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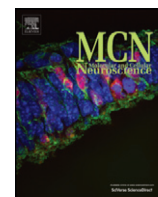

Dendritic remodeling of hippocampal neurons is associated with altered NMDA receptor expression in alcohol dependent rats

Miranda C. Staples, Airee Kim, Chitra D. Mandyam *

Committee on the Neurobiology of Addictive Disorders, The Scripps Research Institute, 10550 North Torrey Pines Road, SP30-2400, La Jolla, CA 92037, USA

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ABSTRACT

Prolonged alcohol exposure has been previously shown to impair the structure and function of the hippocampus, although the underlying structural and biochemical alterations contributing to these deleterious effects are unclear. Also unclear is whether these changes persist into prolonged periods of abstinence. Previous work from our lab utilizing a clinically relevant rodent model of alcohol consumption demonstrated that alcohol dependence (induced by chronic intermittent ethanol vapor exposure or CIE) decreases proliferation and survival of neural stem cells in the hippocampal subgranular zone and hippocampal neurogenesis in the dentate gyrus, implicating this region of the cortex as particularly sensitive to the toxic effects of prolonged ethanol exposure. For this study, we investigated seven weeks of CIE-induced morphological changes (dendritic complexity and dendritic spine density) of dentate gyrus (DG) granule cell neurons, CA3, and CA1 pyramidal neurons and the associated alterations in biochemical markers of synaptic plasticity and toxicity (NMDA receptors and PSD-95) in the hippocampus in ethanol-experienced Wistar rats 3 h (CIE) and 21 days (protracted abstinence) after the last ethanol vapor exposure. CIE reduced dendritic arborization of DG neurons and this effect persisted into protracted abstinence. CIE enhanced dendritic arborization of pyramidal neurons and this effect did not persist into protracted abstinence. The architectural changes in dendrites did not correlate with alterations in dendritic spine density, however, they were associated with increases in the expression of pNR2B, total NR2B, and total NR2A immediately following CIE with expression levels returning to control levels in prolonged abstinence. Overall, these data provide the evidence that CIE produces profound changes in hippocampal structural plasticity and in molecular tools that maintain hippocampal structural plasticity, and these alterations may underlie cognitive dysfunction associated with alcohol dependence. In addition, the compensatory state concurrent with reduced plasticity during protracted abstinence could leave the hippocampus vulnerable to subsequent insult following chronic ethanol exposure.

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1. Introduction

Alcoholism, or the cyclical use, abuse, abstinence, and subsequent relapse of alcohol, is a disorder which has been reported to result in neurobiological deficits in humans. Clinical studies have identified multiple regions of the cortex which are subject to the deleterious effects of chronic alcohol exposure, including the hippocampus, which has been shown to be particularly sensitive to alcohol-induced damage. However, what is less well known is what structural abnormalities, neuronal morphology, and biochemical deviations persist into periods of prolonged abstinence.

Both clinical and pre-clinical studies have clearly demonstrated links between chronic ethanol consumption and impaired hippocampal-sensitive cognitive performance (Brandt et al., 1983; Glenn and Parsons, 1991; Sullivan et al., 2000a,b, 2002), hippocampal morphology (Bengochea and Gonzalo, 1990; Durazzo et al., 2011), and volume (Sullivan et al., 1995; Agartz et al., 1999; Bleich et al., 2003a,b; Beresford et al., 2006). Additionally, the hippocampus has been implicated in multiple aspects of ethanol dependence, including reward and seeking of ethanol (Koob and Volkow, 2010), providing evidence that the hippocampus is not only structurally and functionally damaged by chronic ethanol use, but also demonstrates compromised typical hippocampal plasticity mechanisms including structural and biochemical reorganization.

The hippocampus is a substructure of the mammalian limbic system critically important for numerous aspects of cognitive function, stress, and emotional regulation (reviewed in (Bannerman et al., 2014)), as well as having been identified to be sensitive to chronic exposures to alcohol. This region is comprised primarily of three sub-regions: the Cornu Amonis 1 (CA1), the Cornu Amonis 3 (CA3), and the dentate gyrus (DG). Frontal–cortical structures project to the hippocampus via the entorhinal cortex which send perforant path projections (from layers II and III) directly to the dendritic fields of both the granule cells of the DG and the CA3 pyramidal cells. The DG also projects to the CA3 pyramidal cells via the mossy fiber pathway in addition to the CA3

* Corresponding author.
E-mail address: cmandyam@scripps.edu (C.D. Mandyam).

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pyramidal neurons being highly self-stimulating. The CA3 pyramidal neurons also send Schafer Collateral projections to the CA1 pyramidal neurons, which subsequently send projections primarily back to the entorhinal cortex layer V. While the hippocampal structure and function with regards to chronic alcohol use and exposure has been the focus of numerous studies, as yet, no single study has measured the biochemical and structural changes induced by chronic alcohol and subsequent prolonged abstinence in each subregion independently.

While prevailing evidence suggests a direct relationship between chronic ethanol exposure and gross morphological alterations of the hippocampus, as yet, the potential underlying structural abnormalities of hippocampal neurons following chronic ethanol use have not been well described with only one study noting dendritic retraction in CA1 pyramidal neurons of ethanol exposed animals (McMullen et al., 1984). Functional electrophysiological studies of the hippocampus following chronic ethanol exposure have reported impaired long-term potentiation (LTP; (Fujii et al., 2008)), and altered NMDAR function following ethanol challenge (Sanna and Harris, 1993; Wu et al., 1993; Nelson et al., 2005).

A great emphasis has been placed on understanding the direct impact of choric ethanol on hippocampal structure and function, however, there has been less work aimed at understanding how long these alterations persist into periods of abstinence. While it has been suggested that some impairments in cognitive function are ameliorated over an extended period in human alcoholics, hippocampal-sensitive functions, such as spatial processing, remain perturbed long into the abstinent period (Fein et al., 2006). To date, there has been no work to describe in a detailed manner how neurons in the three distinct regions of the mammalian hippocampus react to both chronic ethanol and prolonged abstinence. Therefore, we hypothesize that CIE exposure will result in hippocampal pyramidal and granule cell structural abnormalities and aberrant glutamatergic receptor expression, changes which will persist into a period of prolonged abstinence from ethanol exposure.

The goal of the present study was to elaborate on the current knowledge of ethanol toxicity in the hippocampus and expand our understanding of the significant and persistent morphological and biochemical deviations resulting from chronic exposures to ethanol and persisting into a period of abstinence. To the best of our knowledge, there has been no study in rats assessing both the effects of chronic ethanol exposures and prolonged abstinence from ethanol on hippocampal cellular morphology nor biochemical correlatives of molecular plasticity. We aim to show that the deviations in DG granule cell and CA3 and CA1 pyramidal cell structure we predict to occur following chronic ethanol exposures will persist into a period of extended abstinence from ethanol exposure, and that these deviations will correspond to aberrant protein expression in the hippocampus.

2. Materials and methods

Experimental procedures were conducted in strict adherence to the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH publication number 85–23, revised 1996) and approved by the Institutional Animal Care and Use Committee of The Scripps Research Institute.

2.1. Animals

Adult male Wistar rats (Charles River), weighing 225–275 g and 8 weeks old at the beginning of the experiments, were housed in groups of 2–3 per cage in a temperature-controlled (22 °C) vivarium on a 12 h/12 h light/dark cycle (lights on at 8:00 P.M.) with ad libitum access to food and water. All procedures were performed during the dark phase of the light/dark cycle. Thirty-three rats began and completed the study (Control: $n = 12$, Acute Withdrawal: $n = 12$, Prolonged Abstinence: $n = 9$). Data on the medial prefrontal cortex from the same animals have been published elsewhere (Kim et al., 2014; Navarro and Mandyam, 2015). Tissue from the hippocampus was stored and analyzed separately for the present study. For each animal, the brain was dissected along the sagittal plane into left and right hemispheres upon sacrifice. For all animals, the right hemisphere was processed for Western blot analysis. For 6 of the control animals, 6 of the 3 h withdrawal animals, and all 9 of the protracted abstinence animals, the left hemisphere was Golgi stained for the structural analysis portion of this study.

2.2. Chronic intermittent ethanol vapor exposure (CIE)

Upon arrival to the animal facility, rats were housed in ethanol vapor chambers for 7 weeks, with ethanol on for 14 h and air only for 10 h, in a reverse light-dark cycle (lights off at 8 am), a cycle of ethanol exposure which has previously been reported as resulting in physical dependence to ethanol (O'Dell et al., 2004; Gilpin et al., 2008; Richardson et al., 2008).

In the chambers, 95% alcohol flows from a large reservoir to a peristaltic pump (model QG-6, FMI Laboratory, Fluid Metering). Alcohol is delivered from the pump to a sidearm flask at a flow rate that can be regulated. The flask is placed on a heater in which the drops of alcohol hitting the bottom of the flask are vaporized. Air flow controlled by a pressure gauge is delivered to the flask and carries the alcohol vapors to the vapor chamber that contains the animal cages. The flow rate was set to deliver vapors that result in blood alcohol levels between 125 and 250 mg% (Fig. 1) or 27.2 and 54.4 mM (Gilpin et al., 2008). In this model, rats exhibit somatic withdrawal signs and negative emotional symptoms reflected by anxiety-like responses, hyperalgesia, and elevated brain reward thresholds (Schulteis et al., 1995; Roberts et al., 2000; Valdez et al., 2002; Rimondini et al., 2003; O'Dell et al., 2004; Zhao et al., 2007; Sommer et al., 2008; Edwards et al., 2012; Vendruscolo et al., 2012). Control rats were not exposed to alcohol vapor. Alcohol naïve animals in this study were housed nearly identically to the alcohol-exposed animals (plastic tub containers of identical construction, color, and size, identical bedding, pair housed, ad lib food [same diet as vapor animals] and water, identical animal maintenance schedule [handling, cage changes, weight measures, etc.], and light schedule) but outside of the exterior vapor chamber. Blood sampling (tail bleedings) was performed immediately after daily bouts of alcohol vapor exposure in CIE animals twice during the first week of vapor exposure and once during each subsequent week of vapor exposure. Plasma (5 μL) was used for measurement of blood alcohol levels using an Analox AM 1 analyzer (Analox Instruments). The reaction is based on the oxidation of alcohol by alcohol oxidase in the presence of molecular oxygen (alcohol + O2 → acetaldehyde + H2O2). The rate of oxygen consumption is directly proportional to the alcohol concentration. Single-point calibrations were done for each set of samples with reagents provided by Analox Instruments (25–400 mg% or 5.4–87.0 mM). When blood samples were outside the target range (125–250 mg%), vapor levels were adjusted accordingly.

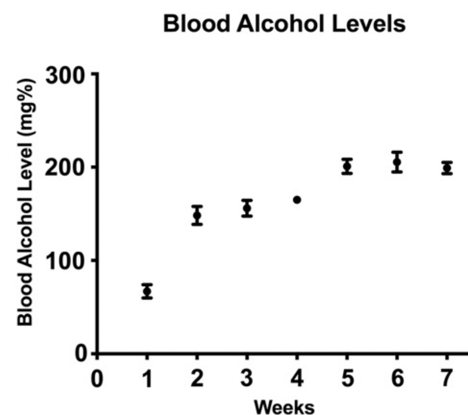

Fig. 1. Blood alcohol levels of rats subjected to seven weeks of chronic intermittent ethanol (CIE). Animals were maintained at blood alcohol levels between 175–250 mg% for the duration of the CIE exposure.

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