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Wheel running reduces ethanol seeking by increasing neuronal activation and reducing oligodendroglial/neuroinflammatory factors in the medial prefrontal cortex

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ABSTRACT

The therapeutic effects of wheel running (WR) during abstinence on reinstatement of ethanol seeking behaviors in rats that self-administered ethanol only (ethanol drinking, ED) or ED with concurrent chronic intermittent ethanol vapor experience (CIE-ED) were investigated. Neuronal activation as well as oligodendroglial and neuroinflammatory factors were measured in the medial prefrontal cortex (mPFC) tissue to determine cellular correlates associated with enhanced ethanol seeking. CIE-ED rats demonstrated escalated and unregulated intake of ethanol and maintained higher drinking than ED rats during abstinence. CIE-ED rats were more resistant to extinction from ethanol self-administration, however, demonstrated similar ethanol seeking triggered by ethanol contextual cues compared to ED rats. Enhanced seeking was associated with reduced neuronal activation, and increased number of myelinating oligodendrocyte progenitors and PECAM-1 expression in the mPFC, indicating enhanced oligodendroglial and neuroinflammatory response during abstinence. WR during abstinence enhanced self-administration in ED rats, indicating a deprivation effect. WR reduced reinstatement of ethanol seeking in CIE-ED and ED rats, indicating protection against relapse. The reduced ethanol seeking was associated with enhanced neuronal activation, reduced number of myelinating oligodendrocyte progenitors, and reduced PECAM-1 expression. The current findings demonstrate a protective role of WR during abstinence in reducing ethanol seeking triggered by ethanol contextual cues and establish a role for oligodendroglia-neuroinflammatory response in ethanol seeking. Taken together, enhanced oligodendroglia-neuroinflammatory response during abstinence may contribute to brain trauma in chronic alcohol drinking subjects and be a risk factor for enhanced propensity for alcohol relapse.

1. Introduction

A major factor contributing to the enduring nature of alcohol relapse is the persistence of subjective responses to contextual cues that were paired with alcohol consumption (O'Brien et al., 1998). These responses could be reduced by behavior-based approaches that have the potential to activate areas of the prefrontal cortex (PFC) associated with voluntary inhibitory control of motivational impulses (Phan et al., 2005; Diekhof and Gruber, 2010).

Growing evidence suggests that the rodent medial prefrontal cortex (mPFC) likely represents a functional homolog of the human medial and dorsolateral PFC (Vertes, 2006), and therefore rodent models of relapse (modeled as reinstatement of ethanol seeking) can be used to uncover neural correlates in the PFC that assist with enhanced propensity for relapse (Martin-Fardon and Weiss, 2013). For example, one such model, namely the extinction-reinstatement model elicits drug seeking in response to ethanol-associated environmental stimuli, such as ethanol context and cues after extinguishing responses to these environmental stimuli in a novel (nondrug-paired) context (Shaham et al., 2003). Using this model, it has been demonstrated that enhanced ethanol seeking triggered by ethanol cues, stress and ethanol itself is associated with alterations in neurotransmitter systems and neuronal activation in the extended amygdala, and that pharmacological manipulations targeting the dysregulated neurotransmitter systems assisted with

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reducing propensity for relapse (Ciccocioppo et al., 2003; Zhao et al., 2006). However, neurobiological correlates in the mPFC in response to ethanol seeking triggered by ethanol context and contextual cues is unknown (Weiss et al., 2001), and could help determine risk factors in a brain region implicated in preoccupation/anticipation or craving stage of addiction (Koob and Volkow, 2010).

We have recently demonstrated that unregulated self-administration of ethanol in animals that experienced chronic intermittent ethanol vapor exposure (CIE-ED; a paradigm that produces alcohol dependence-like behavior) produces profound alterations in the birth and survival of oligodendroglial progenitors (OPCs) in the mPFC compared with regulated ethanol drinking (ED, a paradigm that maintains nondependent drinking; (Richardson et al., 2009; Kim et al., 2015; Somkuwar et al., 2015)). Particularly interesting is the long-lasting effect of withdrawal on OPCs in the mPFC, visualized as increases in proliferation and survival of progenitors in CIE-ED animals that also demonstrated enhanced drinking after prolonged abstinence, suggesting a permanent dysregulation in the oligodendroglial niche maintaining oligodendroglial homeostasis (Somkuwar et al., 2015). Protracted abstinence from CIE is also associated with increases in myelin associated proteins in the mPFC indicating additional compensatory changes in myelinating glia (Navarro and Mandyam, 2015). Such alterations in the expression of oligodendroglia and myelinating glia during withdrawal and abstinence in the mPFC may be regulated by neuroinflammatory response during withdrawal, as interactions between oligodendroglia, myelin, endothelial cells and neuroinflammatory proteins have been demonstrated in models of brain injury, including, stroke and ischemia (Pham et al., 2012; Ortega et al., 2015). For example, proliferation and survival of OPCs is regulated by vascular endothelial cells, such that increase in endothelial response enhances OPC proliferation and survival (Arai and Lo, 2009). Notably, withdrawal from chronic ethanol experience enhances generation of inflammatory mediators such as cytokines in the cortex (Whitman et al., 2013; Harper et al., 2015) and increases in cytokines can upregulate expression of endothelial cell adhesion molecules in endothelial cells, that can in concert support leukocyte emigration via blood-brain barrier disruption (Woodfin et al., 2007; Larochelle et al., 2011). The recruitment of leucocytes by endothelial cell adhesion molecules is directly linked to more tissue damage and an increased release of inflammatory mediators, ultimately leading to uncontrolled inflammation (Privratsky et al., 2010).

In this context, platelet endothelial cell adhesion molecule-1 (PECAM-1, CD31) is known to produce neuroinflammation and blood brain barrier disruption, and accumulating evidence suggests that it could be used as a potential marker for neurological disorders (Losy et al., 1999; Zaremba and Losy, 2002; Hwang et al., 2005; Kalinowska and Losy, 2006; Woodfin et al., 2007; Privratsky et al., 2010; Cheung et al., 2015; Andrews et al., 2016). However, it is not known whether withdrawal from chronic ethanol experience regulates PECAM-1 and if the alterations in PECAM-1 correlate with withdrawal induced increases in oligodendroglia and myelinating glia.

Accumulating evidence demonstrates that behavioral therapies (e.g. physical activity) that augment cognitive flexibility and alter neuroinflammatory responses can be used to enhance recovery from brain injury in models of stroke and ischemia (Hu et al., 2010; Olver et al., 2015). Notably, clinical and preclinical studies also demonstrate an interaction between physical activity (via wheel running in rodents) and alcohol drinking behaviors. Specifically, aerobic exercise in humans and wheel running activity in animals increase drinking behaviors when access to both are not concurrent (Werme et al., 2002; Ozburn et al., 2008; French et al., 2009; Lisha et al., 2011, 2013; Leasure et al., 2015), and lead to reward substitution when both are available concurrently (McMillan et al., 1995). However, it is not clear, at least in animal models of relapse, whether wheel running prevents ethanol seeking triggered by ethanol context and contextual cues after extinguishing ethanol responses in a novel context, and whether the behavioral outcomes correlate with running induced neuroadaptations (Deehan et al., 2011; Li et al., 2015).

The current study tested the hypothesis that wheel running during abstinence prevents ethanol seeking in CIE-ED and ED animals that demonstrate enhanced propensity for relapse. The study also tested the subhypothesis that the reduced ethanol seeking with wheel running is associated with running-induced decreases in oligodendroglia, myelinating glia and PECAM-1, and increases in neuronal activation in the mPFC.

2. Methods

2.1. Animals

Seventy-one adult male Wistar rats (Charles River) completed the study. All rats were 8 weeks old at the beginning of the study, and weighed approximately 220–250 g. The rats were maintained in reverse 12 h light–12 h dark cycle rooms and housed two/cage unless otherwise specified. Food and water were available *ad libitum*. All experimental procedures were carried out 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 were approved by the Institutional Animal Care and Use Committee at The Scripps Research Institute.

2.2. Ethanol self-administration

The behavioral experiments conducted herein are presented as a detailed schematic in Fig. 1a. Forty-seven adult male Wistar experimentally-naïve rats were given two 14-h lever-responding training sessions in the operant conditioning boxes (Med Associates Inc., VT), on an fixed-ratio 1 schedule (FR1; one response resulted in one reinforce delivery), where one press on the available lever resulted in the delivery 0.1 ml of water to a sipper cup mounted on the wall in between the two levers. The operant conditioning boxes were housed inside sound attenuating chambers. During these sessions, the house-light and white noise were turned off (Context A). Then, rats were trained to respond for 0.1 ml of ethanol (10% v/v) over four daily 2-h FR1 sessions; all other conditions remained the same as before. Subsequently, the rats were trained to discriminate between two available levers to obtain 0.1 ml ethanol during daily 30-min FR1 sessions. During these sessions, active (right) lever responding resulted in the delivery of ethanol, while responding on the inactive (left) lever was recorded but had no programmed consequence. Each ethanol delivery was followed by a 4-s time-out during which responding on the active lever did not result in the delivery of ethanol. During this time-out period, the cue-light above the active lever remained on; thus the cue-light was paired with the delivery of ethanol. These 30-min discrimination training sessions continued till stable responding was obtained, where stable responding was defined as less than 10% variation in active lever responding for 3 consecutive 30-min FR1 sessions.

Subsequently, the rats were divided into two groups; one group received chronic intermittent ethanol vapor exposure (CIE-ED rats, n = 21; see procedure below) while the other group was exposed to air in their normal housing condition (ED rats, n = 26; did not experience ethanol vapors) for a duration of 7 weeks. CIE-ED and ED rats continued to experience two 30-min FR1 sessions per week (Tuesdays and Thursdays) during the 7 weeks of vapor exposure