HHS Public Access

Author manuscript

Neuropharmacology. Author manuscript; available in PMC 2016 October 24.

Published in final edited form as:

Neuropharmacology. 2016 August ; 107: 181–188. doi:10.1016/j.neuropharm.2016.03.027.

Role of interleukin-10 (IL-10) in regulation of GABAergic transmission and acute response to ethanol

A. Suryanarayanan[a,*], **J.M. Carter**[c], **J.D. Landin**[c], **A.L. Morrow**[b], **D.F. Werner**[c], and **I. Spigelman**[d,**]

A. Suryanarayanan: a.suryanarayanan@usciences.edu; I. Spigelman: igor@ucla.edu

[a]Department of Pharmaceutical Sciences, University of the Sciences, Philadelphia College of Pharmacy, Philadelphia, PA 19104, USA

[b]Departments of Psychiatry and Pharmacology, University of North Carolina School of Medicine, Chapel Hill, NC 27599, USA

[c]Department of Psychology, Center for Development and Behavioral Neuroscience, Binghamton University, Binghamton, NY 13902, USA

[d]Division of Oral Biology & Medicine, School of Dentistry, 63-078 CHS, University of California, Los Angeles, Los Angeles, CA 90095, USA

Abstract

Mounting evidence indicates that ethanol (EtOH) exposure activates neuroimmune signaling. Alterations in pro-inflammatory cytokines after acute and chronic EtOH exposure have been heavily investigated. In contrast, little is known about the regulation of neurotransmission and/or modulation by anti-inflammatory cytokines in the brain after an acute EtOH exposure. Recent evidence suggests that interleukin-10 (IL-10), an anti-inflammatory cytokine, is upregulated during withdrawal from chronic EtOH exposure. In the present study, we show that IL-10 is increased early (1 h) after a single intoxicating dose of EtOH (5 g/kg, intragastric) in Sprague Dawley rats. We also show that IL-10 rapidly regulates GABAergic transmission in dentate gyrus neurons. In brain slice recordings, IL-10 application dose-dependently decreases miniature inhibitory postsynaptic current (mIPSC) area and frequency, and decreases the magnitude of the picrotoxin sensitive tonic current (I_{tonic}), indicating both pre- and postsynaptic mechanisms. A PI3K inhibitor LY294002 (but not the negative control LY303511) ablated the inhibitory effects of IL-10 on mIPSC area and I_{tonic}, but not on mIPSC frequency, indicating the involvement of PI3K in postsynaptic effects of IL-10 on GABAergic transmission. Lastly, we also identify a novel neurobehavioral regulation of EtOH sensitivity by IL-10, whereby IL-10 attenuates acute EtOH-induced hypnosis. These results suggest that EtOH causes an early release of IL-10 in the brain, which may contribute to neuronal hyperexcitability as well as disturbed sleep seen after binge

*Corresponding author. Department of Pharmaceutical Sciences, University of the Sciences, Philadelphia College of Pharmacy, 600 S 43rd Street, PTC-241, Philadelphia, PA 19104, USA. Tel.: +1 2155967152. **Corresponding author. Division of Oral Biology and Medicine, School of Dentistry, University of California, Los Angeles, 10833 LeConte Avenue, 63-078 CHS, Los Angeles, CA 90095-1668, USA.

Conflicts of interest

The authors declare no competing financial interests.

exposure to EtOH. These results also identify IL-10 signaling as a potential therapeutic target in alcohol-use disorders and other CNS disorders where GABAergic transmission is altered.

Keywords

Interleukin-10; Alcohol; GABAA; Neuroimmune; Anti-inflammatory; mIPSC

1. Introduction

A growing body of evidence from behavioral, molecular, genomic and electrophysiological studies indicates that ethanol (EtOH) exposure activates neuroimmune signaling (Crews et al., 2011; Mayfield et al., 2013; Bajo et al., 2015a). A number of hypotheses exist as to how EtOH causes increased activation of pro-inflammatory cytokines. For example, EtOH-induced liposaccharide (LPS) release into the systemic circulation and increased high-mobility group box 1 (HMGB1) protein release from neurons are implicated, both of which increase toll-like receptor 4 activity (Wang et al., 2010; Zou and Crews, 2014). This activity causes microglial activation, leading to further expression of pro-inflammatory genes, that are associated with increased alcohol consumption (Mayfield et al., 2013). Thus, mice treated with a high EtOH dose show an increase in systemic levels of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNFα) and monocyte chemoattractant protein-1 (MCP-1), accompanied with a persistent increase in pro-inflammatory cytokines in the brain (Norkina et al., 2007; Qin et al., 2008). EtOH preference and amount of EtOH consumed are significantly reduced in mice lacking interleukin-1 receptor antagonist (IL-1ra) and in interleukin-6 (IL-6) knockout mice (Blednov et al., 2012).

In addition to neuroimmune modulation after EtOH exposure, cytokines and their receptors regulate neurotransmission and synaptic plasticity (Vezzani and Viviani, 2015), including ligand-gated ion channel trafficking and function via kinase-dependent mechanisms (Viviani et al., 2003; Yang et al., 2005). TNFα acts on neuronal TNFR1 by a phosphatidylinositol-3-kinase (PI3K) mechanism to exocytose AMPA receptors (AMPAR), thereby increasing excitatory neurotransmission in hippocampal neurons (Stellwagen et al., 2005). Conversely, TNFα causes endocytosis of $GABA_A$ receptors ($GABA_A$Rs) (Stellwagen et al., 2005). Another pro-inflammatory cytokine, interleukin-1β (IL-1β), reduces the frequency of AMPAR-dependent excitatory synaptic currents, but enhances NMDAR-mediated currents by activation of tyrosine kinases (Viviani et al., 2003; Yang et al., 2005). In addition, the IL-1 receptor 1 (IL-1R1) and NMDA receptor families interact with each other (Gardoni et al., 2011). IL-1β also increases recruitment of $GABA_A$Rs to cell-surface via IL-1R1-dependent PI3K activation, causing a delayed enhancement of GABA currents (Serantes et al., 2006). Overall, investigations of $GABA_A$R indicate that the two pro-inflammatory cytokines TNFα and IL-1β diminish and augment inhibitory synaptic transmission, respectively.

In contrast to pro-inflammatory cytokines, less is known about the regulation of neurotransmission and/or modulation of anti-inflammatory cytokines in the brain after acute EtOH exposure. Indeed, recent investigations show that IL-1ra, an endogenous anti-

inflammatory factor, regulates GABAergic transmission in central nucleus of amygdala and EtOH effects on synaptic activity (Bajo et al., 2015b). Also, mice lacking IL-1ra show reduced EtOH consumption (Blednov et al., 2015). In this study, we focused on interleukin-10 (IL-10), an anti-inflammatory cytokine. The −592C > A IL-10 gene polymorphism is associated with alcoholism in Spanish subjects (Marcos et al., 2008). A recent human study showed that after a single binge EtOH exposure, LPS-induced pro-inflammatory state is followed by an anti-inflammatory state in blood samples collected 2–5 h post-binge (Afshar et al., 2015). A single 24 h incubation with EtOH (25 mM) also increases IL-10 production by human monocytes (Norkina et al., 2007). In a 4-day binge EtOH model, hippocampal IL-10 levels were upregulated 7 days after withdrawal (Marshall et al., 2013). Interestingly, no blood-brain-barrier (BBB) disruption or upregulation of hippocampal pro-inflammatory cytokines occurred at this time point. Despite increases peripherally from acute exposure and centrally from chronic exposure, much remains unknown about alterations in anti-inflammatory cytokines in the brain after a single binge EtOH exposure and their possible modulation of GABAergic neurotransmission.

In the current study, we examine the effects of a single intoxicating dose of EtOH on rat hippocampal IL-10 levels. We also examine IL-10 effects on inhibitory tonic and phasic GABAergic currents in hippocampal dentate gyrus (DG) neurons and the involvement of PI3-kinase in these effects. Finally, we examine the behavioral contributions of IL-10 to EtOH's hypnotic responses, which may reflect neuroimmune modulation of sleep. Overall, these results suggest that early increases in brain IL-10 after a single intoxicating dose of EtOH may contribute to disrupted sleep following EtOH exposure.

2. Methods and materials

2.1. Animals

All animal experiments followed the Institutional Animal Care and Use Committee approved protocols. Newborn mixed-sex pups from Sprague Dawley breeding pairs (250–300 g, Harlan) were used for preparation of primary neuron cultures. Male adult Sprague Dawley rats were used for hippocampal slice recordings and behavioral studies (200–250 g; Harlan and Taconic, respectively).

2.2. Cultured cerebral cortical neurons

Primary cultures of rat cerebral cortical neurons were prepared from rat pups on postnatal day 0–1 and maintained for 18–24 DIV (days *in* vitro) before experiments, as described previously (Kumar et al., 2010).

2.3. Chemicals

Unless otherwise stated, all chemicals and ELISA kits were obtained from Sigma-Aldrich. LY294002 and LY303511 were obtained from Tocris Bioscience.

2.4. In vitro and in vivo EtOH exposure

For acute EtOH (Pharmco Products) exposure *in vitro*, primary cultures of rat cerebral cortex were exposed to either vehicle or 50 mM EtOH for 4 h. For EtOH exposure, cultures

were incubated in media containing 50 mM EtOH and placed into an enclosed plastic vapor chamber inside the incubator. A separate beaker of water containing 50 mM EtOH was used to maintain stable EtOH concentrations in this plastic chamber. Control cells had media that did not contain EtOH and were placed in a vapor chamber with a beaker containing only water. For acute EtOH exposure *in vivo*, adult male Sprague Dawley rats were treated with either vehicle or 5 g/kg EtOH by gavage (n = 6/group). Control group was comprised of naive age-matched rats (n = 6). Rats were treated at the same time of the day (10–11:00 a.m.) to control for diurnal variations in cytokine levels. At 1 h post-gavage, rats were anesthetized with isoflurane, euthanized, and hippocampi were extracted and frozen. Hippocampi were homogenized in radio-immunoprecipitation assay buffer and total protein concentrations were measured in a DC protein assay (BioRad, Hercules, CA). All samples were resuspended to a concentration of 2 mg/ml.

2.5. Electrophysiology

Whole-cell voltage clamp recordings from cultured cortical neurons were conducted at room temperature using glass microelectrodes with a resistance of 2–5 MΩ when filled with internal solution composed of (in mM): $CsCl_2$, 150; $MgCl_2$, 3; HEPES, 15; K_2ATP, 2; EGTA, 5; phosphocreatine, 15; and 50 U/ml creatine phosphokinase (adjusted to pH 7.4 with KOH). $GABA_A$R-mediated mIPSCs were pharmacologically isolated by perfusing the neurons with a HEPES-buffered solution composed of (in mM): NaCl 142, HEPES 10, D-glucose 10, KCl 5, $CaCl_2$ 4, $MgCl_2$ 1, pH 7.4 with 300 nM tetrodotoxin and 1 mM kynurenic acid. Cells were voltage-clamped at −70 mV. $GABA_A$R-mediated currents were acquired using pCLAMP10 software (Molecular Devices) and analyzed using pCLAMP10 and the MiniAnalysis (Synaptosoft Inc.) programs.

For slice recordings, 400 μm hippocampal sections were obtained as previously described (Liang et al., 2007). Whole-cell recordings were obtained from DG cells at 34 ± 0.5 °C during perfusion with artificial cerebrospinal fluid (ACSF) composed of (in mM): NaCl, 125; KCl, 2.5; $CaCl_2$, 2; $MgCl_2$, 2; $NaHCO_3$, 26 and D-glucose, 10. The ACSF was continuously bubbled with a 95/5% mixture of O_2/CO_2 to ensure adequate oxygenation of slices and a pH of 7.4. Patch pipettes contained (in mM): $CsCl_2$, 135; $MgCl_2$, 2; $CaCl_2$, 1; ethylene glycol-bis(β-aminoethyl ether)-*N,N,N′,N′*-tetra-acetic acid, 11; *N*-2-hydroxyethylpiperazine-*N′*-2-ethanesulfonic acid, 10; K_2ATP, 2; Na_2GTP, 0.2; pH adjusted to 7.25 with CsOH. $GABA_A$R-mediated mIPSCs were pharmacologically isolated by adding tetrodotoxin (TTX, 0.5 μM), D(−)-2-amino-5-phosphonopentanoate (APV, 40 μM), 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 10 μM), and CGP 54,626 (1 μM) to the ACSF from stock solutions. Cells were voltage-clamped at −70 mV. For the PI3-kinase experiments, LY294002 and LY303511 were dissolved in dimethyl sulfoxide (DMSO) to yield stock solutions of 50 mM PI3K inhibitor LY294002 (10 μM) or LY303511 (10 μM) were included in the recording pipette. The concentration of DMSO in the final internal pipette solution was 0.02%. The kinetics of mIPSCs recorded with control intrapipette solution were compared with those recorded with intrapipette solution containing LY303511 or LY294002.

2.6. Enzyme-linked immunosorbent assay (ELISA)

A rat IL-10 ELISA kit (Invitrogen, Carlsbad, CA) with a sensitivity of <5 pg/ml was used to measure IL-10 content in cortical cell culture supernatants and in adult rat hippocampal tissue.

2.7. Loss of righting reflex (LORR)

Intracerebroventricular (i.c.v.) cannulation surgeries and LORR were performed as we have done elsewhere (Carter et al., 2016). Briefly, animals were anesthetized with isoflurane and a 12.5 mm long steel cannulae were placed with the following stereotaxic measurements from bregma: AP −0.5 mm: ML +1.5 mm, DV −2.5 mm. All subjects were administered buprenorphine as postoperative analgesic and singly housed for one week with enrichment and daily observations and weighing prior to behavioral testing. To measure the effect of IL-10 on EtOH-induced LORR, adult rats were administered IL-10 (300 ng/rat) or ACSF intracerebroventricluarly (i.c.v., 1 μL/min rate) immediately following EtOH administration (3.5 g/kg, intraperitoneally). The selected IL-10 dose was based on studies elsewhere (Knoblach and Faden, 1998; Bluthe et al., 1999). Following completion of drug delivery, the i.c.v. needles were left in place for an additional minute to mitigate backflow into the cannula. Following EtOH and IL-10 administration, rats were placed in a supine position in V-shaped troughs (90° angle) until able to right themselves. The rat was considered to have regained its righting reflex if capable of righting itself three consecutive times within 60 s. As EtOH was administered 5 min prior to IL-10, LORR duration was calculated as the time from EtOH administration until they regained their righting reflex. Trunk blood samples were taken immediately after rats regained the righting reflex and blood alcohol concentration was analyzed using an AM5 Alcohol Analyzer (Analox Instruments, Lunenburg, MA).

2.8. Statistical analyses

Group differences were evaluated by *t*-test or ANOVA where appropriate. For ELISAs, one-way ANOVA-Holm-Sidak method was employed. For electrophysiological data, Dunn's multiple comparison versus control method after application of Kruskal-Wallis one-way ANOVA, or one-way ANOVA-Holm-Sidak method was employed, as appropriate. For LORR data, Student's unpaired *t*-test was employed. $p < 0.05$ was considered statistically significant. Data are expressed as the mean ± SEM.

3. Results

3.1. A single intoxicating dose of EtOH increases hippocampal IL-10 levels

Preliminary *in vitro* studies were carried out in supernatant, i.e. sample media collected from rat primary cultured cortical neurons. EtOH (50 mM) exposure for 4 h increased IL-10 from 82.29 ± 25.19 pg/ml (untreated cultures) to 175.07 ± 60.92 pg/ml (Fig. 1A). IL-10 levels were attenuated after incubation with a rat IL-10 neutralizing antibody (Fig. 1A). Based on these preliminary data we continued our studies in adult rats. We have previously shown that peak plasma [EtOH] of ~60 mM (~275 mg/dL) is reached at 1 h after EtOH (5 g/kg, gavage) administration, a level comparable to that used in our preliminary cultured neuron studies

(Liang et al., 2007). EtOH gavage increased hippocampal IL-10 levels from 60.97 ± 4.86 pg/mg in naïve rats to 89.79 ± 9.17 pg/mg at one hour ($p = 0.025$, n = 8/group) (Fig. 1B). No significant change in IL-10 was observed following water gavage suggesting that the increase in IL-10 seen after EtOH treatment was not an effect mediated by gavage-related stress to the animals.

3.2. IL-10 causes a dose-dependent inhibition of GABAergic mIPSCs

In whole-cell patch clamp recordings, when $GABA_AR$ currents were pharmacologically isolated by blockade of ionotropic glutamate receptors, $GABA_BRs$, and voltage-gated sodium channels, $GABA_AR$ currents could be separated into two types: phasic miniature inhibitory postsynaptic currents (mIPSCs) and tonic current (I_{tonic}) which are mediated by synaptic and extrasynaptic and $GABA_ARs$, respectively (Mody and Pearce, 2004). In preliminary recordings from cultured cortical neurons (18–24 DIV), we observed that application of IL-10 (5–50 ng/ml) dose-dependently inhibited mIPSC total charge transfer and frequency (Fig. 2A and B). This IL-10 inhibition of $GABA_AR$ currents was reversible upon washout. IL-10 (5 ng/ml) also significantly reduced the holding current (I_{hold}), suggesting that IL-10 inhibits the tonic current. Conversely, incubating cultures with the IL-10 neutralizing antibody for 4 h caused a significant increase in mIPSC frequency as compared to untreated sister cultures (Fig. 2C). Decreased mIPSC frequency after IL-10 application and increased mIPSC frequency after IL-10 neutralizing antibody incubation both suggest presynaptic actions of IL-10. In contrast, the IL-10-mediated decrease in mIPSC total charge transfer suggests postsynaptic actions.

To study the actions of IL-10 in adult brain slices, we recorded the effect of bath application of IL-10 on mIPSCs and on the picrotoxin-sensitive I_{tonic} in DG cells of rat hippocampus (Fig. 3). Similar to recordings from cultured neurons, we found that IL-10 (20–50 ng/ml) decreased the mIPSC area to ~ 80% of control (Fig 3B). In agreement with a decrease in mIPSC area, 20–50 ng/ml IL-10 also reduced the peak amplitude to 63% of the control peak amplitude (Fig. 3C, n = 7–13 neurons/6 rats; $p < 0.05$). In addition, IL-10 application caused a reversible decrease in I_{tonic} (Fig. 3A and E). Application of 1–50 ng/ml IL-10 caused a concentration-dependent decrease in I_{tonic} from 46.2 ± 6.9 pA (control) to 5.6 ± 2.6 pA (50 ng/ml) (Fig. 3E, n = 7–13 neurons/6 rats $p < 0.05$). IL-10 also caused a concentration-dependent decrease in mIPSC frequency, 50 ng/ml IL-10 reduced mIPSC frequency to 71% of the control frequency (Fig. 3D, n = 7–13 neurons/6 rats, $p < 0.05$), indicating presynaptic effects of IL-10 (Cagetti et al., 2003). Together, these data suggest both pre- and postsynaptic actions of IL-10 on GABAergic neurotransmission.

3.3. IL-10 inhibits GABAergic neurotransmission via a mechanism involving phosphatidylinositol 3-kinase (PI3K)

Previous studies have shown that neuropeptides such as insulin and brain-derived neurotrophic factor (BDNF) modulate $GABA_AR$ trafficking via diverse mechanisms such as phosphoinositide 3 kinase (PI3K) mediated activation of Akt and PKC-mediated phosphorylation of $GABA_AR$ β3 subunit (reviewed in (Luscher et al., 2011)). It is also known that phosphorylation of a single site of the $GABA_AR$ β subunit can have differential effects on $GABA_AR$ trafficking depending on the kinase involved (Luscher et al., 2011).

Given these observations, we hypothesized that PI3K was involved in IL-10 modulation of postsynaptic $GABA_AR$ function. To test this hypothesis, we examined the effects of IL-10 on $GABA_AR$ currents in DG cells, in the presence/absence of a PI3K inhibitor in the patch pipette (Fig. 4 A–C). We found that IL-10-mediated decreases in mIPSC area and I_{tonic} magnitude were abolished in presence of the PI3K inhibitor LY294002 (10 μM) but not by LY303511 (10 μM), a structurally related negative control compound (Fig. 4A and B, n = 6–8 neurons/3 rats; $p < 0.05$). In the presence of LY303511 in the patch pipette, bath application of 20 and 50 ng/ml IL-10 caused a similar change in mIPSC area (Fig 4A), I_{tonic} magnitude (Fig. 4B) and mIPSC frequency (Fig. 4C), similar to results seen in control recordings with application of IL-10 alone (n = 6–8 neurons/3 rats, $p < 0.05$). However, in the presence of LY294002 in the pipette, 20 and 50 ng/ml IL-10 application no longer caused a decrease in mIPSC area or I_{tonic} magnitude (Fig. 4A and B, n = 6–8 neurons/3 rats, $p > 0.05$). As expected, inhibiting postsynaptic intracellular PI3K activity did not alter IL-10-mediated decrements in mIPSC frequency (Fig. 4C).

3.4. IL-10 administration decreases duration of EtOH-induced LORR

Finally, since EtOH exposure caused an increase in hippocampal IL-10, we investigated if IL-10 administration (300 ng/rat, i.c.v.) affected the obtunding behavioral response to EtOH. IL-10 reduced the duration of EtOH-induced LORR by ~26% (Fig. 5: 189.8 ± 17.1 min for IL-10; 257.1 ± 21.9 for vehicle controls; $t(10) = 2.426$, $p < 0.05$). Analysis of blood EtOH concentrations revealed higher levels in IL-10- versus ACSF-treated rats upon regaining the righting reflex. (IL-10-treated group: 357.2 ± 10.9 mg%, ACSF-treated group: 320.9 ± 15.6 mg%; $t(10) = 1.906$, $p < 0.05$). As IL-10 exposure resulted in shorter LORR duration, despite higher BECs, this suggests that IL-10's behavioral effect is likely due to its central neurochemical activity and not related to altered EtOH metabolism.

4. Discussion

We show that brain content of the anti-inflammatory cytokine IL-10 is increased 1 h after a single intoxicating dose of EtOH. We also identify a novel rapid regulation of GABAergic transmission by IL-10 in cortical and hippocampal neurons, via both pre- and postsynaptic mechanisms. The postsynaptic effect appears to be PI3K-dependent. Reduced sleep quality is one of the most prominent effects of acute EtOH withdrawal and is likely due to hyperexcitability, but the mechanisms involved are unclear. Behavioral evidence presented shows that IL-10 regulates EtOH sensitivity, by reducing acute EtOH-induced hypnosis. Overall, these results suggest that early increases in brain IL-10 after a single intoxicating dose of EtOH may contribute to disrupted sleep following EtOH exposure.

A growing body of evidence implicate several cytokines in modulation of synaptic function and surface expression of ion channels (Vezzani and Viviani, 2015). For example, IL-1β augments $GABA_AR$ function in the brain, an effect that presumably mediates somnogenic and motor-depressant effects of IL-1β (Miller et al., 1991). Conversely, TNF-α shifts the balance between synaptic excitation and inhibition towards excitation via an increase in surface expression of AMPARs and endocytosis of $GABA_ARs$ (Stellwagen et al., 2005). IL-6 has been similarly shown to tilt the balance between excitation and inhibition towards

excitation in the temporal cortex (Atzori et al., 2012). We show that the anti-inflammatory cytokine IL-10 has a direct inhibitory effect on GABAergic mIPSCs recorded from adult rat hippocampal DG neurons. IL-10 causes a concentration-dependent inhibition of mIPSC frequency and amplitude, as well as tonic current magnitude. Previous studies have shown that IL-10 does not affect GluA1 trafficking in hippocampal cultures, so the effects of IL-10 seem to be selective for $GABA_A$Rs (Stellwagen et al., 2005).

Several studies also indicate changes in cytokine production and neuroinflammation after EtOH treatment. For example, Qin et al. have shown that after 10 daily doses of EtOH (5 g/kg) and LPS, pro-inflammatory cytokines are acutely elevated in the brain and remain elevated for a prolonged periods (Qin et al., 2008). The same study also reported that IL-10 expression and peptide levels were elevated in the liver, but decreased in the brain after EtOH and LPS treatment. Here, we show that a single intoxicating dose of EtOH increases IL-10 content in rat hippocampus as well as in primary cultured cortical neurons. Since IL-10 is a cytokine with anti-inflammatory and neuroprotective properties, this observation suggests early adaptive neuroprotective changes after an acute EtOH exposure. Interestingly, other studies report protracted elevations in IL-10 peptide levels 7 days after a 4-day binge EtOH exposure, without a breach in the BBB, as well as after 12-day withdrawal in a chronic EtOH exposure model (Marshall et al., 2013; Schunck et al., 2015). Moreover, it has been shown that IL-10 cannot cross the BBB (Kastin et al., 2003). These observations, along with our data, indicate that: 1) EtOH results in rapid *de novo* IL-10 synthesis in the brain independent of peripheral immune responses; and 2) IL-10's early adaptive effects may contribute to withdrawal hyperexcitability.

We find that hippocampal IL-10 levels are elevated as early as 1 h after an acute binge alcohol exposure and that IL-10 inhibits GABAergic transmission. It is also known that alcohol consumption is associated with increased wakefulness during the second half of sleep (Westermeyer, 1987; Landolt et al., 1996). Moreover, binge-drinking disrupts sleep homeostasis, leading to alcohol-related sleep disorders (Thakkar et al., 2014). Interestingly, central IL-10 administration has been shown to reduce sleep in rats and rabbits (Opp et al., 1995; Kushikata et al., 1999). IL-10 KO mice spend more time in slow-wave sleep and less time in wakefulness than WT mice (Toth and Opp, 2001). In parallel to these observations, our behavioral data show that IL-10 decreases EtOH-induced LORR duration in rats. These observations coupled with the role of $GABA_A$R in sleep suggest that acute EtOH exposure increases brain IL-10 levels, which in turn may inhibit sleep by inhibiting GABAergic transmission. Increased brain IL-10 levels could also play a role in disrupting sleep architecture, a commonly occurring phenomenon in binge alcohol drinkers (Popovici and French, 2013).

Neural structures and systems involved in sleep production and regulation, including the corticothalamocortical network, prefrontal and limbic structures, and hippocampal–cortical communications (Hobson and Pace-Schott, 2002) are highly susceptible to disruption by EtOH (Fadda and Rossetti, 1998) and especially by multiple cycles of chronic EtOH exposure (Becker, 1998; Veatch, 2006) Thus, mice exposed to repeated cycles of EtOH vapor show profound disruptions sleep time and sleep architecture mirror those reported for the human alcoholics (Veatch, 2006). We have previously shown that acute EtOH induces

temporary, but reversible changes in $GABA_AR$ plasticity in DG neurons, and such changes are highly correlated with tolerance to diazepam-induced LORR (Liang et al., 2007), while chronic intermittent EtOH causes persistent $GABA_AR$ plasticity in DG, along with tolerance to EtOH-induced LORR (Liang et al., 2006). Interestingly, neurogenesis in DG cells is reduced after sleep deprivation (Guzman-Marin et al., 2008), while binge alcohol consumption also reduces neurogenesis and cell proliferation in the adult rat DG (Nixon and Crews, 2002). Overall these results implicate the dentate gyrus as an important predictor brain region in behavioral plasticity to EtOH and sleep.

IL-10 has been reported to be produced by microglia (Lim et al., 2013) and astrocytes (Fickenscher et al., 2002). The IL-10 receptor is expressed by glia as well as neurons (Sharma et al., 2011). It has also been shown that IL-10 does not cross the blood-brain-barrier (Kastin et al., 2003), further supporting *de novo* synthesis in the brain, as suggested by our results. IL-10 binds to its cognate cell surface heterotetramer complex consisting of two ligand binding IL-10 receptor 1 (IL-10R1) and two accessory IL-10 receptor 2 (IL-10R2) subunits. Binding of IL-10 to the IL-10R activates IL-10R1-associated Janus kinase 1 (JAK1) and IL-10R2-associated tyrosine kinase 2 (Tyk2), reviewed in (Kwilasz et al., 2015). Activation of these 2 kinases causes IL-10R1 phosphorylation and phosphorylation of STAT3. Ultimately, a variety of downstream signaling events such as cytokine modulation by NF-κB activation, neuroprotection, among others are affected. IL-10 receptors have been shown to be expressed in microglia (Ledeboer et al., 2002) and cortical (Sharma et al., 2011) and hippocampal neurons (Lim et al., 2013). IL-10 is postulated to mediate neuroprotective effects by activation of the survival pathway consisting of PI3K and Akt/PKB and by modulation of intracellular Ca^{2+} levels (Strle et al., 2002; Tukhovskaya et al., 2014). PI3K activation leads to formation of phosphatidylinositol (3,4,5)-trisphosphate (PIP3) at the plasma membrane, causing the recruitment of AKT and its kinase, phosphoinositide-dependent protein kinase 1 (PDK1), to the membrane. With respect to $GABA_ARs$, insulin has been shown to induce surface expression of $GABA_AR$ via activation of PI3K, reviewed in (Luscher et al., 2011). One postulated mechanism is that PI3K P85 subunit forms a complex with $GABA_AR$ and this complex is abundant under basal conditions. On stimulation with insulin, there is a further rapid increase in the abundance of this complex and its association with phosphorylated lipids (PIP3). There is also an increase in the translocation of the $GABA_AR$-PI3K P85 complex to the membrane. Similarly, PI3K plays a role in interleukin-2 receptor (IL-2R) endocytosis (Basquin et al., 2013). In addition, infusion of the PI3K inhibitor, wortmannin in the nucleus accumbens attenuates both alcohol-mediated phosphorylation of AKT and excessive alcohol drinking in rats (Neasta et al., 2011). Collectively, these studies indicate that PI3K is activated after IL-10 binding to IL-10R and implicate PI3K in regulation of membrane $GABA_AR$ expression during binge alcohol intake. Based on these observations and our results that show IL-10-mediated inhibition of GABAergic mIPSCs, we hypothesized that PI3K was involved in IL-10 mediated inhibition of GABAergic transmission. To test this hypothesis, we carried out mIPSC recordings in presence/absence of a PI3K inhibitor in the patch pipette. Our results indicate that PI3K is involved in the postsynaptic effects of IL-10 on $GABA_AR$, since the PI3K inhibitor ablated the inhibitory effects of IL-10 on mIPSC area and tonic current, but not mIPSC frequency. The mechanisms of the intracellular signaling cascade after activation

of IL-10 R and any crosstalk with $GABA_AR$ remain to be elucidated. Similarly, IL-10 could also be altering trafficking of $GABA_AR$.

We also identify a novel neurobehavioral regulation of EtOH sensitivity by IL-10. We show that IL-10 blocks acute EtOH-induced hypnosis. Several other neuroimmune molecules have been implicated in the behavioral response to acute EtOH. For example, inhibition of toll-like receptor 4 (TLR4) signaling by (+) naloxone and null mutations in TLR4 cause a reduction in LORR duration (Wu et al., 2012). A similar effect on has been reported by blocking IL-1R signaling and by using IL-1ra in mice (Wu et al., 2011). It would be interesting to evaluate the behavioral response to EtOH using an IL-10R antagonist, but such a compound is not yet available commercially, albeit a human IL-10R peptide antagonist has been recently described (Naiyer et al., 2013). Given that anti-inflammatory compounds such as minocycline (Agrawal et al., 2011) and the NFκB inhibitor, caffeic acid phenethyl ester (CAPE) have been shown to reduce alcohol consumption (Harris and Blednov, 2013), it would be intriguing to evaluate the effects of IL-10 administration on EtOH consumption and preference.

Overall, our results uncover a novel neuroimmune modulation of GABAergic neurotransmission as well as a novel role for IL-10 in response to acute EtOH intake. We propose that an early increase in IL-10 levels contribute to the complex changes in neuroimmune signaling that occur after acute exposure to EtOH. Further, IL-10 has an inhibitory effect on GABAergic transmission, possibly leading to inhibition of sleep. Targeting IL-10 or signaling molecules such as PI3K may be a novel option for development of therapies in various CNS disorders such as alcohol-use disorders and epilepsy associated with an inflammatory component.

Acknowledgments

This work was supported by Dept. of Pharmaceutical Sciences at University of the Sciences and Milton Lev Memorial Foundation funds (A.S.), Psychology Department, Binghamton University (D.W.), P60-AA11605 (ALM), AA016100, AA022408, and AA022707 (I.S.).

References

Afshar M, Richards S, Mann D, Cross A, Smith GB, Netzer G, Kovacs E, Hasday J. Acute immunomodulatory effects of binge alcohol ingestion. Alcohol. 2015; 49:57–64. [PubMed: 25572859]

Agrawal RG, Hewetson A, George CM, Syapin PJ, Bergeson SE. Minocycline reduces ethanol drinking. Brain Behav Immun. 2011; 25(Suppl 1):S165–S169. [PubMed: 21397005]

Atzori M, Garcia-Oscos F, Mendez JA. Role of IL-6 in the etiology of hyperexcitable neuropsychiatric conditions: experimental evidence and therapeutic implications. Future Med Chem. 2012; 4:2177–2192. [PubMed: 23190106]

Bajo M, Herman MA, Varodayan FP, Oleata CS, Madamba SG, Harris RA, Blednov YA, Roberto M. Role of the IL-1 receptor antagonist in ethanol-induced regulation of GABAergic transmission in the central amygdala. Brain Behav Immun. 2015a; 45:189–197. [PubMed: 25479427]

Bajo M, Varodayan FP, Madamba SG, Robert AJ, Casal LM, Oleata CS, Siggins GR, Roberto M. IL-1 interacts with ethanol effects on GABAergic transmission in the mouse central amygdala. Front Pharmacol. 2015b; 6:49. [PubMed: 25852553]

Basquin C, Malarde V, Mellor P, Anderson DH, Meas-Yedid V, Olivo-Marin JC, Dautry-Varsat A, Sauvonnet N. The signalling factor PI3K is a specific regulator of the clathrin-independent

dynamin-dependent endocytosis of IL-2 receptors. J Cell Sci. 2013; 126:1099–1108. [PubMed: 23345407]

Becker HC. Kindling in alcohol withdrawal. Alcohol Health Res World. 1998; 22:25–33. [PubMed: 15706729]

Blednov YA, Benavidez JM, Black M, Mayfield J, Harris RA. Role of interleukin-1 receptor signaling in the behavioral effects of ethanol and benzodiazepines. Neuropharmacology. 2015; 95:309–320. [PubMed: 25839897]

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; 17:108–120. [PubMed: 21309947]

Bluthe RM, Castanon N, Pousset F, Bristow A, Ball C, Lestage J, Michaud B, Kelley KW, Dantzer R. Central injection of IL-10 antagonizes the behavioural effects of lipopolysaccharide in rats. Psychoneuroendocrinology. 1999; 24:301–311. [PubMed: 10101735]

Cagetti E, Liang J, Spigelman I, Olsen RW. Withdrawal from chronic intermittent ethanol treatment changes subunit composition, reduces synaptic function, and decreases behavioral responses to positive allosteric modulators of GABAA receptors. Mol Pharmacol. 2003; 63:53–64. [PubMed: 12488536]

Carter JM, Landin JD, Gigante ED, Rieger SP, Diaz MR, Werner DF. Inhibitors of calcium-activated anion channels modulate hypnotic ethanol responses in adult Sprague Dawley rats. Alcohol Clin Exp Res. 2016; 40:301–308. [PubMed: 26842249]

Crews FT, Zou J, Qin L. Induction of innate immune genes in brain create the neurobiology of addiction. Brain Behav Immun. 2011; 25(Suppl 1):S4–S12. [PubMed: 21402143]

Fadda F, Rossetti ZL. Chronic ethanol consumption: from neuroadaptation to neurodegeneration. Prog Neurobiol. 1998; 56:385–431. [PubMed: 9775400]

Fickenscher H, Hor S, Kupers H, Knappe A, Wittmann S, Sticht H. The interleukin-10 family of cytokines. Trends Immunol. 2002; 23:89–96. [PubMed: 11929132]

Gardoni F, Boraso M, Zianni E, Corsini E, Galli CL, Cattabeni F, Marinovich M, Di Luca M, Viviani B. Distribution of interleukin-1 receptor complex at the synaptic membrane driven by interleukin-1beta and NMDA stimulation. J Neuroinflammation. 2011; 8:14. [PubMed: 21314939]

Guzman-Marin R, Suntsova N, Bashir T, Nienhuis R, Szymusiak R, McGinty D. Rapid eye movement sleep deprivation contributes to reduction of neurogenesis in the hippocampal dentate gyrus of the adult rat. Sleep. 2008; 31:167–175. [PubMed: 18274263]

Harris, RA.; Blednov, YA. Neuroimmune genes and alcohol drinking behavior. In: Changhai Cui, AN.; Grandison, Lindsey, editors. Neural-immune Interactions in Brain Function and Alcohol Related Disorders. Springer; New York: 2013. p. 425-440.

Hobson JA, Pace-Schott EF. The cognitive neuroscience of sleep: neuronal systems, consciousness and learning. Nat Rev Neurosci. 2002; 3:679–693. [PubMed: 12209117]

Kastin AJ, Akerstrom V, Pan W. Interleukin-10 as a CNS therapeutic: the obstacle of the blood-brain/blood-spinal cord barrier. Brain Res Mol Brain Res. 2003; 114:168–171. [PubMed: 12829328]

Knoblach SM, Faden AI. Interleukin-10 improves outcome and alters proinflammatory cytokine expression after experimental traumatic brain injury. Exp Neurol. 1998; 153:143–151. [PubMed: 9743576]

Kumar S, Suryanarayanan A, Boyd KN, Comerford CE, Lai MA, Ren Q, Morrow AL. Ethanol reduces GABAA alpha1 subunit receptor surface expression by a protein kinase Cgamma-dependent mechanism in cultured cerebral cortical neurons. Mol Pharmacol. 2010; 77:793–803. [PubMed: 20159950]

Kushikata T, Fang J, Krueger JM. Interleukin-10 inhibits spontaneous sleep in rabbits. J Interferon Cytokine Res Off J Int Soc Interferon Cytokine Res. 1999; 19:1025–1030.

Kwilasz AJ, Grace PM, Serbedzija P, Maier SF, Watkins LR. The therapeutic potential of interleukin-10 in neuroimmune diseases. Neuropharmacology. 2015; 96:55–69. [PubMed: 25446571]

Landolt HP, Roth C, Dijk DJ, Borbely AA. Late-afternoon ethanol intake affects nocturnal sleep and the sleep EEG in middle-aged men. J Clin Psychopharmacol. 1996; 16:428–436. [PubMed: 8959467]

Ledeboer A, Breve JJ, Wierinckx A, van der Jagt S, Bristow AF, Leysen JE, Tilders FJ, Van Dam AM. Expression and regulation of interleukin-10 and interleukin-10 receptor in rat astroglial and microglial cells. Eur J Neurosci. 2002; 16:1175–1185. [PubMed: 12405978]

Liang J, Suryanarayanan A, Abriam A, Snyder B, Olsen RW, Spigelman I. Mechanisms of reversible GABAA receptor plasticity after ethanol intoxication. J Neurosci Off J Soc Neurosci. 2007; 27:12367–12377.

Liang J, Zhang N, Cagetti E, Houser CR, Olsen RW, Spigelman I. Chronic intermittent ethanol-induced switch of ethanol actions from extrasynaptic to synaptic hippocampal GABAA receptors. J Neurosci Off J Soc Neurosci. 2006; 26:1749–1758.

Lim SH, Park E, You B, Jung Y, Park AR, Park SG, Lee JR. Neuronal synapse formation induced by microglia and interleukin 10. PLoS One. 2013; 8:e81218. [PubMed: 24278397]

Luscher B, Fuchs T, Kilpatrick CL. GABAA receptor trafficking-mediated plasticity of inhibitory synapses. Neuron. 2011; 70:385–409. [PubMed: 21555068]

Marcos M, Pastor I, Gonzalez-Sarmiento R, Laso FJ. Interleukin-10 gene polymorphism is associated with alcoholism but not with alcoholic liver disease. Alcohol Alcohol. 2008; 43:523–528. [PubMed: 18436572]

Marshall SA, McClain JA, Kelso ML, Hopkins DM, Pauly JR, Nixon K. Microglial activation is not equivalent to neuroinflammation in alcohol-induced neurodegeneration: the importance of microglia phenotype. Neurobiol Dis. 2013; 54:239–251. [PubMed: 23313316]

Mayfield J, Ferguson L, Harris RA. Neuroimmune signaling: a key component of alcohol abuse. Curr Opin Neurobiol. 2013; 23:513–520. [PubMed: 23434064]

Miller LG, Galpern WR, Dunlap K, Dinarello CA, Turner TJ. Interleukin-1 augments gamma-aminobutyric acid A receptor function in brain. Mol Pharmacol. 1991; 39:105–108. [PubMed: 1847488]

Mody I, Pearce RA. Diversity of inhibitory neurotransmission through GABA(A) receptors. Trends Neurosci. 2004; 27:569–575. [PubMed: 15331240]

Naiyer MM, Saha S, Hemke V, Roy S, Singh S, Musti KV, Saha B. Identification and characterization of a human IL-10 receptor antagonist. Hum Immunol. 2013; 74:28–31. [PubMed: 23000375]

Neasta J, Ben Hamida S, Yowell QV, Carnicella S, Ron D. AKT signaling pathway in the nucleus accumbens mediates excessive alcohol drinking behaviors. Biol Psychiatry. 2011; 70:575–582. [PubMed: 21549353]

Nixon K, Crews FT. Binge ethanol exposure decreases neurogenesis in adult rat hippocampus. J Neurochem. 2002; 83:1087–1093. [PubMed: 12437579]

Norkina O, Dolganiuc A, Shapiro T, Kodys K, Mandrekar P, Szabo G. Acute alcohol activates STAT3, AP-1, and Sp-1 transcription factors via the family of Src kinases to promote IL-10 production in human monocytes. J Leukoc Biol. 2007; 82:752–762. [PubMed: 17575268]

Opp MR, Smith EM, Hughes TK Jr. Interleukin-10 (cytokine synthesis inhibitory factor) acts in the central nervous system of rats to reduce sleep. J Neuroimmunol. 1995; 60:165–168. [PubMed: 7642744]

Popovici I, French MT. Binge drinking and sleep problems among young adults. Drug Alcohol Depend. 2013; 132:207–215. [PubMed: 23466223]

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]

Schunck RV, Torres IL, Laste G, de Souza A, Macedo IC, Valle MT, Salomon JL, Moreira S, Kuo J, Arbo MD, Dallegrave E, Leal MB. Protracted alcohol abstinence induces analgesia in rats: possible relationships with BDNF and interleukin-10. Pharmacol Biochem Behav. 2015; 135:64–69. [PubMed: 26013579]

Serantes R, Arnalich F, Figueroa M, Salinas M, Andres-Mateos E, Codoceo R, Renart J, Matute C, Cavada C, Cuadrado A, Montiel C. Interleukin-1beta enhances GABAA receptor cell-surface expression by a phosphatidylinositol 3-kinase/Akt pathway: relevance to sepsis-associated encephalopathy. J Biol Chem. 2006; 281:14632–14643. [PubMed: 16567807]

Sharma S, Yang B, Xi X, Grotta JC, Aronowski J, Savitz SI. IL-10 directly protects cortical neurons by activating PI-3 kinase and STAT-3 pathways. Brain Res. 2011; 1373:189–194. [PubMed: 21138740]

Stellwagen D, Beattie EC, Seo JY, Malenka RC. Differential regulation of AMPA receptor and GABA receptor trafficking by tumor necrosis factor-alpha. J Neurosci Off J Soc Neurosci. 2005; 25:3219–3228.

Strle K, Zhou JH, Broussard SR, Venters HD, Johnson RW, Freund GG, Dantzer R, Kelley KW. IL-10 promotes survival of microglia without activating Akt. J Neuroimmunol. 2002; 122:9–19. [PubMed: 11777539]

Thakkar MM, Sharma R, Sahota P. Alcohol disrupts sleep homeostasis. Alcohol. 2014; 49:299–310. [PubMed: 25499829]

Toth LA, Opp MR. Cytokine- and microbially induced sleep responses of interleukin-10 deficient mice. Am J Physiol Regul Integr Comp Physiol. 2001; 280:R1806–R1814. [PubMed: 11353686]

Tukhovskaya EA, Turovsky EA, Turovskaya MV, Levin SG, Murashev AN, Zinchenko VP, Godukhin OV. Anti-inflammatory cytokine interleukin-10 increases resistance to brain ischemia through modulation of ischemia-induced intracellular Ca(2)(+) response. Neurosci Lett. 2014; 571:55–60. [PubMed: 24796809]

Veatch LM. Disruptions in sleep time and sleep architecture in a mouse model of repeated ethanol withdrawal. Alcohol Clin Exp Res. 2006; 30:1214–1222. [PubMed: 16792570]

Vezzani A, Viviani B. Neuromodulatory properties of inflammatory cytokines and their impact on neuronal excitability. Neuropharmacology. 2015; 96:70–82. [PubMed: 25445483]

Viviani B, Bartesaghi S, Gardoni F, Vezzani A, Behrens MM, Bartfai T, Binaglia M, Corsini E, Di Luca M, Galli CL, Marinovich M. Interleukin-1beta enhances NMDA receptor-mediated intracellular calcium increase through activation of the Src family of kinases. J Neurosci Off J Soc Neurosci. 2003; 23:8692–8700.

Wang HJ, Zakhari S, Jung MK. Alcohol, inflammation, and gut-liver-brain interactions in tissue damage and disease development. World J Gastroenterol. 2010; 16:1304–1313. [PubMed: 20238396]

Westermeyer J. Alcohol and other drug abuse and sleep behavior disorder. Jama. 1987; 258:323.

Wu Y, Lousberg EL, Moldenhauer LM, Hayball JD, Coller JK, Rice KC, Watkins LR, Somogyi AA, Hutchinson MR. Inhibiting the TLR4-MyD88 signalling cascade by genetic or pharmacological strategies reduces acute alcohol-induced sedation and motor impairment in mice. Br J Pharmacol. 2012; 165:1319–1329. [PubMed: 21955045]

Wu Y, Lousberg EL, Moldenhauer LM, Hayball JD, Robertson SA, Coller 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. 2011; 25(Suppl 1):S155–S164. [PubMed: 21276848]

Yang S, Liu ZW, Wen L, Qiao HF, Zhou WX, Zhang YX. Interleukin-1beta enhances NMDA receptor-mediated current but inhibits excitatory synaptic transmission. Brain Res. 2005; 1034:172–179. [PubMed: 15713269]

Zou JY, Crews FT. Release of neuronal HMGB1 by ethanol through decreased HDAC activity activates brain neuroimmune signaling. PLoS One. 2014; 9:e87915. [PubMed: 24551070]

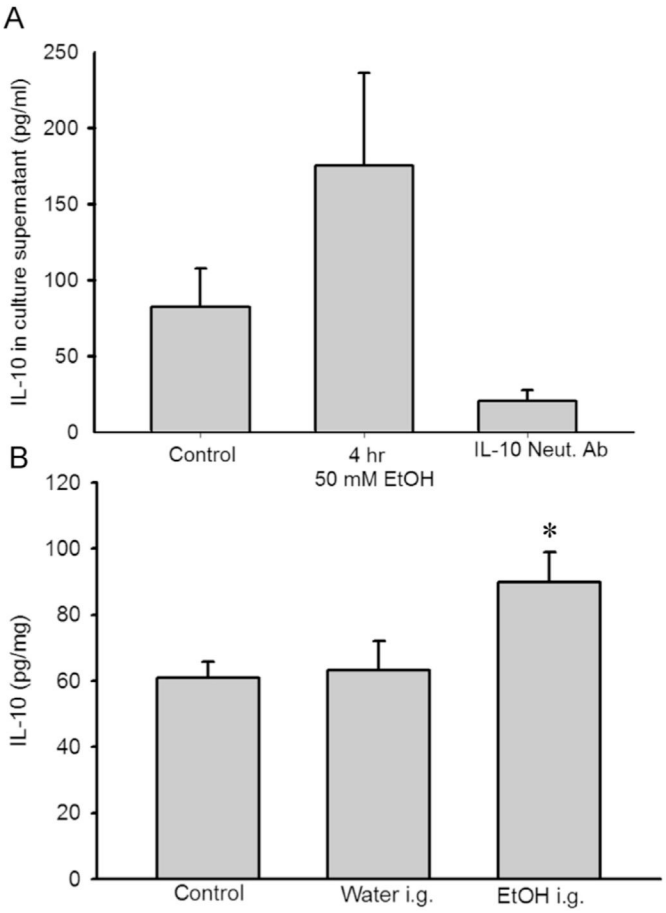

Fig. 1.

A. IL-10 release in primary cultured cortical neurons was potentiated after a 4-h incubation with 50 mM EtOH. IL-10 levels appeared attenuated after incubation with a rat IL-10 neutralizing antibody (n = 4/group). B. Hippocampal IL-10 content in adult rats was significantly increased one hour after a single intoxicating dose of EtOH (5 g/kg, i.g.). *, p = 0.025 (n = 8/group).

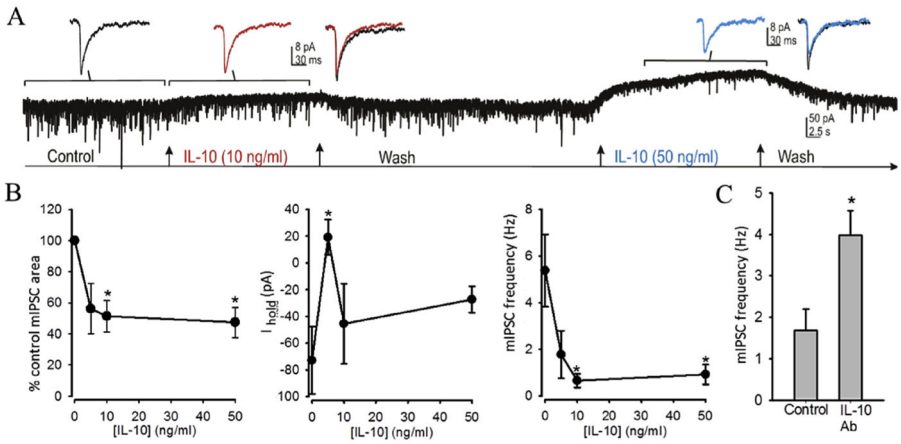

Fig. 2.

A. Application of 10 and 50 ng/ml IL-10 caused a reversible decrease in holding current of mIPSCs recorded from rat cultured cortical neurons (n = 11–13). B. Application of 5–50 ng/ml IL-10 caused a decrease in mIPSC total charge transfer (*, $p < 0.05$), an outward shift in holding current (*, $p < 0.05$) and decreased mIPSC frequency (*, $p < 0.001$), n = 5–13/group. C. Incubation of cultured neurons with a rat IL-10 neutralizing antibody caused a significant increase in mIPSC frequency (*, $p < 0.05$, n = 8/group).

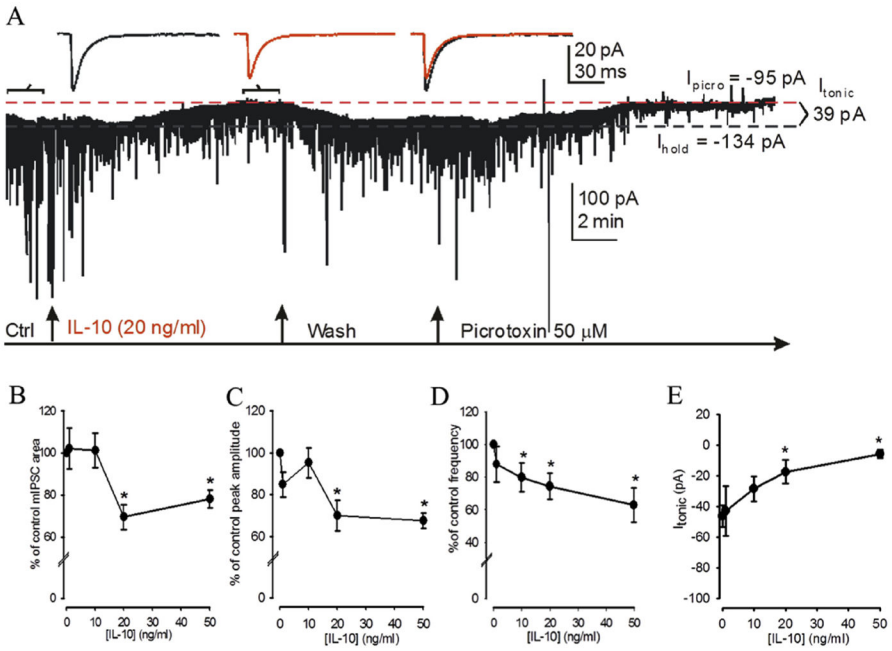

Fig. 3.

A. Application of IL-10 caused a reversible decrease in the picrotoxin-sensitive tonic current (I_{tonic}) recorded from a DG neuron. Application of 1–50 ng/ml IL-10 caused a decrease in B. mIPSC total charge transfer (*, $p < 0.05$). C. peak amplitude (*, $p < 0.05$), D. frequency of mIPSCs (*, $p < 0.001$) and E. tonic current magnitude (*, $p < 0.05$) and n = 7–13.

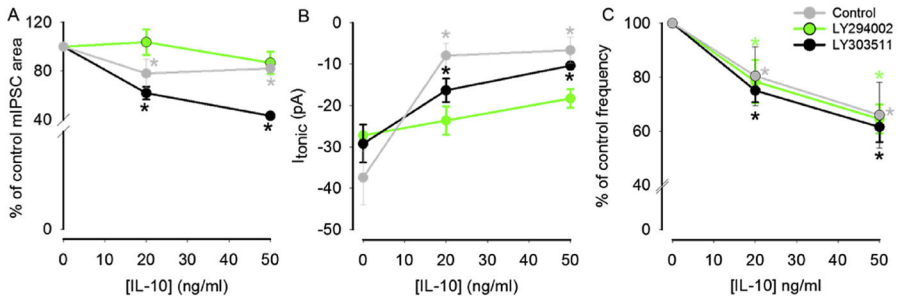

Fig. 4.

Effects of IL-10 (20 and 50 ng/ml) perfusion on A. mIPSC total charge transfer, B. tonic current magnitude, and C. mIPSC frequency. Recordings were made with control ( ), 10 μM LY294002 (, PI3-kinase inhibitor), or 10 μM LY303511 (●, negative control for LY294002) intrapipette solutions. Note that the IL-10-induced decreases in mIPSC area and I_{tonic} magnitude were blocked by the PI3-kinase inhibitor, but not by the negative control. With both LY compounds, the IL-10-induced decreases in mIPSC frequency were similar to recordings with control pipette solution. *, $p < 0.05$, n = 6–8/group.

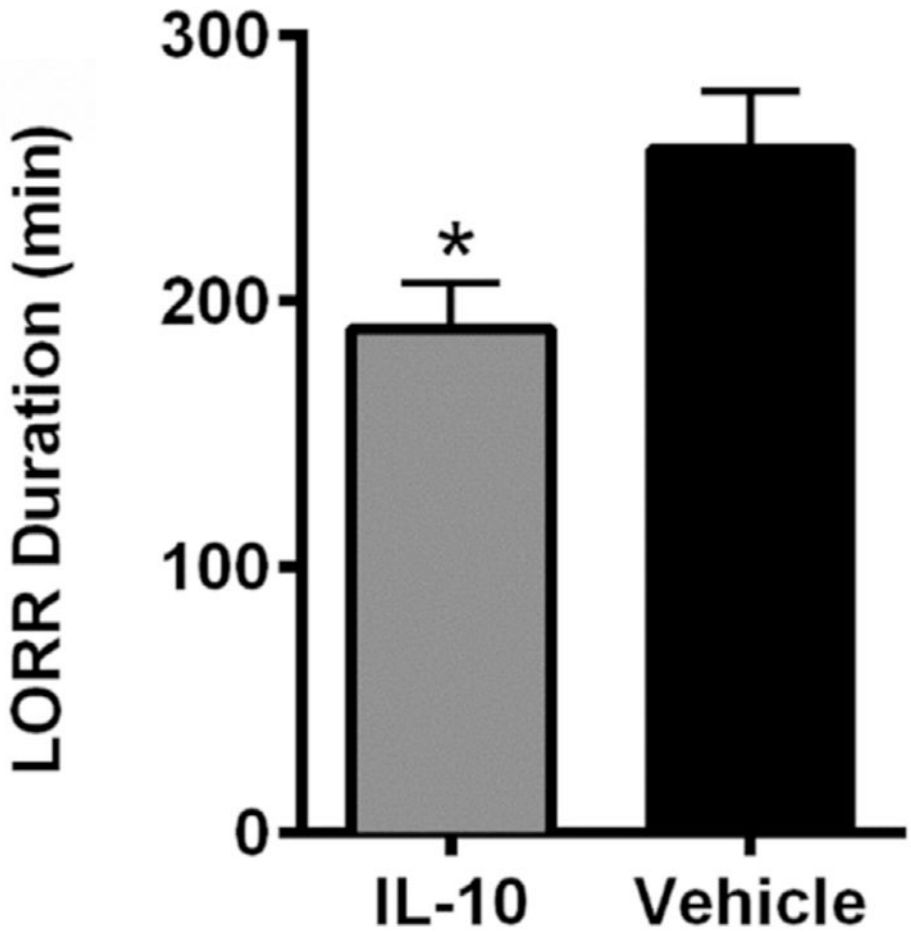

Fig. 5.

Effects of IL-10 administration on EtOH-induced loss of righting reflex. Immediately following a 3.5 g/kg intraperitoneal dose of EtOH, vehicle (ACSF) or IL-10 (300 ng, i.c.v.) was administered in adult rats. *, $p < 0.05$, $n = 6$ per group.