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Chronic intracerebroventricular infusion of monocyte chemoattractant protein – 1 leads to a persistent increase in sweetened ethanol consumption during operant self-administration but does not influence sucrose consumption in Long-Evans rats

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Abstract

Background—Among the evidence implicating neuroimmune signaling in alcohol use disorders are increased levels of the chemokine monocyte chemoattractant protein-1 (MCP-1) in the brains of human alcoholics and animal models of alcohol abuse. However, it is not known whether neuroimmune signaling can directly increase ethanol consumption, and whether MCP-1 is involved in that mechanism. We designed experiments to determine if MCP-1 signaling itself is sufficient to accelerate or increase ethanol consumption. Our hypothesis was that increasing MCP-1 signaling by directly infusing it into the brain would increase operant ethanol self-administration.

Methods—We implanted osmotic minipumps to chronically infuse either one of several doses of MCP-1 or vehicle into the cerebral ventricles of Long-Evans rats and then tested them in the operant self-administration of a sweetened ethanol solution for 8 weeks.

Results—There was a significant interaction between dose of MCP-1 and sweetened ethanol consumed across the first 4 weeks (while pumps were flowing) and across the 8-week experiment. Animals receiving the highest dose of MCP-1 (2 µg/day) were the highest consumers of ethanol during weeks 3 through 8. MCP-1 did not influence the acquisition of self-administration (measured across the first 5 days), the motivation to consume ethanol (time to lever press or progressive ratio), withdrawal-induced anxiety, or the consumption of sucrose alone.

Conclusion—We provide novel evidence that neuroimmune signaling can directly increase chronic operant ethanol self-administration, and that this increase persists beyond the administration of the cytokine. These data suggest that ethanol-induced increases in MCP-1, or increases in MCP-1 due to various other neuroimmune mechanisms, may further promote ethanol consumption. Continued research into this mechanism, particularly using models of alcohol dependence, will help determine if targeting MCP-1 signaling has therapeutic potential in the treatment of alcohol use disorders.

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Keywords

dose-response; NF- κ B; neuroinflammation; neuromodulation; dopamine neurons

Introduction

Recent research has implicated neuroimmune signaling in the neurobiological changes that promote unhealthy alcohol drinking behavior (for a review, see Crews et al., 2011). A variety of experiments have revealed that a specific chemokine, monocyte chemoattractant protein-1 (MCP-1, or CCL2), and its receptor, CCR2, are particularly significant. For example, Zou and Crews (2010) discovered a 1000% increase in MCP-1 in brain slice cultures treated with ethanol, relative to controls. Brain MCP-1 concentrations were increased 2–3 fold in mouse models of sub-chronic or chronic ethanol exposure (Qin et al. 2008, Pascual et al., 2015), in a rat model of chronic ethanol exposure (Ehrlich et al., 2012), as well as in human alcoholic brains post-mortem (He and Crews, 2008). Ehrlich and colleagues (2012) found elevated cortical MCP-1 in rats on a 20% ethanol liquid-only diet for 12 months. Furthermore, manipulating MCP-1 signaling can influence ethanol self-administration in animal models. For example, MCP-1 ligand, receptor, and combined ligand and receptor knockout mice had a robust reduction in ethanol consumption and preference (Blednov et al., 2005). More recently, siRNA-mediated knockdown of neuronal MCP-1 expression in the ventral tegmental area or central amygdala resulted in marked decreases in operant responding for ethanol (June et al., 2015). Additionally, June et al. (2015) found evidence that corticotropin-releasing factor (CRF) modulates MCP-1 expression in neurons. Interestingly, MCP-1 has been previously shown to enhance dopamine neurotransmission through modulation of potassium channels (Guyon et al, 2009; Apartis et al, 2010; Wakida et al., 2014), leading the authors of June et al. (2015) to hypothesize that CRF signaling regulates excessive alcohol drinking through the modulation of MCP-1 expression on dopamine neurons. Altogether, these data suggest that ethanol-induced increases in MCP-1 may facilitate increased ethanol consumption. Experimental manipulation to determine if MCP-1 signaling itself can directly increase ethanol consumption was warranted.

Our hypothesis was that increasing MCP-1 signaling by directly infusing it into the brain would increase the operant self-administration of sweetened ethanol. We designed an experiment that would allow us to independently examine the effect of MCP-1 on several distinct components of ethanol self-administration, including acquisition, motivation, and consumption. We also examined withdrawal-induced anxiety. We implanted subcutaneous osmotic minipumps connected to intracranial cannulae to chronically infuse a wide range of concentrations of MCP-1 into the cerebral ventricles (ICV) of Long-Evans rats, using doses that are substantially below those required to trigger neuroinflammatory mechanisms such as BBB breakdown or leukocyte infiltration (Stamatovic et al., 2005). We provide novel evidence that neuroimmune signaling can directly increase chronic operant ethanol self-administration, and that this increase persists beyond the administration of the cytokine.

Materials and Methods

Timeline

During the 1st week, animals were acclimated and handled. During the 2nd week, animals were trained to lever press. During the 3rd week, MCP-1 was reconstituted and osmotic minipumps were filled and surgically implanted for ICV delivery of MCP-1. During weeks 4 through 11 (8 weeks), animals performed daily operant self-administration sessions of a sucrose-sweetened ethanol solution (Monday-Friday). Pumps delivered MCP-1 continuously for 5 weeks and self-administration started during the 2nd week of delivery. Progressive ratio and withdrawal-induced anxiety tests were performed on the Sunday at the end of the 4th week of self-administration (on the last day of pump delivery of MCP-1). The self-administration of a sucrose solution without ethanol was performed in a separate group of animals and followed this timeline except that the experiment was stopped after 4 weeks of self-administration.

Animals

We used male Long-Evans rats (Charles River Laboratories, Wilmington, MA) weighing an average of 190 grams upon arrival and 350 grams on the first day of ethanol self-administration. The rats were dual-housed at 25 °C on a 12-h light/dark schedule (lights on 7am to 7pm) with ad libitum access to food and water in an AAALAC-accredited facility. All procedures were carried out in compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of the University of Texas at Austin.

Care was given to minimize stress to the animals. Animals were dual-housed immediately upon arrival and throughout the experiment. Rats were handled by every experimenter for at least 4 days prior to operant training for a total of 15–20 minutes each day. Each cage had a Nylabone (Neptune, NJ) for enrichment throughout the study. Bedding was made from wood chips and was replaced weekly. The animal housing room is only occupied by rats used by the Gonzales lab and is adjacent to the room with operant chambers. Animals are carried from their home cage through one open door directly to the operant chambers. Operant chambers are cleaned daily. The two rooms are isolated from other traffic and noises.

Operant Training

Animals were water deprived for 16–22 hours per day and trained to lever press for a 10% sucrose solution using an FR1 schedule of reinforcement. Self-administration took place in standard operant chambers (Med Associates Inc., St. Albans, VT). Each chamber contained a single, retractable lever on the left side (2 cm above the grid floor). Each time the animal pressed the lever, a retractable drinking spout entered the chamber on the right side of the same wall (5 cm above the grid floor). An interior chamber light and a sound-attenuating fan were activated with the start of each operant session. Operant chamber components were controlled by a personal computer running MEDPC software (Med Associates Inc., St. Albans, VT). Initially, 1-hour sessions were used, with session duration declining to 40 minutes and then 20 minutes for individual animals as they made progress. All rats were

trained to lever press successfully within 5 days, with 20 minute sessions occurring during day 5 (or sooner).

MCP-1

Recombinant rat MCP-1 (Peptotech, Rocky Hill, NJ) was dissolved in dH₂O and then diluted in concentrated artificial cerebro-spinal fluid (aCSF) to reach a standard final concentration of 149 mM NaCl, 2.8 mM KCl, 1.2 mM MgCl₂, 1.2 mM CaCl₂, 0.42 mM ascorbic acid, 5.4 mM D-glucose, and 1% rat serum albumin (Sigma-Aldrich, St. Louis, MO). The solution was then serially-diluted in aCSF (with final salt and albumin concentrations) to reach desired MCP-1 concentrations. Alzet osmotic minipumps (model 1004) and brain infusion cannulae ("kit 2", 28 ga) were obtained from Durect Corporation (Cupertino, CA). Pumps were filled with a specific concentration of MCP-1 adjusted to each lot's individual flow rate to yield a final dose of either 0.2 ng/day, 20 ng/day, 2000 ng/day, or vehicle control. An additional group of 2 ng/day pumps were used for the ELISA experiment. The osmotic pumps used were designed to flow at a constant rate of 0.12 μ L/hr for 34.5–35.9 days with a mean fill volume of approximately 100 μ L, leading to an MCP-1 concentration of approximately 54 μ M for the 2000 ng/day dose. MCP-1 was reconstituted and pumps were filled on Mondays and primed in sterile saline at 37°C to ensure reliable flow prior to implantation (which occurred on Tuesdays and Wednesdays), and should have reliably stopped flowing on the 35th day, according to the manufacturer.

The dose of MCP-1 was chosen based on the ability of an acute ICV dose of 20 – 100 ng to induce persistent behavioral effects (Plata-Salaman and Borkoski, 1994; Banisadr et al., 2002; Breese et al., 2008). Based on those experiments, we centered on the delivery of 20 ng over a 24-hour period and then included 100-fold higher and 100-fold lower doses (which do not compromise the blood-brain barrier or lead to leukocyte infiltration when administered ICV (see discussion)). The chronic infusion method was chosen due to the propensity of the interaction between ethanol and neuroimmune signaling to be chronic in nature. We chose the ICV method to simulate the presence of brain-induced MCP-1 and the method's ability to target the whole brain with molecules that don't readily cross the blood-brain barrier (Dzenko et al., 2001). Evidence shows that ICV administration of MCP-1 or other cytokines distributes throughout the brain to initiate receptor-mediated effects (for MCP-1-specific data, see Stamatovic et al., 2005).

Surgery

The cannula tip was aimed at the left lateral ventricle using the following coordinates relative to bregma (mm): anterior/posterior –0.60, medial/lateral +1.50, and dorsal/ventral –3.80, and was then glued to the skull using cyanoacrylate adhesive. The pump was implanted subcutaneously in a pocket just under the skin which was created by sliding needle holders through a head incision to the mid upper-back region. A 5 cm polyethylene tube connected the pump to the L-shaped cannula. The skin was sutured over the cannula once the tab for stereotaxic placement was removed. Bupivacaine (Hospira, Inc., Lake Forest, IL) was administered intradermally, and both bupivacaine and gentamicin (APP Pharmaceuticals, LLC, Schaumburg, IL) were dripped into the wound (2 mg/kg each, in total).

Self-administration

Animals drank either a solution of 10% sucrose (w/v) alone or a solution with both 10% sucrose (w/v) and 10% ethanol (v/v). The solutions were made using 95 % ethanol (Aaper Alcohol and Chemical Co., Shelbyville, KY) and molecular biology grade D-sucrose (Fisher, Hampton, New Hampshire) dissolved in tap water.

Animals began self-administration the week after surgery (5–6 days after implantation). Animals self-administered Monday through Friday for 8 weeks, of which pumps delivered MPC-1 during the first 4 weeks. Self-administration sessions occurred between 5 and 8 hours into the light cycle. Due to the presence of sucrose, intake levels stabilize within a few days. We defined acquisition as the intake values across the first 5 days. The self-administration of 10% sucrose without ethanol was measured in a distinct group of animals and followed these methods, except limited to just the first 4 weeks of self-administration and the highest dose of MCP-1 (2000 ng/day) and controls.

To capture whether MCP-1 influenced the motivation to gain access to ethanol independent of the quantity consumed, we chose an appetitive-consummatory model of self-administration. The appetitive phase required the animal to press a lever 4 times in order to gain access to the ethanol solution, and the time taken to reach this response requirement was recorded. This phase was followed by the consummatory phase, which consisted of 20 minutes of access to the ethanol solution, and the amount of ethanol consumed was recorded. If 20 minutes passed without the animal pressing 4 times, access to the ethanol was automatically given. Ethanol was contained in a retractable sipper tube with a 50 mL conical vial. Dripping was collected in a weigh boat, and both the vial/tube and the dish were weighed before and after the session to the hundredths of an mL.

As a secondary test of motivation, a progressive ratio test was administered on the final day of pump flow (the Sunday of the 5th week). We followed the model of Walker and Koob (2007) and used the following schedule of reinforcement: 2,2,3,3,4,4,5,5,7,7,9,9,11,11,13,13,15,15,18,18. The amount of ethanol consumed and the break point reached were measured.

Since MCP-1 is an inflammatory molecule with unknown effects during chronic administration, animals were closely monitored for illness, including body weight and locomotor activity. Locomotor activity was only recorded during the 20 minutes of ethanol access. Seven of 8 chambers had infrared sensors to measure locomotor activity.

Withdrawal-induced anxiety

Six to 8 hours after the progressive ratio session, animals were tested for withdrawal-induced anxiety by measuring the seconds of social interaction they initiated with an unfamiliar animal with a matched dose of MCP-1 over a 5 minute period. Generally, interaction was defined as contact directed at the other animal (sniffing, grooming, crawling, fighting, etc) and took place in a square open field (60 × 60 cm², with 16 squares marked on the floor for assessing locomotor activity) and under low-lighting. A minimum of two observers, each blind to the treatment conditions, independently scored social interaction and locomotor activity.

Enzyme-Linked Immunosorbent Assay (ELISA)

The stability of MCP-1 under the experimental conditions was unknown. In a separate experiment, rat MCP-1 ELISA kits (Life Technologies, Grand Island, NY) were used to determine the stability of MCP-1 across the infusion duration. We tested the concentration of MCP-1 in the pumps of animals 14 and 28 days after MCP-1 was reconstituted and then diluted to a 2 ng/day dose (after 1 and 3 weeks of drinking, respectively). Pumps were taken from drinking animals that were sacrificed at the day of testing. Pumps were cut open and contents were quantified for MCP-1 according to the manufacturer's instructions.

Histology

After the experiment, animals were sacrificed using CO₂ and then decapitated. Brains were extracted and put into 10% formalin for 3 days before being sliced and immediately examined under a microscope to determine cannula placement using Paxinos and Watson (2007).

Data analysis

Daily values were averaged for each week for each animal. A repeated measures ANOVA with a type I error set to $P < 0.05$ was conducted across 4-week and 8-week data sets (the first four weeks while pumps were flowing, the last four weeks while pumps were stopped, and the eight week combined set), or the first 5 days for the acquisition analysis. A one-way ANOVA was used for one-time experiments (progressive ratio and anxiety). Values are reported as mean $\pm$ SEM.

Results

MCP-1 did not increase the operant self-administration of sucrose alone

Chronic ICV infusion of MCP-1 started 1 week before the initiation of self-administration and continued for 4 weeks of self-administration, for a total of 5 weeks of infusion. MCP-1 did not significantly influence the self-administration of 10% sucrose solution (Fig. 1A, $F_{3,39}=0.04$, $P=0.99$, dose x time interaction, $n=7$ for controls and $n=8$ for MCP-1 group). Only the highest dose of MCP-1 (2000 ng/day) was tested.

MCP-1 did not influence the acquisition of operant self-administration of sweetened ethanol

Chronic ICV infusion of MCP-1 started 1 week before the initiation of self-administration and continued through the acquisition period (5 days). ICV infusion of MCP-1 had no effect on the acquisition of operant self-administration of sweetened ethanol, measured during the first 5 days (Fig. 1B, $F_{12,156}=1.40$, $P=0.17$ dose x time interaction, $N=11/10/11/11$ for control/0.2/20/2000 ng/day doses respectively).

MCP-1 increased consumption during chronic operant self-administration of sweetened ethanol

Chronic ICV infusion of MCP-1 started 1 week before the initiation of self-administration and continued during 4 weeks of self-administration, for a total of 5 weeks of infusion. Self-

administration took place across 8 weeks, the first 4 of which MCP-1 was being delivered. MCP-1 increased the self-administration of sweetened ethanol over 8 weeks with animals receiving the highest dose of MCP-1 (2 µg/day) drinking the most ethanol on average during weeks 3 through 8 (Fig. 1C/D, $F_{21,168} = 1.65$, $P = 0.04$), dose x time interaction, $n = 7/6/7/8$ for control/0.2/20/2000 ng/day doses respectively). The effect was also significant for the first 4 weeks of self-administration alone, while the pumps were flowing ($F_{9,72} = 2.33$, $P = 0.02$, dose x time interaction). The effect was not significant when analyzed over the final 4-week period ($F_{9,72} = 1.566$, $P = 0.14$, dose x time interaction). Of the 43 animals used for the acquisition experiment, 28 were used for the chronic self-administration analyses (12 animals had pumps that stopped prior to 4 weeks, 2 animals were lost due to fighting and 1 due to infection).

MCP-1 did not influence health-related measures

MCP-1 had no effect on the body weight of the animals ($F_{9,72} = 0.85$, $P = 0.58$, dose x time interaction, $n = 7/6/7/8$ for control/0.2/20/2000 ng/day doses respectively) or locomotor activity ($F_{9,60} = 0.85$, $P = 0.58$, dose x time interaction, $n = 6/5/6/7$ for control/0.2/20/2000 ng/day doses respectively) across the first 4 weeks of drinking. A summary of the data averaged across the first 4 weeks of drinking is shown in Fig. 2A and Fig. 2B for the body weight and locomotor activity, respectively. There was also no effect on % baseline body weight, or any of the 8-week analyses (data not shown). No other overt signs of sickness were detected throughout the experiments.

MCP-1 did not increase the motivation to consume sweetened ethanol

There was no effect on the time to reach the response requirement of 4 lever presses to gain access to the ethanol solution across the four weeks of drinking ($F_{9,72} = 0.49$, $P = 0.88$, dose x time interaction, $n = 7/6/7/8$ for control/0.2/20/2000 ng/day doses respectively). A summary of the data averaged across the first 4 weeks of drinking is shown in Fig. 3A. There was also no effect across 8 weeks (data not shown). Animals that took longer than 2 standard deviations from the mean (266 seconds/4.43 min) during a particular day were removed from the analysis (20 instances out of 1120, or 1.8%, across all 8 weeks). These exclusions were distributed across doses, with 6/5/7/2 exclusions for control/0.2/20/2000 ng/day doses respectively.

On the final day of pump flow (35 days after pumps were filled, at the end of the 4th week of ethanol self-administration), a progressive ratio test was administered. There was no effect on the consumption of ethanol during a progressive ratio test performed on the last day of pump delivery of MCP-1 (Fig. 3B, $F_{3,23} = 0.33$, $P = 0.80$, one-way ANOVA, $n = 7/5/7/8$ for control/0.2/20/2000 ng/day doses respectively). There was no effect on the break point reached during the progressive ratio session (Fig. 3C, $F_{3,23} = 0.39$, $P = 0.76$, one-way ANOVA, $n = 7/5/7/8$ for control/0.2/20/2000 ng/day doses respectively). One animal didn't make the progressive ratio analysis because of infection.

MCP-1 did not influence ethanol withdrawal-induced anxiety

On the final day of pump flow, 6–8 hours after the progressive ratio test was administered, animals were tested for ethanol withdrawal-induced anxiety. During the progressive ratio

session, animals had an average intake of 0.83 ± 0.06 g/kg, which was evenly distributed across doses as indicated by Fig. 3B. During the five-minute anxiety test session, animals were paired with an unfamiliar animal matched by dose. There was no effect of dose on social interaction during the test (Fig. 4A, $F_{3,20}=1.11$, $P=0.37$, one-way ANOVA, $n=6/4/6/8$ for control/0.2/20/2000 ng/day doses respectively). Three animals were excluded because they did not have a matched-dose partner at the time of the test. There was no effect of dose on locomotion during the test (Fig. 4B, $F_{3,20}=0.75$, $P=0.54$, one-way ANOVA, $n=6/4/6/8$ for control/0.2/20/2000 ng/day doses respectively).

Stability of MCP-1 during ICV infusion

ELISA was used to determine the concentration of MCP-1 in the pumps after 14 days (one week after drinking began) and 28 days (3 weeks after drinking began). Pumps were taken from drinking animals that were sacrificed at the day of testing (drinking data not reported because they didn't meet the 4-week criteria for the ANOVA). Data are expressed as a percent of the original concentration of 54 nM (2 ng/day pumps). There were detectable levels of MCP-1 in all pumps tested (Fig. 5).

Histology

Histological examination confirmed that cannula broke through the corpus callosum into the lateral ventricle in all but one animal, which was discarded from the analyses. Ten placements in the ethanol experiment and two placements in the sucrose experiment penetrated through the ventricle into the fimbria of the hippocampus or the dorsal striatum and were included in the analyses because flow into the ventricle should not have been significantly impacted.

Discussion

Data suggesting that proinflammatory neuroimmune signaling plays a role in unhealthy drinking behaviors has been mounting, but the mechanisms that may contribute are not completely clear. Our results provide the first evidence of a specific cytokine increasing the self-administration of ethanol. In this experiment, chronic ICV infusion of MCP-1 did not influence the acquisition of sweetened ethanol self-administration (across the first week) but increased consumption across the first 4 weeks (while MCP-1 pumps were flowing) and across the 8-week experiment. The effect of MCP-1 on self-administration developed over several weeks of infusion and several weeks of consumption, with the highest dose of MCP-1 (2 μ g/day) yielding the highest consumption during weeks 3 through 8, an effect that persisted for several weeks after MCP-1 delivery ended. These data suggest that increases in MCP-1, whether due to ethanol or various other neuroimmune mechanisms, promotes further ethanol consumption.

Due to a paucity of published data using ICV MCP-1 infusion, particularly chronic infusion, it was necessary for our experiment to test a wide range of MCP-1 concentrations. Since only the highest dose yielded a sustained effect, our experiments may have been limited by the dose or stability of MCP-1. Experiments with higher doses of MCP-1 are necessary to

confirm and extend our results, with the possibility that higher doses or increased stability would yield a more robust increase in drinking or a longer-lasting effect.

Chemokines, including MCP-1, participate in a variety of “normal” brain functions in addition to inflammation and pathology (for a review, see Réaux-Le Goazigo et al., 2013). The hallmarks of neuroinflammation are microglial activation, leukocyte infiltration, and blood-brain barrier (BBB) permeability, each with potentially deleterious consequences. MCP-1 by itself does not activate microglia but does regulate microglial chemotaxis, leukocyte infiltration, and BBB permeability (Hinojosa et al., 2011; Gunn et al., 1997). MCP-1 alters BBB permeability *in vivo* through direct effects on endothelial CCR2 receptors and subsequent tight junction modification, as well as indirectly through the recruitment of monocytes which can then release additional MCP-1 to alter permeability (Stamatovic et al., 2005; Cushing and Fogelman, 1992; Tieu et al., 2009, Gunn et al., 1997). An acute injection of 1 µg caused a very localized breakdown of the blood-brain barrier in hippocampal tissue (Bell et al., 1996) and a 25 µg ICV bolus injection (but not 5 µg – 20 µg) led to BBB permeability as measured by FITC-albumin leakage and leukocyte infiltration detected throughout the brain (Stamatovic et al., 2005). It is difficult to translate bolus injections quantities to chronic pump infusion rates, but Stamatovic and colleagues (2005) also used chronic ICV administration of 5 µg/h for 3 days or 2.5 µg/hr for 7 days to achieve BBB permeability and leukocyte infiltration to a degree comparable to those seen with the 25 µg bolus, which are 30-fold and 60-fold higher concentrations than we used in our experiment. Thus, it is unlikely that our effect on ethanol consumption was due to neuroinflammatory mechanisms. Although an experiment to determine if using a higher dose of MCP-1 could lead to a stronger effect on ethanol consumption is warranted, measurements of leukocyte infiltration, BBB permeability, microglial density and activation, and brain MCP-1 levels, each at various points in time throughout the study, would help determine if MCP-1 would be facilitating ethanol consumption through normal or inflammatory mechanisms.

We speculate that MCP-1 is having a neuromodulatory effect on the rewarding or aversive properties of ethanol consumption or withdrawal, respectively. One possible “normal” mechanism in line with the concentrations of MCP-1 used in our experiment is the activation of CCR2 receptors on dopamine neurons. An intracranial injection of 50 ng of MCP-1 into the substantia nigra resulted in elevated dopamine levels in the dorsal striatum for 2 hours (measurements taken every 20 minutes; Guyon et al., 2009). The same study also showed an increase in dopaminergic activity in slices exposed to 10 nM MCP-1, through modulation of potassium currents. A 50 ng bolus ICV injection also resulted in an increase in phosphorylated tyrosine hydroxylase levels in the VTA 24 hours later, while a CCR2 antagonist attenuated the conditioned place preference for methamphetamine (Wakida et al., 2014). It will be critical to determine if MCP-1 levels reached in our experiment, or through ethanol administration alone, can influence dopaminergic activity. The modest influence of MCP-1 on self-administration in our experiment parallels the modest influence of MCP-1 on dopamine seen in these studies. These parallel effects are consistent with a dopamine link in the mechanism of MCP-1 on sweetened ethanol consumption.

We used sweetened ethanol in our experiment in order to maximize the success rate of ethanol self-administration induction and to provide consistently high levels of self-administration in a minimal amount of time. Minimizing induction time was critical in order to study both the acquisition and maintenance of self-administration during limited access sessions within the time-frame of MCP-1 delivery through the osmotic minipumps. The higher intake achieved with the addition of sucrose to the solution (1.0 g/kg with sucrose versus 0.6 g/kg without sucrose in a 20-minute limited-access session is common) increases the likelihood of central pharmacological effects of ethanol. We have previously detected increased mesolimbic dopamine release within the first few minutes of intake using this model (Carrillo and Gonzales, 2011; Howard et al., 2009), leading us to believe that the BAC range reached (~0.05 % BAC) is reinforcing. Others have shown that low BAC's are anxiolytic and reinforcing as well (for a review, see Koob 2004). Although we acknowledge the presence of sucrose as a complication, the lack of effect on sucrose self-administration leads us to believe that the interaction was primarily driven by ethanol.

Breese et al. (2008) previously did not show an increase in ethanol intake after two acute ICV injections of 100 ng MCP-1 or other cytokines, or various doses of lipopolysaccharide (LPS). The ethanol intake model was 5 days of 4.5% ethanol liquid-only diet, which corresponds to the timing of (and lack of effect during) our acquisition experiment. We are not surprised by the lack of immediate effect in either study, given the protracted nature of both clinical or animal models of ethanol-induced neuroimmune gene expression or neuroinflammation (Qin et al., 2008; He and Crews, 2008; Valles et al., 2004; Pascual et al., 2007; Pascual et al., 2015; Zou and Crews, 2012; Alfonso-Loeches et al., 2010; Ehrlich et al., 2012) or neuroimmune-induced increases in self-administration (Blednov et al., 2011). However, comparisons of intake between our operant model and the 4.5% ethanol liquid-only diet used by Breese et al. (2008) should be made with caution due to the forced nature of ethanol consumption in the liquid-only model. Further experimentation using chronic dependence models is warranted.

In the same experiment, Breese et al. (2008) showed an increase in withdrawal-induced anxiety after two weekly ICV injections of 100 ng MCP-1 followed by 5 days of a 4.5% ethanol liquid-only diet. Their experiment did not show increased anxiety during withdrawal unless cytokines were administered, and previously showed increased anxiety only after repeated withdrawals (3 cycles) or restraint stress (Breese et al., 2004). They recorded blood ethanol levels at the start of withdrawal from the 5-day ethanol diet between 0.10 – 0.12 % BAC (Breese et al., 2004). The average BAC after our progressive ratio operant session (0.86 g/kg) would be approximately 0.04 % BAC (Carrillo and Gonzales, 2011; Howard et al., 2009), which may have been too low to sensitize withdrawal anxiety. Additionally, our animals were dual-housed, in contrast to the single-housed method employed by Breese and colleagues (2004; 2008). However, our animals were paired with an unfamiliar partner and our control animals matched the anxiety levels of the control animals in Breese et al. (2004; 2008). Taken together, the data from our experiment and Breese et al. (2004; 2008) suggest that neither acute nor chronic MCP-1 cause anxiety, and likely exacerbate alcohol withdrawal-induced anxiety only after moderate or greater BAC. The apparent anxiolytic effect of low-dose MCP-1 in our experiment deserves further exploration, particularly in a model of alcohol dependence.

In summary, we discovered that neuroimmune signaling through a specific cytokine can increase the consumption of sweetened ethanol in Long-Evans rats. Our data suggest that ethanol-induced increases in MCP-1, or increases in MCP-1 due to various other neuroimmune mechanisms, may further promote ethanol consumption. Our data add to a growing body of evidence implicating neuroimmune signaling in alcohol use disorders. Continued research into this mechanism, particularly using models of alcohol dependence, will help determine if targeting MCP-1 signaling has therapeutic potential in the treatment of alcohol use disorders.

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Fig. 1.

The effect of chronic intracerebroventricular (ICV) infusion of monocyte chemoattractant protein – 1 (MCP-1) on operant self-administration. MCP-1 was administered using subcutaneous osmotic minipumps over 5 weeks (1 week of recovery followed by 4 weeks of self-administration with pumps flowing) followed by 4 weeks of self-administration without flow. A) MCP-1 did not influence the self-administration of sucrose alone ($P=0.99$, $n=7$ for controls and $n=8$ for MCP-1 group). B) ICV infusion of MCP-1 had no effect on the first 5 days of operant self-administration of sweetened ethanol ($P=0.17$, $N=11/10/11/11$ for control/0.2/20/2000 ng/day doses respectively). C, D) MCP-1 increased the self-administration of sweetened ethanol over 8 weeks ($P=0.04$, $n=7/6/7/8$ for control/0.2/20/2000 ng/day doses respectively). The data is split across 2 graphs for clarity. The effect was also significant for the first four weeks of self-administration alone, while pumps were flowing ($P=0.02$). MCP-1 did not influence drinking over the final four-week period ($P=0.14$). For clarity, not all error bars are included. The black bar indicates MCP-1 infusion, which began one week prior to the initiation of self-administration.

Fig. 2.

The effect of chronic ICV infusion of MCP-1 on health-related measures. A) Chronic ICV infusion of MCP-1 had no effect on the body weight of the animals across 4 weeks of drinking ($P=0.58$, $n=7/6/7/8$ for control/0.2/20/2000 ng/day doses respectively). B) There was also no effect on locomotor activity during drinking sessions ($P=0.58$, $n=6/5/6/7$ for control/0.2/20/2000 ng/day doses respectively). For both A and B, a summary of the data averaged across the first 4 weeks of drinking is shown.

Fig. 3.

The effect of chronic ICV infusion of MCP-1 on the motivation to consume sweetened ethanol. A) There was no effect on the time to reach the response requirement (4 lever presses to gain access to the ethanol solution) across the four weeks of drinking ($P=0.88$, $n=7/6/7/8$ for control/0.2/20/2000 ng/day doses respectively). A summary of the data averaged across the first 4 weeks of drinking is shown. B) There was no effect on the consumption of ethanol during a progressive ratio test performed at the end of the 4th drinking week ($P=0.80$, $n=7/5/7/8$ for control/0.2/20/2000 ng/day doses respectively). C) There was no effect on the break point reached during the progressive ratio session ($P=0.76$, $n=7/5/7/8$ for control/0.2/20/2000 ng/day doses respectively).

Fig. 4.

The effect of chronic ICV infusion of MCP-1 on withdrawal-induced anxiety. The test was administered 6–8 hours after the progressive ratio self-administration session, at the end of the 4th drinking week. During a five-minute session, animals were paired with an unfamiliar animal matched by dose. A) There was no effect of dose on social interaction during the test ($P=0.37$, $n=6/4/6/8$ for control/0.2/20/2000 ng/day doses respectively). B) There was no effect on locomotion during the test ($P=0.54$, $n=6/4/6/8$ for control/0.2/20/2000 ng/day doses respectively).

Fig. 5.

ELISA was used to determine the concentration of MCP-1 in the pumps after 14 days (one week after drinking began) and 28 days (3 weeks after drinking began). Data is expressed as a percent of the original concentration of 54 nM (2 ng/day pumps). N=4 for each time point.