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Male adolescent rats display blunted cytokine responses in the CNS after acute ethanol or lipopolysaccharide exposure☆

Tamara L. Doremus-Fitzwater *, Anny Gano, Jacqueline E. Paniccia, Terrence Deak

Developmental Exposure Alcohol Research Center (DEARC), Behavioral Neuroscience Program, Department of Psychology, Binghamton University, Binghamton, NY 13902-6000, United States

HIGHLIGHTS

- LPS increased IL-6, IL-1, TNFα, and IκBα expression in hippocampus, PVN, and amygdala.
- Adolescents had lower LPS-related increases in cytokine expression versus adults.
- Plasma endotoxin was increased by LPS exposure in adults, but not adolescents.
- Ethanol did not alter plasma endotoxin, but elevated brain IL-6 and IκBα in both ages.
- Adolescents exhibited attenuated increases in IL-6 and IκBα expression by ethanol.

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ABSTRACT

Alcohol induces widespread changes in cytokine expression, with recent data from our laboratory having demonstrated that, during acute ethanol intoxication, adult rats exhibit consistent increases in interleukin (IL)-6 mRNA expression in several brain regions, while showing reductions in IL-1 and TNFα expression. Given evidence indicating that adolescence may be an ontogenetic period in which some neuroimmune processes and cells may not yet have fully matured, the purpose of the current experiments was to examine potential age differences in the central cytokine response of adolescent (P31–33 days of age) and adult (69–71 days of age) rats to either an acute immune (lipopolysaccharide; LPS) or non-immune challenge (ethanol). In Experiment 1, male Sprague–Dawley rats were given an intraperitoneal (i.p.) injection of either sterile saline, LPS (250 μg/kg), or ethanol (4-g/kg), and then trunk blood and brain tissue were collected 3 h later for measurement of blood ethanol concentrations (BECs), plasma endotoxin, and central mRNA expression of several immune-related gene targets. In Experiment 2, the response to intragastrically (i.g.) administered ethanol was examined and compared to animals given tap water (i.g.). Results showed that LPS stimulated robust increases in expression of IL-1, IL-6, TNFα, and IκBα in the hippocampus, PVN, and amygdala, and that these increases were generally less pronounced in adolescents relative to adults. Following an i.p. ethanol challenge, IL-6 and IκBα expression was significantly increased in both ages in the PVN and amygdala, and adults exhibited even greater increases in IκBα than adolescents. I.g. administration of ethanol also increased IL-6 and IκBα expression in all three brain regions, with hippocampal IL-6 elevated even more so in adults compared to adolescents. Furthermore, assessment of plasma endotoxin concentrations revealed (i) whereas robust increases in plasma endotoxin were observed in adults injected with LPS, no corresponding elevations were seen in adolescents after LPS; and (ii) neither adolescents nor adults demonstrated increases in plasma endotoxin concentrations following i.p. or i.g. ethanol administration. Analysis of BECs indicated that, for both routes of exposure, adolescents exhibited lower BECs than adults. Taken together, these data suggest that categorically different mechanisms are involved in the central cytokine response to antigen exposure versus ethanol administration. Furthermore, these findings confirm once again that acute ethanol intoxication is a potent activator of brain cytokines, and calls for future studies to identify the mechanisms underlying age-related differences in the cytokine response observed during ethanol intoxication.

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* Corresponding author at: Behavioral Neuroscience Program, Department of Psychology, Binghamton University-State University of New York, Binghamton, NY 13902-6000, United States.

E-mail address: tlfitz@binghamton.edu (T.L. Doremus-Fitzwater).

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1. Introduction

Ethanol exposure has been recognized as significantly influencing a variety of neurotransmitter systems, including but not limited to GABA, glutamate, dopamine, serotonin, and endocannabinoid systems (for review see [1,2]). Yet, research has now demonstrated that ethanol can also profoundly impact inflammatory-related processes, both peripherally as well as in the brain. Historically, the role of inflammatory pathways in alcohol-related liver disease and organ damage has been the focus of research, with studies in this area commonly examining the effects of acute or chronic ethanol exposure on expression of immune factors after an antigen challenge. Results from experiments such as these have shown a complex relationship between ethanol exposure and the immune response, with ethanol sometimes dampening cytokine expression in response to an antigen [e.g., 3–6], yet exacerbating the cytokine response to bacterial challenge in other instances [e.g., 7–9].

Studies of this nature have been crucial in elucidating the mechanisms responsible for organ damage under conditions of chronic ethanol consumption, as well as an enhanced susceptibility to infection that is often observed among alcoholics (for review see [10]). More recently, however, evidence indicates that ethanol can also dramatically alter inflammatory-related factors in the absence of an immune challenge. In humans, for example, elevations in several plasma cytokines were observed during withdrawal following an acute alcohol challenge [11,12]. Additionally, post-mortem examination of the brains of alcoholics demonstrated that monocyte chemotactic protein (MCP)-1, a chemokine, was increased in the ventral tegmental area, substantia nigra, hippocampus, and amygdala, with microglial markers also increased in certain brain regions [13]. In parallel, chronic ethanol administration or long term ethanol consumption has been shown to significantly elevate expression of a variety of cytokines in the brain, including interleukin (IL)-1β, IL-6, tumor necrosis factor alpha (TNFα), and MCP-1 in both rats [14,15] and mice [8,16,17]. Furthermore, increased expression of these inflammatory factors has been observed across several different brain regions such as the hippocampus [e.g., 14], cortex [e.g., 16], and cerebellum [e.g., 18].

While studies such as those described above have consistently demonstrated that chronic ethanol exposure can influence inflammatory factors, other research has shown that manipulation of neuroimmune pathways/processes can alter ethanol intake, ethanol responsivity, and ethanol reward/reinforcement. Studies utilizing knock-out mice have indicated that deletion of several immune-related genes resulted in significant reductions in ethanol intake and preference when compared to wild-type mice, while also increasing sensitivity to ethanol-induced conditioned taste aversion [19,20]. Furthermore, when mice were injected with minocycline (a putative microglia inhibitor), ethanol intake was similarly reduced [21]. In contrast, however, stimulation of immune processes by lipopolysaccharide (LPS) administration led to increased ethanol intake in mice [22]. Moreover, acute ethanol-induced sedation and motor impairment were also affected by alterations in cytokine signaling, as administration of minocycline or interleukin-1 receptor antagonist (IL-1ra) to adult mice differentially impacted these measures of ethanol responsivity [23].

Given evidence that alterations in neuroimmune pathways have been shown to influence ethanol responsivity and intake, it is not surprising that the effects of ethanol exposure during adolescence on immune-related factors have now begun to receive attention. Indeed, adolescence is now known to be an ontogenetic period characterized by elevated ethanol consumption [24,25], as well as a unique sensitivity to ethanol (for review see [26]). For example, using an animal model of adolescence (for review see [27]), studies have shown that adolescent rodents are less sensitive to many consequences of ethanol exposure, including the sedative/hypnotic [28], motor impairing [29], hangover [30], aversive [31], and social inhibitory [32] effects. Yet, on the other hand, adolescents are seemingly more sensitive to other consequences of ethanol exposure, such as ethanol-induced memory impairment [33], ethanol-related deficits in hippocampal LTP [34], and the social stimulatory effects of acute ethanol exposure [32]. As these studies identified the sensitivity of the hippocampus to adolescent ethanol exposure, several research groups have begun to explore the effects of adolescent binge ethanol administration on the hippocampus, as well as other brain regions known to undergo profound neurodevelopmental changes during adolescence. For instance, McClain et al. (2011) reported that adolescent binge ethanol exposure led to an upregulation of partially activated microglia (the resident macrophage of the brain) in the hippocampus [35]. Additionally, BrdU/Iba-1 co-labeling demonstrated that adolescent binge ethanol led to increased proliferation of new microglia in this brain region, with these microglia remaining up to 30 days after ethanol exposure [35]. Crews and colleagues have also reported that a similar adolescent binge ethanol exposure upregulated RAGE/TLR-4 and HMGB1 in the PFC of adolescent rats, as well as gene expression of numerous other neuroimmune factors, and these effects persisted well into adulthood [36,37]. Notably, a recent study [38] would suggest that some of these effects of adolescent binge ethanol administration are specific to exposure during adolescence, as an ethanol-related up-regulation of expression of TLR-2, TLR-4, IL-1, and TNFα in the PFC was only observed in adolescent but not adult mice under these circumstances.

While studies such as these have demonstrated that adolescent rodents exhibit significant alterations in neuroimmune processes following chronic binge-like ethanol exposure, there have been few studies in which adolescents and adults have been directly compared, and even fewer studies investigating effects of acute ethanol exposure. We have recently shown that marked changes in cytokine gene expression are apparent in the adult brain during the first ethanol exposure, with these changes dose-, time-, and regionally-dependent in nature [39]. More specifically, an acute 4 g/kg ethanol challenge consistently and significantly increased expression of IL-6 during intoxication in the paraventricular nucleus of the hypothalamus (PVN), hippocampus, cerebellum, and amygdala, whereas decreased expression of IL-1 and TNFα was generally observed in these same structures during intoxication [39]. In contrast, withdrawal from an acute ethanol challenge was not shown to result in marked alterations in cytokine expression, with IL-1 expression sometimes elevated relative to non-ethanol-exposed controls.

As (i) adolescence is a developmental period during which the first exposure to alcohol typically occurs, (ii) acute ethanol exposure has been shown to significantly alter cytokine expression in adults, and (iii) binge ethanol exposure during adolescence results in significant alterations in immune processes, the primary purpose of the current series of experiments was to directly compare central cytokine responses of adolescent and adult rats to an acute ethanol challenge. In doing so, it was deemed necessary to include a more fundamental comparison of antigen exposure in both adolescents and adults to help inform how neuroimmune responses to naturally occurring immunogens might vary in these two age groups. Thus, rats of both ages were exposed to either LPS, ethanol, or vehicle in a controlled series of experiments, and potential age differences in plasma endotoxin responses evoked by LPS or ethanol were examined. Data from these animals also served as a positive verification of cytokine detection procedures employed in subsequent analyses of ethanol-exposed rats. Considering recent findings that showed ethanol administration via gastric gavage increased apparent endotoxin concentration in blood obtained from the hepatic portal vein [40,41], separate groups of rats received an acute ethanol challenge via either an i.p. or i.g. route of administration. These groups provided a key comparison between intra-lumenal (i.g.) versus abdominal (i.p.) ethanol as potential drivers of endotoxin transit into blood, and whether such responses might differentially impact the action of ethanol on brain cytokines following these two commonly used modes of ethanol administration. Our a priori hypothesis was that ethanol exposure (regardless of route or age) would evoke central cytokine changes consistent with