Published in final edited form as:

Alcohol Clin Exp Res. 2014 August ; 38(8): 2186–2198. doi:10.1111/acer.12481.

Intoxication- and withdrawal-dependent expression of central and peripheral cytokines following initial ethanol exposure

Tamara L. Doremus-Fitzwater, Hollin M. Buck, Kelly A. Bordner, Laura Richey, Megan E. Jones, and Terrence Deak*

Behavioral Neuroscience Program, Department of Psychology, Binghamton University—SUNY, Binghamton NY 13902-6000

Abstract

Background—Evidence has emerged demonstrating that ethanol influences cytokine expression within the CNS, although most studies have examined long-term exposure. Thus, the cytokine response to an acute ethanol challenge was investigated, in order to characterize profiles of cytokine changes following acute exposure.

Methods—Rats pups were injected intraperitoneally (i.p.) with 2-g/kg ethanol and IL-1 mRNA and protein assessed 0, 60, 120, 180, and 240 min post-injection (Exp. 1). In Exps. 2-5, the expression of several cytokines was examined in adult male rats during acute intoxication (3 hr after 4-g/kg ethanol), as well as withdrawal (18 hr post-injection), after i.p. and intragastric (i.g.) ethanol administration.

Results—Early in ontogeny, acute ethanol significantly decreased brain IL-1 mRNA and protein. Subsequently, when adult rats were examined, significant and temporally dynamic alterations in central and peripheral cytokines were observed following acute i.p. ethanol exposure (4-g/kg). Although cytokine- and region-dependent, central IL-6 expression was generally increased and TNF α decreased during intoxication, whereas IL-1 expression exhibited increases during withdrawal. In the periphery, acute i.p. ethanol elevated expression of all cytokines, with the response growing in magnitude as the time post-injection increased. Following acute i.g. ethanol (4-g/kg), intoxication-related increases in IL-6 expression were again observed in the PVN, although to a lesser extent. Long-term, voluntary, intermittent ethanol consumption resulted in tolerance to the effects of an i.g. ethanol challenge (4-g/kg) on PVN IL-6 expression, whereas these same elevations in IL-6 expression were still seen in the amygdala in rats with a history of moderate ethanol intake. Treatment with minocycline did not significantly attenuate i.p. or i.g. ethanol-induced changes in central cytokine expression.

Conclusions—Together, these studies provide a foundation for understanding fluctuations in central and peripheral cytokines following acute ethanol as potential contributors to the constellation of neural and behavioral alterations observed during ethanol intoxication and withdrawal.

*Address Correspondence to: Terrence Deak, Ph.D., Behavioral Neuroscience Program, Department of Psychology, Binghamton University-State University of New York, Binghamton, NY 13902-6000, tdeak@binghamton.edu, Phone: +1 (607) 777-5918, Fax: +1 (607) 777-4890.

The authors have no conflicts of interest to declare.

Keywords

ethanol; rat; cytokine; brain; minocycline

Introduction

Given the number of people that struggle with alcohol use and abuse disorders (e.g., Grant et al., 2004), it is imperative that mechanisms contributing to the onset of use, transition to problem drinking, and maintenance of abusive behaviors be identified, such that preventative and therapeutic strategies may be improved. One possible pathway through which the neural and behavioral consequences of alcohol exposure may be influenced is alcohol-related alterations in cytokines. Cytokines are a classification of small proteins, including chemokines, interleukins, and lymphokines, which are known to be important for initiation of the immune response to infection or damage. Several cell types within the CNS such as microglia, perivascular and meningeal macrophages (van Dam et al., 1992), astrocytes (Martinez et al., 1992), and neurons (Tringali et al., 1996), have been shown to produce cytokines, with cytokine receptors identified on nearly all cell types within the brain (Vitkovic et al., 2000). Importantly, however, cytokines have been recognized as having roles outside of neuroinflammation, as they are expressed in both neurons and glia during non-pathological conditions (for review see Vitkovic et al., 2000), and are also recruited in response of non-immunogenic stimuli, such as stress (Hueston et al., 2011).

While there is a vast literature demonstrating an interaction between ethanol and immune pathways following antigen exposure, growing evidence suggests ethanol-related alterations in peripheral or brain cytokines in the absence of an immunological challenge (for review see Deak et al., 2013). For example, chronic ethanol administration or voluntary consumption in rats significantly elevated cytokine expression in the hippocampus and cortex (Tiwari et al., 2009; Valles et al., 2004). Similarly, studies using mice have demonstrated that acute (Qin et al., 2008) and chronic (Alfonso-Loeches and Guerri, 2011; Kane et al., 2014; Qin et al., 2008) ethanol exposure can significantly impact central cytokines. Furthermore, other research indicates that modifications to inflammatory-related pathways may alter ethanol responsiveness, as well as ethanol's rewarding/reinforcing effects. In mice, administration of minocycline (a putative microglia inhibitor) reduced ethanol intake when compared to controls (Agrawal et al., 2011), whereas immune stimulation via systemic injection of lipopolysaccharide (LPS) resulted in long-term increases in ethanol consumption (Blednov et al., 2011). These effects were linked to toll-like receptor (TLR) 4 signaling, since null mutant mice lacking CD14 (a pathogen recognition receptor and TLR 4 co-receptor) did not exhibit LPS-related increases in ethanol intake (Blednov et al., 2011). Furthermore, mice lacking inflammatory-related genes such as CD14, IL-6, and IL-1ra, as well as genes for several chemokines and chemokine receptors, demonstrated reduced ethanol consumption and preference, and increased sensitivity to ethanol-induced conditioned taste aversion (Blednov et al., 2005; Blednov et al., 2012). Behavioral responsiveness to acute ethanol was also affected by alterations to cytokine signaling, as administration of minocycline or IL-1ra to adult mice differentially impacted acute sedative and motor-impairing effects of an ethanol challenge (Wu et al., 2011).

While many of these effects appear to occur during intoxication, ethanol withdrawal has also been associated with alterations in central cytokine expression. For example, when healthy adult male subjects were asked to drink 1.5 g/kg of alcohol, elevated levels of plasma IL-10, IL-12, and interferon (IFN) γ were observed 13 hr later during acute withdrawal (Kim et al., 2003). In another study, serum IL-8 levels were significantly increased in healthy non-alcoholic volunteers 36 hr after an acute alcohol challenge (Gonzalez-Quintela et al., 2000). Ethanol withdrawal-related changes in cytokines have been observed in animal models as well, with hypothalamic IL-1, IL-6 and tumor necrosis factor (TNF) α protein levels significantly increased 48 hr post-ethanol injection in adult male mice (Emanuele et al., 2005). Other data has revealed that severity of ethanol withdrawal can be impacted by cytokines. Using a model of social anxiety, Breese and colleagues demonstrated that central injection of several cytokines (e.g., IL-1, TNF α) augmented ethanol withdrawal-associated anxiety, similar to peripheral administration of LPS (Breese et al., 2008), likely through CRF-related mechanisms (Knapp et al., 2011).

Given these indications that ethanol interacts with central inflammatory pathways, the purpose of the current series of experiments was to characterize the time-course of ethanol-induced alterations in central cytokine expression following acute ethanol challenge, as there is a dearth of information regarding in vivo central cytokine changes in ethanol-naïve animals. To do this, ethanol-induced alterations in several cytokines were first examined following intraperitoneal (i.p.) administration of a 4-g/kg ethanol challenge across multiple time points, brain regions, and peripheral organs, followed by a targeted comparison to ethanol-induced changes in hypothalamic cytokine expression with intragastrically administered ethanol. Ethanol delivered by either route resulted in profound changes in several cytokines, which were dependent upon time point, region of interest, and cytokine examined. Given previously described evidence of microglial involvement in ethanol responsivity and intake, microglial activation was hypothesized to be a potential mechanism through which ethanol could impact cytokine expression. Thus, we explored whether minocycline might also block the effects of acutely delivered ethanol on brain cytokine expression. Lastly, we expanded our focus beyond acute exposure, and investigated the effects of long-term voluntary ethanol intake on ethanol-induced changes in central cytokine expression evoked by an acute ethanol challenge.

Materials and Methods

General Methods

Subjects—Early postnatal male Sprague-Dawley pups were obtained from a breeding colony at Binghamton University (Bordner et al., 2008), with the day of birth deemed postnatal day (P) 0 and all experimental procedures conducted on P4. Adult male Sprague-Dawley rats (350-450 g; P65-P70) used in Exps. 2-4 were purchased from Harlan Laboratories and acclimated to the colony for 1-2 weeks. Rats were briefly handled (3-5 min) for 2 days prior to the start of an experiment. Colony conditions were maintained at 22 $\pm$ 1°C on a 14:10 light:dark cycle (lights on 06:00h). Animals were pair-housed in standard Plexiglas bins with *ad libitum* access to food and water. In all experiments, animals were maintained and treated in accordance with the guidelines set forth by the Institute of

Laboratory Animal Resources (1996), and with protocols approved by the IACUC committee at Binghamton University.

Drugs—For i.p. administration, ethanol (95%) was diluted fresh daily with pyrogen-free physiological saline to a final concentration of 20% (v/v), and sterile saline alone used as vehicle. When delivered i.g., ethanol was mixed with tap water (20% v/v), with tap water alone delivered isovolumetrically to the ethanol intubations as the vehicle. In Exp. 3, minocycline (Sigma Corporation) was diluted fresh daily with pyrogen-free physiological saline (37°C) and administered at a final dose of 40 mg/kg (i.p.), whereas minocycline was mixed directly with the ethanol solution and administered i.g. at 60 mg/kg in Exp. 5.

Blood and tissue collection—Animals were rapidly decapitated (unanesthetized) at the noted time points and trunk blood collected into EDTA-coated Vacutainers. Plasma was separated through refrigerated centrifugation and stored at -20°C until time of assay. In Exps. 1 and 2, brain and peripheral tissues were quickly removed and dissected on a cold plate, with tissue used for assessment of protein flash frozen and stored at -80°C, and tissue used for assessment of mRNA expression stored in RNALater at -20°C. In Exps. 3 - 5, brain structures were microdissected from 2mm coronal slices and stored in RNALater at -20°C (see Blandino et al., 2013).

Reverse-transcription Polymerase Chain Reaction—In Exp. 1, RT-PCR was conducted using procedures previously described (Blandino et al., 2009; Deak et al., 2005). Real-time RT-PCR was acquired and subsequently used in Exps. 2–5 (e.g., Blandino et al., 2009; Hueston et al., 2011). Please refer to supplemental text for more detailed methodology concerning measurement of gene expression.

Measurement of IL-1 β protein—For the assessment of IL-1 β protein in Exp. 1, a commercially available ELISA kit (R&D Systems, Minneapolis, MN) was used (Deak et al., 2005). Total protein content was measured in each sample using the method of Bradford (Bradford, 1976). Data were expressed as pg of IL-1/100 μ g total protein.

Corticosterone (CORT) Concentrations—Ethanol exposure has been shown to elicit a robust CORT response and, importantly, corticosteroids have a profound impact on cytokine expression (Busillo and Cidlowski, 2013). Thus, given that the HPA axis response is a potential mechanism by which ethanol exposure might influence cytokine expression, plasma CORT measurements were taken in most studies. Plasma CORT was measured via radioimmunoassay (RIA) in Exp. 2 (Deak et al., 2003; Deak et al., 2005), and via an enzyme immunoassay (EIA) kit (Assay Designs; Ann Arbor, MI) in Exp. 4 (Buck et al., 2011).

Blood Ethanol Concentrations (BECs)—In Exps. 1 and 2, BECs were assessed using gas chromatography (see Varlinskaya et al., 2010), whereas BECs were determined using an Analox AM-1 alcohol analyzer in Exps. 3-5 (Analox Instruments, Lunenburg, MA) (as described elsewhere Buck et al., 2011).

Experiment 1: Alterations in expression of cytokine protein and mRNA in rat pups following a 2-g/kg ethanol challenge—While ethanol-induced changes in

peripheral immune responses have been consistently demonstrated, only more recently have ethanol-related alterations in central immune factors been observed (e.g., Kane et al., 2014; Qin et al., 2008; Valles et al., 2004). As an initial examination of acute ethanol effects on central cytokine expression, rat pups (P4; n = 8/group; N = 40) were injected with acute ethanol (2-g/kg i.p.) or saline and brain IL-1 protein and mRNA expression measured in Experiment 1. Trunk blood and brain tissues were collected 30, 60, 120 or 240 min after injection. Brains were hemisected sagittally at the midline, with half used for protein measurement and the other half used to assess mRNA expression.

Experiment 2: Time-course of cytokine changes in adult rats after a 4-g/kg ethanol challenge—Experiment 1 demonstrated attenuated levels of IL-1 message and protein within the CNS of rat pups during acute intoxication. Experiment 2, therefore, examined whether acute ethanol exposure would impact cytokine expression in adult brain. Thus, acute ethanol intoxication- and withdrawal-associated changes in cytokine expression were examined within several brain regions (hypothalamus, hippocampus, cerebellum), as well as in the periphery (spleen and liver), in adult rats. More specifically, adults (n = 8/group; N = 40) were injected with 4-g/kg ethanol (i.p.) and returned to their home cages until the time of tissue harvest (3, 9, 15, or 18 hr following injection). To minimize animal use, saline-injected (n = 8) rats were evenly distributed across the 4 time points of tissue collection and collapsed into one control group for analysis.

Experiment 3: Effect of minocycline on cytokine changes during ethanol intoxication and withdrawal—Experiment 2 revealed significant alterations in expression of several cytokines across multiple time points, and in a variety of peripheral and brain sites, following acute ethanol exposure. Although the cell type in which these cytokines were expressed remains unclear, functional studies using putative inhibitors of microglial activity, such as minocycline, have implicated microglia as important mediators of neuroinflammatory-like consequences of chronic ethanol exposure (e.g., Fernandez-Lizarbe et al., 2013; Qin and Crews, 2012), as well as stress (Blandino et al., 2006; Blandino et al., 2009). Thus Experiment 3 investigated whether acute ethanol-related alterations in cytokine expression would be blocked by minocycline, thereby implicating microglia in the cytokine response evoked by acute ethanol.

Rats (n = 8-10/group; N = 50) were injected with 40 mg/kg of minocycline (i.p.) or saline at a volume of 1 ml/kg. One hr later, animals were challenged with ethanol (4-g/kg, i.p.) or saline. Tissue was harvested either 3 or 18 hr following ethanol administration—time points that corresponded with maximal alterations in cytokine expression in Exp. 2. Rats challenged with saline on their second drug exposure were killed either 3 or 18 hr later, and then ultimately collapsed into one control group. In order to gain greater spatial resolution, the paraventricular nucleus of the hypothalamus (PVN) was examined. In prior studies, the PVN has been shown to be a key hypothalamic nucleus that is responsive to stress-induced alterations in central cytokines (Hueston et al., 2011), with minocycline effectively blocking stress-related increases in PVN IL-1 expression (Blandino et al., 2006). Thus, given the sensitivity of the hypothalamus demonstrated in Exp. 1, and the responsivity of this structure to minocycline, analyses were focused on cytokine changes within this brain region.

Experiment 4: Time-course of cytokine changes after intragastric challenge

with ethanol—Results from Experiment 2 demonstrated dynamic changes in the expression of several cytokines during both intoxication and withdrawal from an acute ethanol injection delivered i.p. After observing these ethanol-induced effects specifically within the PVN in Exp. 3, we then assessed acute ethanol effects on central cytokine expression following i.g. delivery of an ethanol challenge. Again, analyses were focused on a single, highly responsive brain structure (PVN).

Rats (N = 32) were administered an i.g. ethanol challenge (4 g/kg) and killed 3 (n = 6), 10 (n = 6) or 14 (n = 6) hr following intubation. A control group of water-exposed animals were counterbalanced across time-points and collapsed into a single group for comparison (n = 14). Trunk blood was collected and later analyzed for BECs. Given the sensitivity of hypothalamus (more specifically, PVN) to ethanol-induced alterations in cytokine expression using the i.p. route of administration, we focused exclusively on the PVN.

Experiment 5: The impact of long-term voluntary ethanol consumption on acute ethanol-induced alterations in central cytokine expression

—Given that ethanol-naïve animals exhibited profound and consistent alterations in central cytokine expression following acute ethanol challenge, in Experiment 5, we sought to examine whether long-term voluntary ethanol consumption would alter the cytokine response evoked later by a controlled, acute, ethanol challenge.

Long-term ethanol intake procedure—A voluntary ethanol consumption procedure originally introduced by Wise (1973), and more recently utilized by others (e.g., Carnicella et al., 2009; Simms et al., 2008), was used. Rats (N = 24) were given unsweetened ethanol (20% v/v in tap water) every Monday, Wednesday and Friday. Each rat was individually presented with 24-hr continuous-access to a 2-bottle choice between water and ethanol in graduated, glass, drinking tubes (Ancare, Bellmore, NY) with ball-bearing spouts. Ethanol solutions were prepared fresh daily, with the positions of the water and ethanol bottles alternated each day. Ad libitum food was available throughout. At the start of a drinking session, pair-housed rats were weighed and then separated by a perforated divider (e.g., Vetter-O'Hagen et al., 2011). After 24 hr, the divider was removed and 2 bottles of water were provided. Dividers were only inserted into the cage during times of ethanol access. In total, rats received 30 24-hr presentations of the ethanol solution. As 3 sessions were conducted per week, the intermittent ethanol drinking procedure lasted for 10 weeks. An additional group of rats (n = 8) experienced similar treatment (handling, weighing, bottle changes/measurements, etc.) as the ethanol groups, but were only given access to 2 bottles containing water.

Acute ethanol challenge—After the final intake session, rats drinking ethanol were assigned to one of three groups: (1) administered an acute i.g. 4-g/kg ethanol challenge (E-E); (2) intubated with tap water (E-V); or (3) given an i.g. ethanol challenge containing 60-mg/kg minocycline (E-E/M), such that average ethanol intake was comparable for all groups. The ethanol-naïve rats (given only water during the intake phase) also received an i.g. ethanol intubation on the challenge day (W-E). The drug challenge was administered 72

hr after the last intake session, with brains harvested and trunk blood collected 3 hr later. Bilateral PVN, dorsal hippocampus, and amygdala samples were later microdissected.

Results

Experiment 1

Data were analyzed with 1-way ANOVAs, with Fisher's LSD used to determine the locus of significant effects. Ethanol administration significantly increased BECs at all time points [$F(4,34) = 118.62, p = 0.00001$]. Peak concentrations were observed at 30-min, with BECs declining thereafter (Figure 1a). Analysis of IL-1 (Figure 1b) revealed significant reductions in mRNA expression 30 and 60 min after ethanol when compared to saline controls [$F(4,29) = 4.36, p = 0.01$]. A similar trend for ethanol-induced reduction in IL-1 protein (Figure 1c) was observed (although not significant), with protein and mRNA expression significantly correlated ($r = +0.511, n = 34, p < .01$). Protein levels were likely at the floor of assay sensitivity, however, thereby precluding detection of treatment-induced reductions. Thus, ethanol-exposed animals were collapsed across time points and compared to saline controls. While there are certainly limitations to this approach, results revealed that IL-1 protein was significantly reduced relative to controls [$t(37) = 2.26, p = 0.05$].

Experiment 2

All data were analyzed using 1-way ANOVAs. Fisher's LSD post hoc tests identified significant group differences.

Central mRNA Expression—In the hypothalamus (Figure 2A, D, G), IL-6 was elevated 3 and 9 hr after ethanol, whereas TNF α was significantly decreased at these times compared to controls. 15 and 18 hr following ethanol injection, however, IL-1 was significantly elevated, as was TNF α at 18 hr post-administration. IL-6 expression was unchanged at these later time points relative to saline-exposed rats [IL-1: $F(4,35) = 6.18, p = 0.001$; IL-6: $F(4,34) = 6.81, p = 0.001$; TNF α : $F(4,33) = 18.69, p = 0.000001$].

In the hippocampus (Figures 2B, E, H), IL-1 was significantly elevated during withdrawal (15 hr), whereas IL-6 was increased during intoxication (3 and 9 hr) [IL-1: $F(4,33) = 2.86, p = 0.05$; IL-6: $F(4,35) = 18.25, p = 0.00001$]. In this structure, IL-6 was also significantly increased at 18 hr. In contrast, TNF α expression was generally suppressed by ethanol administration (i.e., at 3, 9, and 18 hr) [$F(4,34) = 19.21, p = 0.00001$].

When examining the cerebellum, ethanol significantly suppressed IL-1 and TNF α at 3 hr and at 3 and 9 hr, respectively (Figures 2C, I) [IL-1: $F(4,33) = 8.02, p = 0.001$; TNF α : $F(4,34) = 11.60, p = 0.00001$]. Additionally, IL-6 (Figure 2F) was significantly increased 9 hr after the ethanol bolus, with a trend for increased IL-6 expression at 3 hr post-injection ($p = 0.051$) [$F(4,31) = 4.12, p = 0.01$].

Peripheral mRNA Expression—IL-1 expression in both spleen [$F(4,33) = 40.61, p = 0.000001$] and liver [$F(4,34) = 10.32, p = 0.0001$] (Figure 3A, B respectively) was significantly increased 9, 15, and 18 hr after ethanol administration when compared to controls. Significant increases in IL-6 were seen at 15 and 18 hr in the spleen (Figure 3C)

[$F(4,35) = 9.37, p = 0.0001$] and at 18 hr in the liver (Figure 3D) [$F(4,25) = 3.82, p = 0.05$]. Withdrawal-related increases in TNF α expression were also observed at 18 hr in the spleen (Figure 3E) [$F(4,32) = 6.06, p = 0.001$] and at both 15 and 18 hr in the liver (Figure 3F) [$F(4,34) = 9.24, p = 0.0001$].

BECs and CORT—Ethanol significantly elevated BECs at 3, 9, and 15 hr relative to saline-exposed rats, with BECs comparable to controls by the 18 hr time point [$F(4,34) = 220.76, p = 0.001$] (Figure 3G). In the analysis of plasma CORT (Figure 3H), ethanol significantly increased CORT 3 hr post-injection when compared to saline controls [$F(4,35) = 7.18, p = 0.001$] and, although there was also a trend for CORT to remain elevated 9, 15, and 18 hr after ethanol, these increases were not statistically significant.

Experiment 3

PVN data were analyzed using a 2 (Drug: minocycline vs. vehicle) $\times$ 3 (Ethanol Condition: saline vs. 3 hr vs. 18 hr post-ethanol) factorial ANOVA design. No significant main effects or interactions involving minocycline emerged in these analyses. A main effect of Ethanol Condition was observed for IL-6, IL-1 and TNF α [$F(2,42) = 21.42, p = 0.00001$; $F(2,42) = 10.02, p = 0.001$; and $F(2,42) = 98.99, p = 0.00001$, respectively], with IL-6 (Figure 4A) significantly elevated at 3 hr and not different than controls by 18 hr post-injection. When IL-1 and TNF α were examined (Figures 4B, C), both exhibited significant decreases in expression at 3 hr, with levels returning to baseline by 18 hr. Analysis of BECs (Figure 4D) revealed significantly elevated BECs at 3 hrs, and no statistical differences at 18 hr [$F(2,44) = 187.29, p = 0.00001$].

Experiment 4

Data were analyzed using 1-way ANOVA, with Fisher's LSD post hoc tests. As seen in Table 1, BECs were increased, yet returned to baseline by 10 and 14 hr [$F(3,27) = 52.29, p = 0.0001$]. In the PVN, IL-6 was significantly increased 3 hr after intubation [$F(3,25) = 5.704, p = .01$], whereas IL-1 and TNF α were unchanged at all times examined.

Experiment 5

Ethanol Intake—Ethanol intake significantly increased across the 30 drinking sessions when assessed on both a g/kg and percent preference basis. After 24 hr of access (Figures 5B, D, F), rats were drinking approximately 4-g/kg of ethanol by the last intake day—roughly 25 ml/kg of ethanol. Water intake (Figure 5D) conversely decreased throughout the study, resulting in increased preference from 10% to over 30%. When intake was examined during the first 30-min of access (Figures 5A, C, and E), data revealed that rats were consuming approximately 0.5 g/kg (or 4 ml/kg) of ethanol by the final drinking day, with preference nearly 80% during these first few minutes of access.

Central Cytokine Expression—Compared to rats that received water intubation on the challenge day (E-W), animals with no drinking history that were given an acute ethanol challenge (W-E) exhibited a significant increase in IL-6 expression in the PVN (Figure 6D), with E-E and E-E/M rats not showing this elevation in IL-6 [$F(3,28) = 4.12, p = 0.05$]. In contrast, all groups administered an ethanol challenge (i.e., W-E, E-E, E-E/M) demonstrated

significant reductions in IL-1 expression in the PVN (Figure 6A) relative to rats that were given a tap water intubation (E-W) [$F(3,28) = 4.19, p = 0.05$], with a trend for a reduction in TNF α after ethanol intubation ($p = 0.078$) also observed (Figure 6G). In the hippocampus, an acute ethanol challenge decreased IL-1 expression [$F(3,26) = 5.63, p = 0.01$] in the W-E group relative to the E-W group (Figure 6B), while also decreasing TNF α expression [$F(3,28) = 7.03, p = 0.01$] in all ethanol-challenged groups (W-E, E-E, E-E/M) compared to the water-treated controls (Figure 6H). IL-6 expression, however, was increased in this structure [$F(3,28) = 3.25, p = 0.05$] for animals in the E-E/M group compared to water-intubated controls (E-W rats; Figure 6E). Examination of the amygdala demonstrated that IL-1 was generally decreased (W-E, E-E, and E-E/M groups; Figure 6C) [$F(3,27) = 11.96, p = 0.0001$], whereas IL-6 was increased (W-E and E-E groups) [$F(3,27) = 3.32, p = 0.05$] by ethanol challenge compared to acute water exposure (E-W group; Figure 6F).

BECs and CORT—CORT concentrations following ethanol challenge were comparable across all 3 ethanol-exposed groups, with these animals also exhibiting significantly greater CORT than rats that received a water challenge (E-W group) [$F(3,28) = 6.75, p = 0.01$] (Figure 6J). Although there was a trend for a history of ethanol consumption (E-E and E-E/M groups) to decrease BECs relative to control animals (W-E rats), no statistically significant differences in BECs were observed among ethanol-challenged rats (see Figure 6K),

Discussion

The results of the current series of experiments demonstrated that acute ethanol administration and withdrawal were both potent activators of central and peripheral cytokines. The pattern of cytokine expression following ethanol exposure and subsequent withdrawal was both site- and time-specific (as summarized in Table 2), with, overall, structures in the CNS appearing to exhibit intoxication-related increases specifically in IL-6 expression. In contrast, cytokine expression was generally elevated during withdrawal in the periphery, and for all cytokines examined. While the current results would suggest that ethanol-induced alterations in brain cytokine expression were driven by cells other than microglia—as acute minocycline administration did not fully reverse many of these ethanol-related changes—this possibility cannot be fully excluded since a more complete examination of doses and timing of injections has not yet been examined, and we cannot be certain that minocycline would specifically inhibit production of cytokines from microglia. A long-term history of ethanol consumption resulted in tolerance to intoxication-induced increases in IL-6 in the PVN, with elevated IL-6 in the amygdala not altered by an ethanol intake history, again demonstrating that these ethanol effects were structure- and target-dependent.

The present results add to a growing body of literature indicating ethanol-related alterations in central cytokine expression in the absence of an immune challenge (Deak et al., 2013). Few previous studies have examined brain cytokine changes during an initial exposure to ethanol (e.g., Emanuele et al., 2005; Qin et al., 2008; Whitman et al., 2013), however, with the current series of studies further characterizing the time-course of cytokine expression across multiple brain sites, cytokine targets, and routes of administration. During acute

withdrawal, we observed increased TNF α and IL-1, similar to what others have reported during the ethanol withdrawal phase (Emanuele et al., 2005; Qin et al., 2008). Three hours after injection, however, intoxication was consistently associated with increased IL-6 expression, whereas TNF α and IL-1 were either unchanged or reduced. Although IL-6 is classically considered a pro-inflammatory cytokine, other evidence suggests that IL-6 might have anti-inflammatory properties under some conditions (Scheller et al., 2011; Xing et al., 1998), thus raising the possibility that early elevations in IL-6 may be responsible for suppression of some cytokines, while also potentially initiating processes that lead to later induction of inflammatory-related factors. Furthermore, it is likely that elevated CORT observed during the intoxication phase in several studies at least partially contributed to suppressed expression of IL-1 and TNF α , as glucocorticoids have been shown to markedly alter cytokine expression (Busillo and Cidlowski, 2013). While the mechanisms responsible for these ethanol-related alterations in cytokines remain unclear, it is possible that acute ethanol exposure represents a non-pathogenic challenge that induces a sterile inflammatory response via activation of danger-associated molecular patterns (DAMPs), such as heat-shock proteins (e.g., hsp72) or high-mobility group box 1 (HMBG-1) (Crews et al., 2013; Whitman et al., 2013). While the results of the current experiments are primarily based on expression of mRNA, it is notable that these effects were highly reproducible across a diverse range of methods, with mRNA and protein significantly correlated and demonstrating good coherence when simultaneously assessed. Nevertheless, current studies in our laboratory are aimed at investigating protein changes following alcohol exposure, and will be of critical importance when ultimately interpreting these results.

Interleukins, and in particular IL-1 and IL-6, are cytokines that are released from immune-related cells during times of sickness and infection, with secretion of these factors leading to a variety of behavioral and physiological responses regarded as “sickness behaviors” (Dantzer, 2001). Comprised of fever, reduced food and water intake, reduced activity, and reduced social and sexual behavior (Dantzer, 2004), these behaviors are strikingly similar to those exhibited by rodents during acute withdrawal (Becker, 2000; Buck et al., 2011; Richey et al., 2012). Given significant alterations in these cytokines in hypothalamic and hippocampal sites, it seems plausible that some of the behavioral consequences of acute ethanol exposure are a result of increased cytokine signaling. Since microglia are the primary resident immune cells of the CNS, and are thought to be a principal source of proinflammatory cytokines, minocycline (a putative microglia inhibitor) was administered prior to ethanol exposure in an attempt to block ethanol-induced alterations in cytokines. In both Exps. 3 and 5, however, minocycline generally did not attenuate ethanol's effects on central cytokine expression. While these data would suggest that microglia are seemingly not responsible for alterations in cytokine expression, other studies have demonstrated that microglia are likely involved in ethanol-related behaviors and effects (e.g., Agrawal et al., 2011; Wu et al., 2011). Clearly, more studies are needed in order to fully characterize the role that microglia may play in ethanol-associated effects on inflammatory factors.

In order to investigate how these acute ethanol-induced alterations in central cytokines might be influenced in animals that were not naïve to ethanol, a long-term intermittent ethanol consumption paradigm was utilized, which encouraged moderate levels of intake that slowly escalated throughout the experiment. Indeed, intake measurements and

preference levels indicated that pharmacologically relevant BECs were likely achieved during drinking, as previously reported in another study using this intermittent ethanol access procedure with Sprague-Dawley rats (Bito-Onon et al., 2011). Although animals without a drinking history demonstrated typical intoxication-related increases in IL-6 following an acute ethanol challenge, a long-term history of intermittent ethanol consumption diminished this ethanol effect in the PVN, but not the amygdala, while seemingly not influencing ethanol-associated decreases in IL-1 expression. Other recent data from our laboratory (Gano et al., submitted) has indicated unique patterns of tolerance to ethanol-induced alterations in brain cytokines across 6 consecutive 4-g/kg ethanol intubations, thus further demonstrating that ethanol history is an important variable to consider when examining the effects of ethanol on inflammatory-related factors.

Taken together, ethanol-related changes in cytokines were generally similar regardless of age or route of ethanol administration, but with distinct differences apparent in the pattern of cytokine alterations in the periphery versus the brain. A dissociation between central and peripheral effects would suggest that the observed ethanol-associated alterations in brain cytokines are not merely an organism-wide enhancement or suppression of inflammatory-related factors, but rather a unique response specific to the CNS. While the results of these experiments have focused on changes in three specific cytokines that are classically thought to have “proinflammatory” actions, there are of course multitudes of other cytokines which were not examined in these studies, particularly those that typically stimulate “anti-inflammatory” processes. Ultimately, an unbiased array approach will identify a more extensive and exhaustive list of key cytokines that are involved in the response to acute ethanol exposure, and indeed, these studies are currently on going. In sum, these experiments have begun to identify early-term (acute), dynamic changes in cytokines in relation to alcohol, as well as how such effects might transform over the course of developmental history of the subject or in response to a growing number of alcohol exposures across the lifetime. Such studies will be instrumental in forging a link between initial alcohol exposure and the ultimate, adverse consequences of lifetime alcohol abuse.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Supported by NIH grant number R21AA016305-01 to T.D., the Developmental Exposure Alcohol Research Center (DEARC; P50AA017823), and the Center for Development and Behavioral Neuroscience at Binghamton University. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of the above stated funding agencies.

References

- Agrawal RG, Hewetson A, George CM, Syapin PJ, Bergeson SE. Minocycline reduces ethanol drinking. *Brain Behav Immun* 25 Suppl. 2011; 1:S165–9.
- Alfonso-Loeches S, Guerri C. Molecular and behavioral aspects of the actions of alcohol on the adult and developing brain. *Crit Rev Clin Lab Sci*. 2011; 48(1):19–47. [PubMed: 21657944]
- Becker HC. Animal models of alcohol withdrawal. *Alcohol Res Health*. 2000; 24(2):105–13. [PubMed: 11199277]

- Bito-Onon JJ, Simms JA, Chatterjee S, Holgate J, Bartlett SE. Varenicline, a partial agonist at neuronal nicotinic acetylcholine receptors, reduces nicotine-induced increases in 20% ethanol operant self-administration in Sprague-Dawley rats. *Addict Biol.* 2011; 16(3):440–9. [PubMed: 21392178]
- Blandino P Jr, Barnum CJ, Deak T. The involvement of norepinephrine and microglia in hypothalamic and splenic IL-1 β responses to stress. *J Neuroimmunol.* 2006; 173(1-2):87–95. [PubMed: 16386803]
- Blandino P Jr, Barnum CJ, Solomon LG, Larish Y, Lankow BS, Deak T. Gene expression changes in the hypothalamus provide evidence for regionally-selective changes in IL-1 and microglial markers after acute stress. *Brain Behav Immun.* 2009; 23(7):958–68. [PubMed: 19464360]
- Blandino P Jr, Hueston CM, Barnum CJ, Bishop C, Deak T. The Impact of Ventral Noradrenergic Bundle Lesions on Increased IL-1 in the PVN and Hormonal Responses to Stress in Male Sprague Dawley Rats. 0013-7227. 2013; 154(7):2489–500. [PubMed: 23671261]
- Blednov YA, Benavidez JM, Geil C, Perra S, Morikawa H, Harris RA. Activation of inflammatory signaling by lipopolysaccharide produces a prolonged increase of voluntary alcohol intake in mice. *Brain Behav Immun* 25 Suppl. 2011; 1:S92–S105.
- Blednov YA, Bergeson SE, Walker D, Ferreira VM, Kuziel WA, Harris RA. Perturbation of chemokine networks by gene deletion alters the reinforcing actions of ethanol. *Behav Brain Res.* 2005; 165(1):110–25. [PubMed: 16105698]
- Blednov YA, Ponomarev I, Geil C, Bergeson S, Koob GF, Harris RA. Neuroimmune regulation of alcohol consumption: behavioral validation of genes obtained from genomic studies. *Addict Biol.* 2012; 17(1):108–20. [PubMed: 21309947]
- Bordner KA, Molina JC, Spear NE. Analysis of ethanol reinforcement in 1-day-old rats: assessment through a brief and novel operant procedure. *Alcohol Clin Exp Res.* 2008; 32(4):580–92. [PubMed: 18366451]
- Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem.* 1976; 72:248–54. [PubMed: 942051]
- Breese GR, Knapp DJ, Overstreet DH, Navarro M, Wills TA, Angel RA. Repeated lipopolysaccharide (LPS) or cytokine treatments sensitize ethanol withdrawal-induced anxiety-like behavior. *Neuropsychopharmacology.* 2008; 33(4):867–76. [PubMed: 17551540]
- Buck HM, Hueston CM, Bishop C, Deak T. Enhancement of the hypothalamic-pituitary-adrenal axis but not cytokine responses to stress challenges imposed during withdrawal from acute alcohol exposure in Sprague-Dawley rats. *Psychopharmacology (Berl).* 2011; 218(1):203–15. [PubMed: 21735074]
- Busillo JM, Cidlowski JA. The five Rs of glucocorticoid action during inflammation: ready, reinforce, repress, resolve, and restore. *Trends Endocrinol Metab.* 2013; 24(3):109–19. [PubMed: 23312823]
- Carnicella S, Ahmadiantehrani S, Janak PH, Ron D. GDNF is an endogenous negative regulator of ethanol-mediated reward and of ethanol consumption after a period of abstinence. *Alcohol Clin Exp Res.* 2009; 33(6):1012–24. [PubMed: 19302086]
- Crews FT, Qin L, Sheedy D, Vetreno RP, Zou J. High mobility group box 1/Toll-like receptor danger signaling increases brain neuroimmune activation in alcohol dependence. *Biol Psychiatry.* 2013; 73(7):602–12. [PubMed: 23206318]
- Dantzer R. Cytokine-induced sickness behavior: mechanisms and implications. *Ann N Y Acad Sci.* 2001; 933:222–34. [PubMed: 12000023]
- Dantzer R. Cytokine-induced sickness behaviour: a neuroimmune response to activation of innate immunity. *Eur J Pharmacol.* 2004; 500(1-3):399–411. [PubMed: 15464048]
- Deak T, Bellamy C, D'Agostino LG. Exposure to forced swim stress does not alter central production of IL-1. *Brain Res.* 2003; 972(1-2):53–63. [PubMed: 12711078]
- Deak T, Bordner KA, McElderry NK, Barnum CJ, Blandino P Jr, Deak MM, Tammariello SP. Stress-induced increases in hypothalamic IL-1: a systematic analysis of multiple stressor paradigms. *Brain Res Bull.* 2005; 64(6):541–56. [PubMed: 15639551]
- Deak, T.; Doremus-Fitzwater, TL.; Okrainets, A. Mechanisms of stress-dependent neuroinflammation and their implications for understanding consequences of alcohol exposure. In: Cui, C.; Grandison,

- L.; Noronha, A., editors. Neural-immune interactions in brain function and alcohol related disorders. Springer; New York: 2013. p. 133-166.
- Emanuele N, LaPaglia N, Kovacs EJ, Emanuele MA. Effects of chronic ethanol (EtOH) administration on pro-inflammatory cytokines of the hypothalamic-pituitary-gonadal (HPG) axis in female rats. *Endocr Res.* 2005; 31(1):9–16. [PubMed: 16238187]
- Fernandez-Lizarbe S, Montesinos J, Guerri C. Ethanol induces TLR4/TLR2 association, triggering an inflammatory response in microglial cells. *J Neurochem.* 2013; 126(2):261–73. [PubMed: 23600947]
- Gonzalez-Quintela A, Dominguez-Santalla MJ, Perez LF, Vidal C, Lojo S, Barrio E. Influence of acute alcohol intake and alcohol withdrawal on circulating levels of IL-6, IL-8, IL-10 and IL-12. 1043-4666. 2000; 12(9):1437–40. [PubMed: 10976010]
- Grant BF, Dawson DA, Stinson FS, Chou SP, Dufour MC, Pickering RP. The 12-month prevalence and trends in DSM-IV alcohol abuse and dependence: United States, 1991-1992 and 2001-2002. *Drug Alcohol Depend.* 2004; 74(3):223–34. [PubMed: 15194200]
- Hueston CM, Barnum CJ, Eberle JA, Ferraioli FJ, Buck HM, Deak T. Stress-dependent changes in neuroinflammatory markers observed after common laboratory stressors are not seen following acute social defeat of the Sprague Dawley rat. *Physiol Behav.* 2011; 104(2):187–98. [PubMed: 21406198]
- Institute of Laboratory Animal Research CoLS. National Research Council. Guide for the Care and Use of Laboratory Animals. National Academy Press; Washington, D.C: 1996.
- Kane CJ, Phelan KD, Douglas JC, Wagoner G, Johnson JW, Xu J, Phelan PS, Drew PD. Effects of ethanol on immune response in the brain: region-specific changes in adolescent versus adult mice. *Alcohol Clin Exp Res.* 2014; 38(2):384–91. [PubMed: 24033454]
- Kim DJ, Kim W, Yoon SJ, Choi BM, Kim JS, Go HJ, Kim YK, Jeong J. Effects of alcohol hangover on cytokine production in healthy subjects. 0741-8329. 2003; 31(3):167–70. [PubMed: 14693266]
- Knapp DJ, Whitman BA, Wills TA, Angel RA, Overstreet DH, Criswell HE, Ming Z, Breese GR. Cytokine involvement in stress may depend on corticotrophin releasing factor to sensitize ethanol withdrawal anxiety. *Brain Behav Immun* 25 Suppl. 2011; 1:S146–54.
- Martinez F, Abril ER, Earnest DL, Watson RR. Ethanol and cytokine secretion. *Alcohol.* 1992; 9(6): 455–8. [PubMed: 1472299]
- Qin L, Crews FT. Chronic ethanol increases systemic TLR3 agonist-induced neuroinflammation and neurodegeneration. *J Neuroinflammation.* 2012; 9:130. [PubMed: 22709825]
- Qin L, He J, Hanes RN, Pluzarev O, Hong JS, Crews FT. Increased systemic and brain cytokine production and neuroinflammation by endotoxin following ethanol treatment. *J Neuroinflammation.* 2008; 5:10. [PubMed: 18348728]
- Richey L, Doremus-Fitzwater TL, Buck HM, Deak T. Acute illness-induced behavioral alterations are similar to those observed during withdrawal from acute alcohol exposure. *Pharmacol Biochem Behav.* 2012; 103(2):284–94. [PubMed: 22921768]
- Scheller J, Chalaris A, Schmidt-Arras D, Rose-John S. The pro- and anti-inflammatory properties of the cytokine interleukin-6. *Biochim Biophys Acta.* 2011; 1813(5):878–88. [PubMed: 21296109]
- Simms JA, Steensland P, Medina B, Abernathy KE, Chandler LJ, Wise R, Bartlett SE. Intermittent access to 20% ethanol induces high ethanol consumption in Long-Evans and Wistar rats. *Alcohol Clin Exp Res.* 2008; 32(10):1816–23. [PubMed: 18671810]
- Tiwari V, Kuhad A, Chopra K. Suppression of neuro-inflammatory signaling cascade by tocotrienol can prevent chronic alcohol-induced cognitive dysfunction in rats. *Behav Brain Res.* 2009; 203(2): 296–303. [PubMed: 19464322]
- Tringali G, Mancuso C, Mirtella A, Pozzoli G, Parente L, Preziosi P, Navarra P. Evidence for the neuronal origin of immunoreactive interleukin-1 beta released by rat hypothalamic explants. *Neuroscience Letters.* 1996; 219(3):143–146. [PubMed: 8971800]
- Valles SL, Blanco AM, Pascual M, Guerri C. Chronic ethanol treatment enhances inflammatory mediators and cell death in the brain and in astrocytes. *Brain Pathol.* 2004; 14(4):365–71. [PubMed: 15605983]

- van Dam AM, Brouns M, Louisse S, Berkenbosch F. Appearance of interleukin-1 in macrophages and in ramified microglia in the brain of endotoxin-treated rats: a pathway for the induction of non-specific symptoms of sickness? *Brain Res.* 1992; 588(2):291–6. [PubMed: 1393581]
- Varlinskaya EI, Doremus-Fitzwater TL, Spear LP. Repeated restraint stress alters sensitivity to the social consequences of ethanol in adolescent and adult rats. *Pharmacol Biochem Behav.* 2010; 96(2):228–35. [PubMed: 20478326]
- Vitkovic L, Bockaert J, Jacque C. “Inflammatory” cytokines: neuromodulators in normal brain? 0022-3042. 2000; 74:457–471. [PubMed: 10646496]
- Whitman BA, Knapp DJ, Werner DF, Crews FT, Breese GR. The Cytokine mRNA Increase Induced by Withdrawal from Chronic Ethanol in the Sterile Environment of Brain is Mediated by CRF and HMGB1 Release. *Alcohol Clin Exp Res.* 2013
- Wise RA. Voluntary ethanol intake in rats following exposure to ethanol on various schedules. *Psychopharmacologia.* 1973; 29(3):203–10. [PubMed: 4702273]
- Wu Y, Lousberg EL, Moldenhauer LM, Hayball JD, Robertson SA, Collier JK, Watkins LR, Somogyi AA, Hutchinson MR. Attenuation of microglial and IL-1 signaling protects mice from acute alcohol-induced sedation and/or motor impairment. *Brain Behav Immun* 25 Suppl. 2011; 1:S155–64.
- Xing Z, Gauldie J, Cox G, Baumann H, Jordana M, Lei XF, Achong MK. IL-6 is an antiinflammatory cytokine required for controlling local or systemic acute inflammatory responses. *J Clin Invest.* 1998; 101(2):311–20. [PubMed: 9435302]

Figure 1.

(A) Blood ethanol concentration (BEC), (B) Interleukin (IL)-1 mRNA, and (C) IL-1 protein was measured in postnatal day (P) 4 rat pups either 30, 60, 90, or 120 min after a 2-g/kg intraperitoneal ethanol challenge. Pups in the control group were given a saline injection (counterbalanced across time points). All data are expressed as the mean $\pm$ S.E.M. Asterisks (*) indicate $p < .05$ relative to saline-injected controls, whereas the plus sign (+) denotes a significant difference between all ethanol-treated groups (collapsed) compared to saline-treated controls.

Figure 2.

Central expression of interleukin-1 (IL-1) (A, B, C), interleukin- 6 (IL-6) (D, E, F) and tumor necrosis factor alpha (TNFα) (G, H, I) mRNA in the hypothalamus, hippocampus and cerebellum after an intraperitoneal 4-g/kg ethanol or saline injection was measured using RT-PCR. Cytokine expression was examined either during intoxication (3 or 9 hr post-injection; black bars) or acute withdrawal (15 or 18 hr; gray bars), with all data expressed as mean $\pm$ S.E.M. Asterisks (*) indicate $p < .05$ relative to saline-injected controls.

Figure 3.

Peripheral expression of interleukin-1 (IL-1) (A, B), interleukin- 6 (IL-6) (C, D) and tumor necrosis factor alpha (TNFα) (E, F) mRNA in the spleen and liver after an intraperitoneal 4-g/kg ethanol or saline injection was measured using RT-PCR. Cytokine expression was examined either during intoxication (3 or 9 hr post-injection; black bars) or acute withdrawal (15 or 18 hr; gray bars). Blood ethanol concentrations (G) and plasma Corticosterone (H) were also measured at these same time points. All data are expressed as mean ± S.E.M. Asterisks (*) indicate p < .05 relative to saline-injected controls.

Figure 4.

In Experiment 3, animals were injected intraperitoneally (i.p.) with either saline or 4-g/kg ethanol and then blood and tissue collected 3 or 18 hr later. One hour prior to the ethanol challenge, rats received an i.p. injection of either vehicle (white bars) or minocycline (40mk/kg; black bars). Blood ethanol concentrations were measured from plasma collected at these post-ethanol time points (D). Expression of interleukin-6 (IL-6) (A), interleukin-1 (IL-1) (B) and tumor necrosis factor alpha (TNFα) (C) mRNA in the paraventricular nucleus of the hypothalamus was measured using RT-PCR. All data are expressed as mean $\pm$ S.E.M, with an asterisk (*) over a black bar indicating a main effect of ethanol exposure relative to saline-injected controls at a particular time point ($p < .05$).

Figure 5.

Rats were provided the opportunity to consume unsweetened ethanol (20% v/v) using a 2-bottle choice, intermittent access paradigm. Intake was measured either 30-min or 24-hr after presentation of the bottles, with rats given a total of 30 drinking sessions. Ethanol intake is presented on a g/kg basis in panels (A) and (B), whereas both water (white squares) and ethanol (black circles) intake on a ml/kg basis is shown in panels (C) and (D). Ethanol preference was also calculated at 30-min (E) and 24-hr (F) after presentation of the bottles.

Figure 6.

Central expression of interleukin-1 (IL-1) (A, B, C), interleukin-6 (IL-6) (D, E, F) and tumor necrosis factor alpha (TNFα) (G, H, I) mRNA was measured using RT-PCR in the paraventricular nucleus of the hypothalamus (PVN), hippocampus and amygdala 3 hr after an intragastric challenge with either 4-g/kg ethanol or vehicle (tap water). Rats given the ethanol challenge were either ethanol naïve (W-E), or had a long-term history of ethanol intake (E-E). An additional group of ethanol-drinking rats was also given minocycline (60 mg/kg) in their acute ethanol intubation (E-E/M). All data are expressed as mean ± S.E.M.,

with asterisks (*) indicating a significant difference ($p \leq .05$) relative to vehicle-intubated controls (E-V), which also had a history of long-term ethanol intake. Plasma corticosterone (CORT) and blood ethanol concentrations (BECs) were also assessed in these groups 3 hr after the ethanol or vehicle challenge (panels J and K, respectively).

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Table 1

Cytokine Gene Expression (calculated as percent of control) and Blood Ethanol Concentration (mg/dL) Following Intragastric Intubation of 4-g/kg Ethanol. Adult male rats were administered an intragastric 4-g/kg ethanol challenge, and tissue and trunk blood collected 3, 10, or 14 hr after intubation. Additionally, a control vehicle (VEH) group was given tap water, with these animals counterbalanced across time post-challenge. Expression of interleukin (IL)-1, IL-6, and tumor necrosis factor alpha (TNF α) mRNA in the paraventricular nucleus of the hypothalamus (PVN) was assessed (expressed as percent of control), as well as blood ethanol concentration (BEC; mg/dL) at the time of sacrifice. Values represent the mean, with standard error of the mean listed in parentheses. Bolded numbers represent a significant elevation relative to vehicle-treated rats.

	VEH	3 HR	10 HR	14 HR
PVN IL-1	108.2 (9.2)	102.1 (36.3)	111.0 (8.5)	93.9 (11.3)
PVN IL-6	107.0 (11.5)	217.2 (62.3)	73.8 (10.2)	76.3 (10.0)
PVN TNF α	112.4 (14.7)	123.9 (45.1)	129.1 (29.8)	144.1 (15.3)
BEC	16.53 (2.05)	175.12 (20.76)	35.22 (14.04)	20.18 (3.44)

Table 2
Summary of Observed Changes in Cytokines Across Experiments 1 through 5

Results from Experiments 1 through 5 are summarized, with respect to age at time of testing [postnatal day (P) 4 versus adulthood], dose of ethanol delivered, route of administration [intraperitoneal (i.p.) versus intragastric (i.g.)], brain region examined, method used to measure cytokines, time points assessed relative to ethanol challenge, and cytokine target of interest [interleukin-1 (IL-1), interleukin-6 (IL-6), or tumor necrosis factor alpha (TNFα)].

Experiment	Age	Dose	Route	Structures Examined	Method	Time Points Examined	Target	Findings
1	P4	2-g/kg	i.p.	Whole brain	PCR	0.5, 1, 2, 4 hr	IL-1 mRNA	↓ mRNA expression at 30, 60 min
					ELISA		IL-1 protein	↓ protein (collapsed across time)
2	Adult	4-g/kg	i.p.	Hypothalamus	RT-PCR	3, 9, 15, 18 hr	IL-1	↑ at 15, 18 hr
							IL-6	↑ at 3, 9 hr
							TNFα	↓ at 3, 9 hr; ↑ at 18 hr
				Hippocampus	RT-PCR	3, 9, 15, 18 hr	IL-1	↑ at 15 hr
							IL-6	↑ at 3, 9, 18 hr
							TNFα	↓ at 3, 9, 18 hr
				Cerebellum	RT-PCR	3, 9, 15, 18 hr	IL-1	↓ at 3 hr
							IL-6	↑ at 9 hr
							TNFα	↓ at 3, 9 hr
				Spleen	RT-PCR	3, 9, 15, 18 hr	IL-1	↑ at 9, 15, 18 hr
							IL-6	↑ at 15, 18 hr
							TNFα	↑ at 18 hr
				Liver	RT-PCR	3, 9, 15, 18 hr	IL-1	↑ at 9, 15, 18 hr
							IL-6	↑ at 18 hr
							TNFα	↑ at 15, 18 hr
3	Adult	4-g/kg	i.p.	PVN	RT-PCR	3 or 18 hr	IL-1	↓ at 3 hr
							IL-6	↑ at 3 hr
							TNFα	↓ at 3 hr
4	Adult	4-g/kg	i.g.	PVN	RT-PCR	3, 10, 14 hr	IL-1	No change
							IL-6	↑ at 3 hr
							TNFα	No change
5	Adult	10 wks voluntary consumption + 4 g/kg	i.g.	PVN	RT-PCR	3 hr	IL-1	↓ at 3 hr
							IL-6	↑ at 3 hr

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Experiment	Age	Dose	Route	Structures Examined	Method	Time Points Examined	Target	Findings
							TNFα	No change
							IL-1	↓ at 3 hr
				Hippocampus		3 hr	IL-6	No change
							TNFα	↓ at 3 hr
							IL-1	↓ at 3 hr
				Amygdala		3 hr	IL-6	↑ at 3 hr
							TNFα	Trend for ↓ at 3 hr