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ALCOHOL VAPOR EXPOSURE DIFFERENTIALLY IMPACTS MESOCORTICOLIMBIC CYTOKINE EXPRESSION IN A SEX-, REGION-, AND DURATION-SPECIFIC MANNER

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Abstract—Alcohol exposure elicits the production of cytokines that regulate the host response to infection, immunity, inflammation, and trauma. Although increased production of pro-inflammatory cytokines has been linked to symptoms of alcoholism, few studies have evaluated whether cytokine expression changes across the development of alcohol dependence, or whether these changes are region and/or sex specific. In the present study, we subjected adult male and female rats to different regimens of alcohol vapor exposure (acute, subchronic, or chronic) and measured relative mRNA expression for tumor necrosis factor alpha (TNFα), interleukin-6 (IL-6), and chemokine (C–C motif) ligand 2 (CCL2) in reward-related brain regions. Results indicated that acute alcohol exposure increased TNFα mRNA expression in the basolateral amygdala (BLA), nucleus accumbens (NAc), and ventral tegmental area (VTA), whereas IL-6 expression was increased in the VTA, NAc, and ventromedial prefrontal cortex (vmPFC) only in males. After subchronic exposure (1 week daily intermittent exposure, 14 h on:10 h off), TNFα expression remained elevated in the BLA, NAc, and VTA, while IL-6 expression was reduced in the male vmPFC. Chronic alcohol exposure (6 week daily intermittent exposure, 14 h on: 10 h off) increased TNFα mRNA expression in the NAc and increased IL-6 mRNA in the vmPFC and NAc. Interestingly, chronic alcohol exposure also robustly increased CCL2 mRNA expression in the BLA and VTA in males but not females. Thus, alcohol vapor exposure elicits sex-, region-, and duration-specific cytokine alterations that may contribute to differences in the manifestation and progression of symptoms of alcohol dependence in male and female populations. Published by Elsevier Ltd on behalf of IBRO.

Key words: alcohol, vapor, rat, cytokine, sex differences, mesocorticolimbic circuit.

INTRODUCTION

Alcohol misuse is a leading risk factor for premature death and disability globally, and creates an estimated economic burden of over $249 billion in the U.S. alone (Sacks et al., 2015). The underlying neurobiology of alcohol use disorders has been extensively studied in both clinical and preclinical settings, with alterations in the mesocorticolimbic reward/stress circuit serving a permissive role in disease progression (see Koob, 2013 for review). Repeated cycles of alcohol intoxication and withdrawal lead to significant alterations within this circuit, which gives rise to negative affective states that drive compulsive alcohol seeking during periods of abstinence. Moreover, chronic alcohol abuse results in significant neurodegeneration and neuroinflammation throughout the brain (Crews and Nixon, 2009), which has been suggested to play a key role in the manifestation of alcohol-related cognitive, appetitive, and emotional deficits (Crews and Boettiger, 2009; Crews et al., 2011). Thus, understanding the neuroinflammatory effects of alcohol consumption, particularly within the mesocorticolimbic circuit, is crucial for the identification of novel prevention and treatment strategies.

The neuroinflammatory response induced by alcohol exposure is signified by an increase in the expression of pro-inflammatory cytokines and chemokines that regulate the innate immune response (Crews et al., 2006; He and Crews, 2008; Qin et al., 2008). For instance, preclinical studies have shown that chronic alcohol administration in rodents significantly increases the expression of several cytokines, including tumor necrosis factor alpha (TNFα) and interleukin-6 (IL-6) (McClain and Cohen, 1989; Khoruts et al., 1991; Emanuele et al., 2005; Laso et al., 2007; Gonzalez-Quintela et al., 2008; Doremus-Fitzwater et al., 2014). In fact, these cytokines have recently been proposed as diagnostic biomarker candidates for identifying alcoholism in the human population (Achur et al., 2010).

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Abbreviations: BLA, basolateral amygdala; CCL2, chemokine (C–C motif) ligand 2; IL-6, interleukin-6; NAc, nucleus accumbens; TNFα, tumor necrosis factor alpha; vmPFC, ventromedial prefrontal cortex; VTA, ventral tegmental area.

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Chemotactic cytokines (termed 'chemokines') are also implicated in the inflammatory response to alcohol (Fisher et al., 1999; Blednov et al., 2005; He and Crews, 2008). For instance, chemokine (C–C motif) ligand 2 (CCL2; also known as MCP-1), which causes the migration and activation of microglia (McManus et al., 2000), plays a significant role in the motivational properties of alcohol. CCL2 knockout mice show a lower preference for alcohol and consume smaller amounts of alcohol compared to wild-type mice (Blednov et al., 2005). Moreover, in human alcoholics, CCL2 concentrations are significantly elevated in the ventral tegmental area (VTA), substantia nigra, amygdala, and hippocampus post-mortem (He and Crews, 2008), which further supports the notion that alterations in chemokine expression within the mesocorticolimbic pathway could contribute to alcohol-induced pathology and increased susceptibility to dependence.

However, the effects of alcohol on cytokine production are tissue specific and vary widely according to the duration and intensity of alcohol exposure (Crews et al., 2006). For example, whereas some cytokines such as TNFα remain persistently elevated in plasma even following periods of sustained abstinence, others such as IL-6 are more dynamic in nature and their expression varies over the course of dependence induction and withdrawal (Khoruts et al., 1991). Thus, in order to capture the progression of cytokine expression in response to alcohol, it is important to study both the short- and long-term effects of alcohol exposure on inflammatory markers and how they may contribute to alcohol dependence/withdrawal.

Additionally, important sex differences exist with respect to the prevalence, drinking patterns, and health risks associated with alcohol use disorders (Becker and Koob, 2016). Alcohol use disorders are more prevalent in men, but women who drink excessively develop more medical issues associated with alcohol abuse (Devaud and Chadda, 2001; Hommer, 2003; Epstein et al., 2007; Erol and Karpyak, 2015). There are also appreciable sex differences with respect to cytokine activation in response to inflammatory or immune challenges, with females showing heightened pro-inflammatory activity and an increased likelihood for developing autoimmune disorders (Whitacre, 2001; O'Connor et al., 2007; Chapman et al., 2009; Voskuhl, 2011; Moieni et al., 2015). Thus, it is possible that sex differences in the manifestation of alcohol use disorders could be due in part to differential recruitment of pro-inflammatory cytokines. However, there is currently a paucity of research directly comparing the neuroinflammatory response to alcohol exposure in males and females.

The objective of the current study was to evaluate the effects of acute, subchronic, and chronic alcohol exposure on TNFα, IL-6 and CCL2 mRNA expression in the VTA, nucleus accumbens (NAc), ventromedial prefrontal cortex (vmPFC), and basolateral amygdala (BLA) of male and female rats. To accomplish this objective, we used intermittent alcohol vapor administration, which produces robust alcohol dependence and withdrawal symptoms following chronic exposure while affording experimenter control over the dose, duration, and pattern of alcohol exposure (Gilpin et al., 2008). Our results indicate that cytokine mRNA expression is differentially altered in a sex-, region-, and duration-specific manner. Specifically, we show herein that TNFα mRNA expression is elevated in the NAc, BLA, and VTA following acute and subchronic alcohol vapor exposure and remains persistently elevated in the NAc following 6 weeks of intermittent exposure. Moreover, male rats are particularly susceptible to acute and chronic alcohol-induced elevations in IL-6 and CCL2 mRNA expression, respectively. Thus, sex-specific alterations in pro-inflammatory cytokines/chemokines may contribute to the manifestation and progression of alcohol use disorders in males and females.

EXPERIMENTAL PROCEDURES

Animals

Male and female Wistar rats (n = 4–8/sex/group) were obtained from Simonsen Laboratories (Santa Clara, CA, USA) and arrived at 60–65 days old. Animals were group-housed according to sex, with 2–3 animals per cage, given food and water access *ad libitum* and weighed and handled daily. All procedures were performed according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved by the Washington State University Institutional Animal Care and Use Committee.

Alcohol vapor exposure

Three schedules of alcohol vapor exposure were used for this experiment: acute (single 14-h exposure), subchronic (1-week intermittent exposure), chronic (6-week intermittent exposure). These time points were chosen to provide a temporal indication of dependence development. For rats subjected to subchronic or chronic alcohol vapor exposure, we used a chronic intermittent alcohol vapor exposure regimen as described previously (Gilpin et al., 2008). This method induces phenotypic and neurobiological changes reflective of alcohol dependence (see Vendruscolo and Roberts, 2014 for review). Intermittent alcohol vapor exposure produces symptoms of alcohol dependence in as little as 2 weeks (O'Dell et al., 2004), while 4–8 weeks typically produces strong dependence and withdrawal symptoms (Rimondini et al., 2003; Funk et al., 2007). With this in mind, we examined cytokine expression after 1-week and 6-week exposure to make comparisons pre- and post-dependence induction. The alcohol vapor exposure paradigm (14 h on/10 h off; 7 days/week) was implemented as described previously (Williams et al., 2012; Vendruscolo and Roberts, 2014; Henricks et al., 2016). The amount of alcohol that rats were exposed to was adjusted by altering the rate of vaporized 95% alcohol introduced into the airflow. Desired blood alcohol level (BAL) range was 175–250 mg%, and was determined daily during the first week by collecting blood via a tail nick (~50 μl). Thereafter, BALs were measured 1–2 times per week, as well as on testing days. Blood samples were centrifuged, after which alcohol content in plasma frac-