et al., 2015; Tritsch et al., 2012). Fast-scan cyclic voltammetry (FSCV) is often used in ex vivo slice preparations to pharmacologically probe terminal receptor regulation of DA release and how terminal activity may be altered following chronic drug and alcohol administration (Ferris et al., 2013; Siciliano et al., 2015b; Calipari et al., 2015). However, relatively few of these investigations probe terminal fields in the medial NAc shell, partially due to the technical difficulties in obtaining robust, reliable DA release in this region. That is, a single pulse-stimulated DA signal is low in amplitude compared to neighboring regions such as the NAc core and dorsal striatum (Jones et al., 1996). This can be overcome by applying multiple pulses in a stimulation train; however, electrical stimulation trains recruit modulation of DA terminals from concurrent excitation of the surrounding non-dopaminergic neuronal types within the tissue (Melchior et al., 2015). This recruitment often distorts the stimulated DA signal and makes assessments of whether heteroreceptor modulation is occurring through direct (terminal receptors) or indirect (multi-synaptic) mechanisms difficult to determine. Here we use optogenetic tools to selectively light-stimulate DA terminals in the NAc, without concurrent electrical excitation of non-dopaminergic cells in the tissue, providing an isolated DA signal ex vivo.

The goal of these studies was to extend our investigations of chronic ethanol-induced alterations of DA signaling in the ventral striatum (Karkhanis et al., 2015; Rose et al., 2016; Siciliano et al., 2015a) across three novel parameters. 1) We measured DA signals in the NAc shell to provide a direct comparison to NAc core DA terminal fields in order to determine whether chronic ethanol exposure results in differential adaptations between regions. 2) We used targeted light stimulation, avoiding concurrent modulation of DA release by excitation of non-dopaminergic cells in the DA terminal field, to provide greater resolution with regard to direct vs indirect ethanol-induced DA terminal heteroreceptor adaptations. 3) We applied 5 pulse stimulation trains to approximate the phasic spikes in DA neurons that occur in response to discrete environmental cues in vivo.

2. Methods

2.1. Animals

Male Tyrosine Hydroxylase (TH)-internal ribosome entry site (IRES)-Cre Recombinase knock-in mice on a C57Bl/6J background (TH:Cre) were bred, genotyped for positive cre recombinase expression and maintained in group housing in the mouse colony. All animals were maintained according to the National Institutes of Health guidelines and all experimental protocols were approved by the Institutional Animal Care and Use Committee at Wake Forest University School of Medicine.

At 8–12 weeks of age mice were anesthetized with 100 mg/kg ketamine and 8 mg/kg xylazine and placed in a stereotaxic frame. A custom-made glass micropipette (80 μm outer diameter) was inserted directly above the VTA (coordinates from Bregma in mm: −3.3 AP, ± 0.5 ML, −4.3 DV). Microinjections were administered using an air pressure injection system and consisted of applying small pulses of pressure (30 psi, 40–80 msec duration) to the infusion pipette. Individual injections were performed on each side of the midline resulting in bilateral VTA infusions, with each hemisphere of VTA receiving approximately 0.4 μl of AAV5-EF1α-hChR2(H134R)-eYFP (5.5 × 1012 virus molecules/ml; Virus Vector Core, University of North Carolina). Following surgery, mice were returned to the mouse colony, single housed, and maintained for a minimum of 14 days to recover from surgery and allow expression of channelrhodopsin-2 (ChR2).

2.2. Histology

Immunohistochemistry was used to verify ChR2 expression in dopamine axons in the nucleus accumbens as previously described (Melchior et al., 2015). Mice were anesthetized with ketamine (100 mg/kg) and xylazine (8 mg/kg) and transcardially perfused with phosphate-buffered saline (PBS) followed by 10% buffered formalin phosphate (Fischer Scientific, Waltham, MA, USA). Brains were then removed, submerged in 10% buffered formalin phosphate for an additional 24–48 h, and subsequently transferred to 30% sucrose in PBS for 72 h. Sections (40 μm) were obtained on a microtome (American Optical Company, Buffalo, NY, USA) and stored in PBS for immunohistochemistry.

Sections were permeabilized in 0.3% triton (Sigma, St Louis, MO, USA) in PBS (PBS-Tx) for 2 h, blocked in 5% normal goat serum (Vector Laboratories, Burlingame, CA, USA) in PBS-Tx, and incubated in primary antibody in the blocking solution for 24–48 h. Primary antibodies include chicken anti-GFP (1 μg: 500 μl; Aves labs, Tigard, OR, USA) and rabbit anti-tyrosine hydroxylase (1 μg: 1000 μl; Cell Signaling, Danvers, MA, USA). Sections were rinsed and transferred to secondary antibody in blocking solution for 1.5 h. Secondary antibodies include fluorescein-labeled goat anti-chicken IgY (Aves labs, 1 μg: 250 μl) and goat anti-rabbit alexa fluor 594 IgG (1 μg: 250 μl; Molecular Probes, Eugene, OR, USA). Sections were mounted on 1 mm slides with Vectashield (Vector Labs) mounting medium and images were obtained with an Olympus BX-51 Microscope and Optronics Microfire digital camera (Goleta, CA, USA). Images were processed in Adobe Photoshop.

2.3. CIE

ChR2 expressing mice were administered the chronic intermittent ethanol (CIE) exposure protocol outlined on the website for the INIA-Stress research consortium (http://iniastress.org/dependence). Briefly, mice were injected with a 'loading' dose of ethanol (1.6 g/kg) and pyrazole (1 mmol/kg), an alcohol dehydrogenase inhibitor that stabilizes and maintains blood ethanol concentrations, and exposed to