Published in final edited form as:

J Neurovirol. 2018 February ; 24(1): 88–97. doi:10.1007/s13365-017-0597-x.

Effects of docosahexaenoic acid on locomotor activity in ethanol-treated HIV-1 transgenic rats

Jianlin He^{1,2,3}, Wenfei Huang^{2,4}, Shizhong Zheng¹, Michael Vigorito^{2,5}, and Sulie L. Chang^{2,4}

¹Department of Pharmacology, School of Pharmacy, Nanjing University of Chinese Medicine, Nanjing 210029, People's Republic of China

²Institute of NeurolImmune Pharmacology, Seton Hall University, 400 South Orange Avenue, South Orange, NJ 07079, USA

³State Oceanic Administration, Third Institute of Oceanography, Xiamen 361005, People's Republic of China

⁴Department of Biological Sciences, Seton Hall University, South Orange, NJ 07079, USA

⁵Department of Psychology, Seton Hall University, South Orange, NJ 07079, USA

Abstract

Binge drinking affects the onset and progression of human immunodeficiency virus (HIV)-associated neurological disorders. The HIV-1 transgenic (HIV-1Tg) rat was created with a *gag*- and *pol*-deleted HIV-1 viral genome to mimic HIV-infected patients receiving combination anti-retroviral therapy (cART). Docosahexaenoic acid (DHA) is a marine compound that modulates inflammatory responses. Using HIV-1Tg rats subjected to binge exposure to ethanol (EtOH), this study examined whether DHA could reduce the detrimental neurological effects of EtOH and HIV proteins. Young adult male HIV-1Tg and F344 control rats received 4 mL/kg/day saline as a control (Saline group), 20 mg/kg/day DHA (DHA group), 4.8 g/kg/day 52% w/v EtOH (EtOH group), or 4.8 g/kg/day 52% w/v EtOH and 20 mg/kg/d DHA (DHA + EtOH group) by gavage for 5 weeks ($n = 6$ per group). EtOH was administered on days 5, 6, and 7 of each week. Locomotor activity (LMA) was assessed using open field tests before and 45, 90, 135, and 180 min after each treatment. Repeated binge EtOH exposure gradually decreased LMA measured before daily treatments in HIV-1Tg and F344 rats, an effect that was reversed by DHA only in the HIV-1Tg rats. Decreased LMA of rats after treatment and under the influence of EtOH was less pronounced, and the reversal effect of DHA did not reach statistical significance. The plasma endotoxin level was significantly higher in HIV-1Tg rats than in F344 rats. IL-6 and IL-18 expression in the striatum was significantly higher in the HIV-1Tg EtOH group than in the F344 EtOH group. DHA

Correspondence to: Shizhong Zheng; Sulie L. Chang.

Authors' contributions SLC and JH conceived and designed the experiments. JH and WH performed the lab work. SLC provided experimental animals/reagents/materials/analysis tools. JH, WH, MV, and SLC analyzed the data. JH, WH, SZ, MV, and SLC wrote the manuscript.

Compliance with ethical standards The experimental protocol was approved by the Institutional Animal Care and Use Committee (IACUC) at Seton Hall University, South Orange, NJ.

Conflict of interest The authors declare that they have no conflicts of interest.

significantly decreased the high levels of IL-6, IL-18, and NF- κ B expression observed in the HIV-1Tg EtOH group. DHA appears to ameliorate inflammation and consequently lessen the reductions in LMA produced by the combination of EtOH and HIV-1 viral proteins.

Keywords

Docosahexaenoic acid; HIV; Ethanol; Locomotor activity; Striatum

Introduction

Alcohol is the most widely used addictive substance in the world. Alcohol consumption raises considerable public health concerns and is a leading cause of suffering globally (Davoren et al. 2016). Alcohol abuse can produce significant alterations in brain structure and ultimately lead to neuropsychological disorders and behavioral dysfunction (Sanhueza et al. 2011; Yang et al. 2014). Binge drinking, particularly of hard liquor (more than 40% alcohol by volume), is common among college students. It can lead to learning and cognitive deficits and seriously impact a student's academic performance (Johnston et al. 2014; Siegel et al. 2013; Siegel et al. 2011). College students fall within the period of late adolescence to young adulthood when critical structural and functional maturation of the central nervous system (CNS) occurs (Silveri 2012). Ethanol (EtOH) is the key component in alcoholic beverages that produces alcohol's effects. As EtOH can infuse into all cells, alcohol consumption can potentially affect all systems within the body, including the immune system and the CNS (Prendergast and Little 2007). Therefore, alcohol use and abuse during college years, particularly binge drinking, is a major concern.

The striatum is a key brain region responsible for voluntary motor control, which can be experimentally assessed by evaluating locomotor activity (LMA) (Baez-Mendoza and Schultz 2013). The striatum is also part of the brain's reward circuit, and levels of various mediators in the striatum, such as cyclic AMP (cAMP), nerve growth factors, and inflammatory cytokines, have been suggested to integrate actions with rewards, as may occur with drinking alcohol (Aarli 2003; Baez-Mendoza and Schultz 2013; Bie et al. 2012; Crews and Vetreno 2016; Wrona 2006). About two decades ago, Meng et al. reported that modulation of cAMP in the striatum was involved in acute EtOH-induced motor impairment (Meng et al. 1998). Modulation of cAMP can affect many biological and pathological responses, including various inflammatory responses (Pociecha et al. 2015; Shu et al. 2016; Wang et al. 2003; Wang et al. 2017). Inflammation within the CNS, including the striatum, has been identified as one of the mechanisms underlying the neuronal dysfunction that can lead to behavioral disorders (Aarli 2003; Covacu and Brundin 2015).

Furthermore, the prevalence of alcohol use and alcohol abuse in specific populations, such as human immunodeficiency virus (HIV)-infected individuals, is much higher than that of the general population in the USA (Chang et al. 2014). Numerous studies have shown that EtOH and HIV-1 viral proteins have synergistic effects on inflammation in the CNS (Acheampong et al. 2002; Flora et al. 2005; Nair et al. 2015). HIV is a lentivirus that replicates by infecting and destroying primarily CD4⁺ T cells, a subset of lymphocytes

essential for normal function of the immune system. The end-stage of HIV viral progression is acquired immune deficiency syndrome (AIDS) (Kaplan et al. 2009). HIV-1 gp120 and Tat proteins alter tight junctions and brain endothelial cell permeability (Kanmogne et al. 2005). HIV-1 Nef protein produces neurotoxicity associated with cognitive deficits. Abundant expression of HIV-1 Nef and Rev proteins in brain astrocytes in vivo has been associated with dementia (Ranki et al. 1995). Treatment with combination anti-retroviral therapy (cART), which specifically inhibits assembly of HIV viral proteins and the activity of reverse transcriptase, has successfully limited the progression of HIV-1 to AIDS.

An HIV-1 transgenic (HIV-1Tg) rat model was created about two decades ago (Reid et al. 2001). The HIV-1Tg rat carries the *env* gene and six supplementary genes—*tat*, *rev*, *vif*, *vpr*, *vpu*, and *nef*—but the *gag* and *pol* genes, which are essential for assembly and reverse transcription, are deleted (Reid et al. 2001). Thus, the HIV-1Tg rat mimics HIV patients receiving cART and has been used for investigating the effects of long-term HIV-1 viral protein exposure in HIV-1-associated neuroinflammation and behavioral alterations (Vigorito et al. 2015). Despite a lack of viral replication, viral proteins are continually expressed throughout the animal's life (Abbondanzo and Chang 2014; Peng et al. 2010).

Docosahexaenoic acid (DHA) is a long-chain polyunsaturated fatty acid considered essential for maturation of the developing brain, retina, and other organs in newborn infants (Anderson et al. 1990; Jasani et al. 2017; Simopoulos 1991). DHA concentration is reported to be affected by estrogens (Giltay et al. 2004). Thus, women reportedly have a higher circulating DHA concentration than men (Giltay et al. 2004). During pregnancy, the maternal DHA concentration increases (Otto et al. 2001; Postle et al. 1995). DHA is poorly synthesized de novo (Menard et al. 1998) and a dietary supplement is necessary to substantially increase levels. Dietary DHA has been shown to exert beneficial effects on neuronal development, as well as neuroprotective properties (Dyall 2015; Lauritzen et al. 2016). Depletion of DHA is associated with various neurological abnormalities, such as anxiety, irritability, susceptibility to stress, impaired memory and cognitive functions, and extended reaction times (Cardoso et al. 2016). However, the modulatory effects of DHA on voluntary motor control or LMA have not been previously studied.

Based on this information, we hypothesized that daily treatment with DHA could reverse the effects of binge exposure to EtOH on LMA in the presence of HIV viral proteins. To avoid interference due to estrogen, male rats were used in this study. We first examined how repeated binge EtOH exposure affects LMA of HIV-1Tg and control F344 adolescent rats. Then, we studied whether concurrent treatment with DHA can reverse the effects of EtOH on LMA and alter the inflammatory cytokine profile in the striatum of these animals. Our data show that daily DHA consumption, possibly via its antiinflammatory effects, can partially reverse the adverse effects of binge EtOH exposure on behavioral performance.

Materials and methods

Animals

To avoid the interference of sex hormones on DHA, male rats were used in the study. Male F344 (control) and HIV-1Tg rats were purchased from Harlan Inc. (Indianapolis, IN, USA).

The rats were housed with four animals per ventilated plastic rat cage (Animal Care Systems Inc., Centennial, CO) and maintained in a temperature- and humidity-controlled environment. They were maintained in a 12-h light/dark cycle and fed a standard rat diet. The experimental protocol was approved by the Institutional Animal Care and Use Committee (IACUC) at Seton Hall University, South Orange, NJ.

Ethanol and docosahexaenoic acid administration

Treatment began when the animals were 7 weeks old. The rats were randomly divided into four groups ($n = 6$) for each strain and treated by intragastric administration of saline as a control (Saline group), DHA (DHA group), EtOH (EtOH group), or DHA combined with EtOH (DHA + EtOH group). The DHA solution was prepared by dissolving DHA (Nu-chek Prep, Elysian, MN) in saline. The DHA solution was shaken vigorously for 2 min before use. The Saline group was treated with 4 mL/kg saline daily. The DHA group was treated with 20 mg/kg DHA daily. The EtOH group was treated with 4 mL/kg/day saline on day 1 to day 4 from week 1 to week 5. In the first week, the dosage of EtOH was gradually increased from 1 g/kg on day 5 to 2 g/kg on day 6 and 4.8 g/kg on day 7 to allow the rats to adapt to high-dose EtOH. The EtOH dosage remained at 4.8 g/kg from week 2 to week 5, administered on day 5 to day 7 of each week (Fig. 1a). The DHA + EtOH group was administered 20 mg/kg/d DHA on day 1 to day 7, and, on day 5 to day 7, EtOH was administered 1 h after DHA injection using the same dosage escalation schedule as in the EtOH group. At the end of 5 weeks of treatment, the rats were sacrificed (at 12 weeks of age). Whole blood was collected via cardiac puncture and stored in blood collection tubes with lithium heparin (BD, Franklin Lakes, NJ). The brains were microdissected, and the striatum regions were used for analysis of gene expression using quantitative reverse-transcriptase-polymerase chain reaction (qRT-PCR).

Locomotor activity measurement

LMA in the open field apparatus was measured using a video tracking software (Stoelting ANY-maze, Wood Dale, IL) for a 2-min period prior to and at 45, 90, 135, and 180 min, respectively, after EtOH injection on every EtOH treatment day (Fig. 1b). The animals were given the intragastric treatments on the day of sacrifice and placed in the open field apparatus for 10 min prior to sacrifice.

Plasma endotoxin determination

Plasma was prepared by centrifuging the whole blood for 10 min at $2000\times g$ at 4 °C. The supernatant was transferred immediately to endotoxin-free tubes by serological pipettes and stored at -80 °C before use. Endotoxin in the plasma of each sample was measured using the Pyrogen-5000 Kinetic Turbidimetric Assay (Lonza, Walkersville, MD), according to the manufacturer's instruction.

RNA isolation and cDNA preparation

Total RNA was extracted from the striatum using an RNeasy Mini Kit (Qiagen, Valencia, CA), followed by RNase-free DNase (Qiagen, Valencia, CA) to remove contaminating DNA. RNA quality and quantity were determined using the ND1000 Nanodrop

spectrophotometer (Thermo Scientific, Waltham, MA). An equal amount of RNA (200 ng) from each sample was then converted to cDNA using a RT² First Strand Kit (SABiosciences, Frederick, MD), according to the manufacturer's instructions.

Real-time quantitative polymerase chain reaction analysis

Quantification of gene expression was performed using RT² SYBR Green Fluorescein qPCR Master Mix (SABiosciences, Frederick, MD), according to the manufacturer's instructions. Real-time polymerase chain reaction (PCR) was performed using the ABI Prism 7900HT Fast Detection System (Applied Biosystems, Foster, CA). The PCR mix was denatured at 95 °C for 10 min before the first PCR cycle. The thermocycler parameters were 95 °C for 10 min, followed by 40 cycles at 95 °C for 15 s and 60 °C for 1 min. β -actin (*ACTB*) and splicing factor, arginine/serine-rich 4 (*SFRS4*) were used as housekeeping genes. The primer sequences are shown in Table 1.

Statistical analysis

A preliminary review of the LMA data revealed that the distance traveled during the 2 min before daily treatments and the total distances traveled after the daily treatments displayed different patterns of behavior change over weeks. For that reason, the two LMA data sets were analyzed separately using the same statistical analysis as described below.

Due to occasional difficulty with intragastric administration, data points were not available for all rats on every day of treatment. In addition, some rats died before they could complete the full 5 weeks of treatment. Therefore, to analyze the change in LMA with repeated treatment, the LMA data were collapsed into three treatment blocks: block 1 (week 1), block 2 (weeks 2 and 3, Wk2&3), and block 3 (weeks 4 and 5, Wk4&5). Five rats were dropped from the analysis because data points were not available for all three blocks. No more than one rat was dropped from a group (Table 2). The final number of rats per groups was: HIV-1Tg Saline ($n = 5$), HIV-1Tg DHA ($n = 6$), HIV-1Tg EtOH ($n = 5$), HIV-1Tg DHA + EtOH ($n = 5$), F344 Saline ($n = 6$), F344 DHA ($n = 5$), F344 EtOH ($n = 5$), and F344 DHA + EtOH ($n = 6$).

For each genotype, two groups received EtOH (EtOH group and DHA + EtOH group) and two groups did not receive EtOH (Saline group and DHA group). In addition for each genotype, each group received DHA (DHA group and DHA + EtOH group) and two groups did not receive DHA (Saline group and EtOH group). Thus, the data were analyzed as a Genotype (2) $\times$ EtOH (2) $\times$ DHA (2) $\times$ blocks (3) mixed ANOVA with blocks as the repeated measure and the three other factors as between-groups factors, followed by three-way and two-way ANOVAs and Tukey post hoc t tests where appropriate. Plasma endotoxin concentrations were analyzed using two-way ANOVA.

Results

Distance traveled during the 2 min before treatment reveals long-term effects of EtOH on locomotor activity in both F344 and HIV-1Tg and a DHA effect in HIV-1Tg rats

Prior to each EtOH administration including Wk1, each rat was placed in the open field apparatus for 2 min to determine their LMA. Distance traveled during the 2 min prior to each EtOH treatment from Wk2 revealed long-term effects of EtOH and DHA treatment on LMA. Travel distances were analyzed as a Genotype (2) $\times$ EtOH (2) $\times$ DHA (2) $\times$ blocks (3) mixed ANOVA with blocks as the repeated measure and the three other factors as between-groups factors. The HIV-1Tg and F344 groups treated with EtOH showed a decrease in distance traveled over blocks whereas the groups not receiving EtOH did not show this decline (blocks $\times$ EtOH interaction: $F(2, 70) = 17.05$, $p < 0.001$) (Fig. 2). However, this effect of EtOH on distance traveled over blocks was prevented by DHA treatment in the HIV-1Tg rats but not the F344 rats as indicated by a statistically significant four-way interaction, $F(2, 70) = 3.63$, $p = 0.03$ (Fig. 2). To further evaluate this four-way interaction, separate three-way ANOVAs (genotype $\times$ EtOH $\times$ DHA) were conducted on the three blocks separately. The three-way interaction was statistically significant during the third block (Wk4&5), $F(1, 35) = 9.75$, $p = 0.004$, but not during the second (Wk2&3), $F(1, 35) = 0.15$, $p = 0.70$, and the first (Wk1) blocks, $F(1, 35) = 0.19$, $p = 0.67$. Additional two-way ANOVA conducted on the third block data for the genotypes separately yielded a significant EtOH $\times$ DHA interaction in the HIV-1Tg rats, $F(1, 17) = 7.85$, $p = 0.01$, but not in the F344 rats, $F(1, 18) = 2.31$, $p = 0.15$. Tukey post hoc t tests comparing the HIV-1Tg groups further revealed that the DHA + EtOH group differed significantly from the EtOH group ($p = 0.03$), but not from the DHA group ($p = 0.29$) and Saline group ($p = 0.10$).

Total distance traveled under the influence of EtOH reveals increased and decreased locomotor activity in F344 and HIV-1Tg rats

Locomotor activity (LMA) in the presence of EtOH was measured for 2 min at 45, 90, 135, and 180 min after EtOH treatment for a total of 8 min per treatment (Fig. 1b). The distance traveled data for the three blocks (Fig. 3) were analyzed with a four-way ANOVA as in the previous analysis. A genotype (2) $\times$ EtOH (2) $\times$ DHA (2) $\times$ blocks (3) mixed ANOVA did not yield any significant effects of genotype or DHA treatment. The only significant interaction was EtOH $\times$ blocks, $F(2, 70) = 6.69$, $p = 0.002$.

This two-way interaction (Fig. 4) reflected a decrease in distance traveled from the Wk1 block to the Wk2&3 block ($p < 0.001$) and the Wk4&5 block ($p = 0.02$) in the EtOH-treated groups only. Moreover, the EtOH-treated groups traveled a greater distance than No-EtOH-treated groups during Wk1 ($p = 0.04$), but traveled less than the No-EtOH groups during the Wk2&3 block ($p = 0.02$); the EtOH-treated and No-EtOH-treated groups did not differ during the Wk4&5 block ($p = 0.77$) (Fig. 4).

Thus, EtOH treatment significantly increased locomotor activity the first week of treatment in rats regardless of genotype and DHA treatment, but subsequently decreased locomotor activity with further treatment. The tendency for DHA to reverse the instant effects of EtOH in the HIV-1Tg (Fig. 3) did not reach statistical significance.

Plasma endotoxin concentration is higher in HIV-1Tg rats

Plasma endotoxin concentration was tested after 5 weeks of treatment (Fig. 5). A two-way ANOVA showed no significant interactions between treatment and genotype on plasma endotoxin concentration. No significant differences between groups receiving saline, EtOH, DHA, or DHA + EtOH treatment were observed in either F344 or HIV-1Tg rats. However, there was a statistically significant difference in plasma endotoxin between strains, $F(1,31) = 8.859$, $p = 0.0056$.

IL-6, IL-18, and NF- κ B gene expression were altered in the striatum

To further explore the molecular mechanism underlying the EtOH and DHA effects, mRNA expression of cytokines IL-6 and IL-18, as well as transcription factor NF- κ B, were determined using real-time quantitative PCR. A two-way ANOVA showed no significant interactions between treatment and genotype on IL-6, IL-18, or NF- κ B gene expression. As shown in Fig. 6a, b, in HIV-1Tg rats, IL-6 and IL-18 gene expression in the EtOH group were significantly higher than in the DHA group and DHA + EtOH group. NF- κ B gene expression was higher in the HIV-1Tg EtOH group than in the HIV-1Tg DHA group or the HIV-1Tg DHA + EtOH group (Fig. 6c). Notably, the expression of both IL-6 and IL-18 was higher in HIV-1Tg rats given EtOH than in F344 rats treated with EtOH.

Discussion

Epidemiological studies have revealed that people living with HIV (PLWH) in the USA have a high prevalence of alcohol use and abuse (Chang et al. 2014). HIV-1Tg rats mimic people living with HIV who are receiving cART (Vigorito et al. 2015). While HIV virus replication is inhibited via cART, HIV viral proteins persist in the body. This produces systemic inflammation, including neuroinflammation, which ultimately leads to the behavioral disorders associated with HIV infection (Vigorito et al. 2015). Because DHA is known to be neuro-protective (Dyall 2015; Lauritzen et al. 2016), we studied whether daily administration of low-dose DHA could reduce the detrimental effects on locomotor activity (LMA) of binge exposure to both EtOH and persistent HIV viral proteins.

DHA concentration is higher in females than in males (Giltay et al. 2004). During the biosynthesis of DHA from precursors, the endogenous conversion of ^{13}C -labeled ALA into DHA is greater in women than in men (Burdge et al. 2002; Burdge and Wootton 2002). In addition, DHA is reported to be regulated by sex hormones, such as ethinyl estradiol (Giltay et al. 2004). The role of DHA has been studied in both males and females (Burdge et al. 2002; Burdge and Wootton 2002). In this study, we explored the effects of DHA in male rats exposed to binge EtOH to avoid potential interference from sex hormones.

LMA data measured prior to EtOH administrations revealed a decrease in distance traveled over blocks in EtOH-treated groups, but not in non-EtOH-treated groups, in both F344 rats (Fig. 2a) and HIV-1Tg rats (Fig. 2b). This effect of EtOH on travel distance was significantly reduced in EtOH-treated HIV-1Tg rats that also received DHA but not in the F344 rats treated with DHA and EtOH (Fig. 2). This gradual decrease in LMA was seen in the rats when they were not under the influence of EtOH since the behavioral measures were made

before the intragastric treatment for the day, indicating that long-term behavioral changes induced by binge EtOH treatment is reversed only in HIV-1Tg rats.

When HIV-1Tg and F344 rats were tested under the influence of EtOH (after intragastric treatment) there was an initial increase in LMA during the first week (block 1) followed by reduced LMA during weeks 2 and 3 (block 2, Wk2&3). This difference in binge EtOH treatment across blocks may have been partly due to the lower EtOH doses administered during the first 2 days of the first week of treatment to allow the animals to adapt to the high-dose EtOH. The reversal action of DHA on LMA before treatment was also apparent in the HIV-1Tg rats when under the influence of EtOH (Fig. 3), but this effect failed to reach statistical significance.

DHA was previously reported to exert anti-viral action against HIV by activating the endogenous KCNK channels (Hsu 2009). In addition, DHA was found to be involved in the regulation of Nrf2-mediated detoxification mechanisms (Pall and Levine 2015). Thus, we inferred that LMA was impaired by EtOH and that this detrimental effect was exaggerated by the presence of HIV proteins. This may explain why the HIV-1Tg EtOH group had a significantly shorter travel distance than the F344 EtOH group (Fig. 2). The antagonistic actions of DHA on the effects of HIV viral proteins might account for DHA's reversal of the combined effects of binge exposure to EtOH and persistent HIV viral proteins. These therapeutic effects of DHA were not evident in F344 rats (Figs. 2 and 3), presumably because EtOH alone was involved in the decreased LMA in F344 rats.

Binge exposure to EtOH has been reported to increase systemic levels of endotoxin. Repeated binge exposure to EtOH disrupts the intestinal barrier, allowing resident gut bacteria to be released via the intestinal cell walls to enter the bloodstream (Bjarnason et al. 1984). We found that the plasma endotoxin level of HIV-1Tg rats was significantly higher than that of F344 rats (Fig. 6). Plasma endotoxin can enhance the penetration of HIV and its proteins, such as gp120, across the blood-brain barrier (BBB) (Banks et al. 1999; Dohgu and Banks 2008). Peng et al. previously reported that basal expression of HIV viral proteins in brain tissues increases with age (Peng et al. 2010). The high plasma endotoxin level in HIV-1Tg rats might be the mechanism underlying high expression of HIV proteins in brain tissue. Through both paracellular and transcellular mechanisms, Tat and gp120 HIV-1 viral proteins can alter BBB integrity and function (McRae 2016), and these effects might allow binge EtOH-induced elevations of plasma endotoxin to leak into brain tissues. Endotoxin in the brain can be degraded into fatty acid and glucosamine residues, which can penetrate into brain tissues (Kim et al. 2014). Thus, there might be a "positive feedback" mechanism underlying how persistent HIV-1 proteins promote elevated plasma endotoxin leakage into the brain tissues, particularly following repeated binge exposure to EtOH. Binge exposure to EtOH also increases the BBB permeability to HIV-1 proteins (Shiu et al. 2007). Ultimately, the persistence of HIV protein levels and repeated EtOH bingeing lead to inflammation within the brain.

The striatum plays a prominent role in regulating basic motor functions (Grillner et al. 2005), and impaired LMA implicates striatum dysfunction. The striatum neurons receive input from the cortex, as well as other brain structures including the thalamus, and then

project to the output nuclei and other components of the basal ganglia. Neurons in the output nuclei of the basal ganglia in turn project to neurons in the mesencephalic locomotor region (Kiehn and Dougherty 2013). Therefore, because the striatum is a major input station for the basal ganglia, striatum dysfunction might be involved in the impaired LMA.

Neuroinflammation and neuroimmune signaling have been reported to be associated with neuronal dysfunction (Hong et al. 2016; Park et al. 2017). Thus, we tested inflammation-related gene expression in the striatum. Cytokines, such as IL-6 and IL-18, are markers of inflammation that are secreted by brain cells and known to be related to behavioral dysfunction. In the presence of HIV viral proteins, cytokines are elevated. NB- κ B is a protein complex that controls transcription of DNA, cytokine production, and cell survival. When stimulated by IL-18, it subsequently modulates immune-related gene transcription (Alboni et al. 2010), including transcription of the IL-6 gene (Libermann and Baltimore 1990). In the present study, neuroinflammation might be related to functional disorders in the striatum following repeated binge exposure to EtOH for 5 weeks. Such prolonged EtOH intoxication from adolescence to early adulthood may cause dysfunction of the striatum, leading to impaired LMA. This speculation is supported by the binge EtOH-induced elevation of immune-related gene expression, including that of IL-18, IL-6, and NB- κ B.

IL-18, a proinflammatory cytokine elevated in HIV-1 infected patients (Ahmad et al. 2002), is produced by and active in brain microglial cells (Prinz and Hanisch 1999). Increased IL-18 production in the brain is also associated with motor and cognitive dysfunction, leading to the development of HIV-associated dementia (Iannello et al. 2009; von Giesen et al. 2004). EtOH has also been reported to activate the NLRP3-inflammasome in astroglial cells, which stimulates caspase-1 cleavage and IL-1 β and IL-18 production (Alfonso-Loeches et al. 2016). IL-6 is a major cytokine in the CNS that is secreted by various brain cells and has a number of physiological functions (Erta et al. 2012), including behavior regulation (Erta et al. 2015). Moreover, IL-6 expression was previously reported to be induced by HIV-1 Tat, gp120, and Nef (Del Como et al. 2014; Liu and Kumar 2015; Nookala and Kumar 2014). Its level in the CNS is also increased by EtOH exposure (Doremus-Fitzwater et al. 2015; Gano et al. 2016). The role of NF- κ B and its relation to IL-18 and IL-6 was noted above. Furthermore, NF- κ B expression correlates with the severity of the AIDS Dementia Complex, a complication of late HIV infection (Rostasy et al. 2000). In HIV-1Tg rats, IL-6, IL-18, and NF- κ B mRNA expression levels in the striatum were highest in the EtOH group (Fig. 6). These high expression levels (Fig. 6) were associated with low LMA (Fig. 2b). As expected, binge EtOH-induced elevations of IL-6, IL-18, and NF- κ B mRNA were reversed by treatment with DHA. However, these reversal effects of DHA were not observed in F344 control animals, similar to the results observed for LMA.

Taken together, our results suggest that HIV-1 proteins and high levels of endotoxin in HIV-1Tg rats, particularly when exposed to high-dose EtOH on an intermittent basis to mimic binge alcohol use, might lead to increased expression of inflammation-related genes, such as IL-6, IL-18, and NF- κ B, in brain areas including the striatum, which plays a critical role in execution of motor functions. Persistence of HIV viral proteins and binge exposure to EtOH interacted, possibly via neuroinflammation and neuroimmune signaling, to impair LMA. This LMA deficit was partially reversed by treatment with DHA, which attenuated

the elevated expression of inflammatory genes, such as IL-6, IL-18, and NF- κ B, in the striatum of HIV-1Tg rats.

In conclusion, our research showed that DHA supplements might be a novel way to alleviate the interactive effects of HIV infection and binge drinking through their ability to reduce elevated IL-6, IL-18, and NF- κ B expression in the striatum.

Acknowledgements

This work was partially supported by the National Institutes of Health (NIH) grants AA023172 and DA036175 to Sulie L. Chang, as well as the Oversea Study Fellowship from the China Scholarship Council to Jianlin He, No. 201400100091. We thank Andrew Dieterich for his dedication to the animal care.

References

- Aarli JA (2003) Role of cytokines in neurological disorders. *Curr Med Chem* 10:1931–1937 [PubMed: 12871095]
- Abbondanzo SJ, Chang SL (2014) HIV-1 transgenic rats display alterations in immunophenotype and cellular responses associated with aging. *PLoS One* 9:e105256
- Acheampong E, Mukhtar M, Parveen Z, Ngoubilly N, Ahmad N, Patel C, Pomerantz RJ (2002) Ethanol strongly potentiates apoptosis induced by HIV-1 proteins in primary human brain microvascular endothelial cells. *Virology* 304:222–234 [PubMed: 12504564]
- Ahmad R, Sindhu ST, Toma E, Morisset R, Ahmad A (2002) Elevated levels of circulating interleukin-18 in human immunodeficiency virus-infected individuals: role of peripheral blood mononuclear cells and implications for AIDS pathogenesis. *J Virol* 76:12448–12456 [PubMed: 12438570]
- Alboni S, Cervia D, Sugama S, Conti B (2010) Interleukin 18 in the CNS. *J Neuroinflammation* 7:9 [PubMed: 20113500]
- Alfonso-Loeches S, Urena-Peralta J, Morillo-Bargues MJ, Gomez-Pinedo U, Guerri C (2016) Ethanol-induced TLR4/NLRP3 Neuroinflammatory response in microglial cells promotes leukocyte infiltration across the BBB. *Neurochem Res* 41:193–209
- Anderson GJ, Connor WE, Corliss JD (1990) Docosahexaenoic acid is the preferred dietary n-3 fatty acid for the development of the brain and retina. *Pediatr Res* 27:89–97 [PubMed: 2136947]
- Baez-Mendoza R, Schultz W (2013) The role of the striatum in social behavior. *Front Neurosci* 7:233 [PubMed: 24339801]
- Banks WA, Kastin AJ, Brennan JM, Vallance KL (1999) Adsorptive endocytosis of HIV-Igpl20 by blood-brain barrier is enhanced by lipopolysaccharide. *Exp Neurol* 156:165–171 [PubMed: 10192787]
- Bie B, Wang Y, Cai YQ, Zhang Z, Hou YY, Pan ZZ (2012) Upregulation of nerve growth factor in central amygdala increases sensitivity to opioid reward. *Neuropsychopharmacology* 37:2780–2788 [PubMed: 22871918]
- Bjarnason I, Peters TJ, Wise RJ (1984) The leaky gut of alcoholism: possible route of entry for toxic compounds. *Lancet* 1:179–182 [PubMed: 6141332]
- Burdge GC, Jones AE, Wootton SA (2002) Eicosapentaenoic and docosapentaenoic acids are the principal products of alpha-linolenic acid metabolism in young men. *Br J Nutr* 88:355–363 [PubMed: 12323085]
- Burdge GC, Wootton SA (2002) Conversion of alpha-linolenic acid to eicosapentaenoic, docosapentaenoic and docosahexaenoic acids in young women. *Br J Nutr* 88:411–420 [PubMed: 12323090]
- Cardoso C, Afonso C, Bandarra NM (2016) Dietary DHA and health: cognitive function ageing. *Nutr Res Rev* 29:281–294 [PubMed: 27866493]
- Chang SL, Connaghan KP, Wei Y, Li MD (2014) NeuroHIV and use of addictive substances. *Int Rev Neurobiol* 118:403–440 [PubMed: 25175871]

- Covacu R, Brundin L (2015). Effects of neuroinflammation on neural stem cells. *Neuroscientist*
- Crews FT, Vetreno RP (2016) Mechanisms of neuroimmune gene induction in alcoholism. *Psychopharmacology* 233:1543–1557 [PubMed: 25787746]
- Davoren MP, Demant J, Shiely F, Perry IJ (2016) Alcohol consumption among university students in Ireland and the United Kingdom from 2002 to 2014: a systematic review. *BMC Public Health* 16:173 [PubMed: 26895824]
- Del Como M, Donninelli G, Varano B, Da Sacco L, Masotti A, Gessani S (2014). HIV-1 gpl20 activates the STAT3/interleukin-6 axis in primary human monocyte-derived dendritic cells. *J Virol* 88: 11045–11055 [PubMed: 25008924]
- Dohgu S, Banks WA (2008) Lipopolysaccharide-enhanced transcellular transport of HIV-1 across the blood-brain barrier is mediated by the p38 mitogen-activated protein kinase pathway. *Exp Neurol* 210:740–749 [PubMed: 18295207]
- Doremus-Fitzwater TL, Gano A, Paniccia JE, Deak T (2015) Male adolescent rats display blunted cytokine responses in the CNS after acute ethanol or lipopolysaccharide exposure. *Physiol Behav* 148:131–144 [PubMed: 25708278]
- Dyall SC (2015) Long-chain omega-3 fatty acids and the brain: a review of the independent and shared effects of EPA, DPA and DHA. *Front Aging Neurosci* 7:52 [PubMed: 25954194]
- Erta M, Giralt M, Esposito FL, Fernandez-Gayol O, Hidalgo J (2015) Astrocytic IL-6 mediates locomotor activity, exploration, anxiety, learning and social behavior. *Horm Behav* 73:64–74 [PubMed: 26143620]
- Erta M, Quintana A, Hidalgo J (2012) Interleukin-6, a major cytokine in the central nervous system. *Int J Biol Sci* 8:1254–1266 [PubMed: 23136554]
- Flora G, Pu H, Lee YW, Ravikumar R, Nath A, Hennig B, Toborek M (2005) Proinflammatory synergism of ethanol and HIV-1 Tat protein in brain tissue. *Exp Neurol* 191:2–12 [PubMed: 15589507]
- Gano A, Doremus-Fitzwater TL, Deak T (2016) Sustained alterations in neuroimmune gene expression after daily, but not intermittent, alcohol exposure. *Brain Res* 1646:62–72 [PubMed: 27208497]
- Giltay EJ, Gooren LJ, Toorians AW, Katan MB, Zock PL (2004) Docosahexaenoic acid concentrations are higher in women than in men because of estrogenic effects. *Am J Clin Nutr* 80:1167–1174 [PubMed: 15531662]
- Grillner S, Hellgren J, Menard A, Saitoh K, Wikstrom MA (2005) Mechanisms for selection of basic motor programs—roles for the striatum and pallidum. *Trends Neurosci* 28:364–370 [PubMed: 15935487]
- Hong H, Kim BS, Im HI (2016) Pathophysiological role of neuroinflammation in neurodegenerative diseases and psychiatric disorders. *Int Neurol J* 20:S2–S7 [PubMed: 27230456]
- Hsu K (2009) Long-chain polyunsaturated fatty acids as anti-HIV supplementation during breastfeeding. *Taiwan J Obstet Gynecol* 48:65–68 [PubMed: 19346195]
- Iannello A, Samarani S, Debbeche O, Tremblay C, Toma E, Boulassel MR, Routy JP, Ahmad A (2009) Role of interleukin-18 in the development and pathogenesis of AIDS. *AIDS Rev* 11:115–125 [PubMed: 19654853]
- Jasani B, Simmer K, Patole SK, Rao SC (2017) Long chain polyunsaturated fatty acid supplementation in infants born at term. *Cochrane Database Syst Rev* 3:CD000376 [PubMed: 28281303]
- Johnston LD, O'Malley PM, Miech RA, Bachman JG, Schulenberg JE (2014) Monitoring the future national results on drug use: 1975–2013: overview. Findings on Adolescent Drug Use, Key
- Kanmogne GD, Primeaux C, Grammas P (2005) HIV-1 gpl20 proteins alter tight junction protein expression and brain endothelial cell permeability: implications for the pathogenesis of HIV-associated dementia. *J Neuropathol Exp Neurol* 64:498–505 [PubMed: 15977641]
- Kaplan JE, Benson C, Holmes KK, Brooks JT, Pau A, Masur H (2009) Guidelines for prevention and treatment of opportunistic infections in HIV-infected adults and adolescents: recommendations from CDC, the National Institutes of Health, and the HIV Medicine Association of the Infectious Diseases Society of America. *MMWR Recomm Rep* 58(RR-4): 1–207
- Kiehn O, Dougherty K (2013). *Locomotion: circuits and physiology*. Springer New York

- Kim CS, Ross IA, Sapienza PP, Hanes DE, Johnson W, Hutter JC (2014) Distribution and pharmacokinetics of doubleradiolabeled endotoxin in the rat brain and peripheral organs. *Toxicol Ind Health* 30:432–441 [PubMed: 22933553]
- Lauritzen L, Brambilla P, Mazzocchi A, Harslof LB, Ciappolino V, Agostoni C (2016) DHA effects in brain development and function. *Nutrients* 8
- Liebermann TA, Baltimore D (1990) Activation of interleukin-6 gene expression through the NF-kappa B transcription factor. *Mol Cell Biol* 10:2327–2334 [PubMed: 2183031]
- Liu X, Kumar A (2015) Differential signaling mechanism for HIV-1 Nef-mediated production of IL-6 and IL-8 in human astrocytes. *Sci Rep* 5:9867 [PubMed: 26075907]
- McRae M (2016) HIV and viral protein effects on the blood brain barrier. *Tissue Barriers* 4:e1 143543
- Menard CR Goodman KJ, Corso TN, Brenna JT, Cunnane SC (1998) Recycling of carbon into lipids synthesized de novo is a quantitatively important pathway of alpha-[U-13C]linolenate utilization in the developing rat brain. *J Neurochem* 71:2151–2158 [PubMed: 9798942]
- Meng ZH, Pennington SN, Dar MS (1998) Rat striatal adenosinergic modulation of ethanol-induced motor impairment possible role of striatal cyclic AMP. *Neuroscience* 85:919–930 [PubMed: 9639284]
- Nair M, Maria JM, Agudelo M, Yndart A, Vargas-Rivera ME (2015) Platelets contribute to BBB disruption induced by HIV and alcohol. *J Alcohol Drug Depend* 3:182 [PubMed: 26501067]
- Nookala AR, Kumar A (2014) Molecular mechanisms involved in HIV-1 Tat-mediated induction of IL-6 and IL-8 in astrocytes. *J Neuroinflammation* 11:214 [PubMed: 25539898]
- Otto SJ, van Houwelingen AC, Badart-Smook A, Homstra G (2001) Changes in the maternal essential fatty acid profile during early pregnancy and the relation of the profile to diet. *Am J Clin Nutr* 73:302–307 [PubMed: 11157328]
- Pall ML, Levine S (2015) Nrf2, a master regulator of detoxification and also antioxidant, anti-inflammatory and other cytoprotective mechanisms, is raised by health promoting factors. *Sheng Li Xue Bao* 67: 1–18 [PubMed: 25672622]
- Park G, Lee SH, Oh DS, Kim YU (2017). Melatonin inhibits neuronal dysfunction-associated with neuroinflammation by atopic psychological stress in NC/Nga atopic-like mouse models. *J Pineal Res*
- Peng J, Vrgorito M, Liu X, Zhou D, Wu X, Chang SL (2010) The HIV-1 transgenic rat as a model for HIV-1 infected individuals on HAART. *J Neuroimmunol* 218:94–101 [PubMed: 19913921]
- Pociecha K, Przejczowska-Pomiemy K, Cios A, Wyska E (2015) Contemporary concepts in studies on cyclic AMP and its role in the inflammatory reaction. *Postepy Hig Med Dosw (Online)* 69: 777–798 [PubMed: 26206993]
- Postle AD, Al MD, Burdge GC, Homstra G (1995) The composition of individual molecular species of plasma phosphatidylcholine in human pregnancy. *Early Hum Dev* 43:47–58 [PubMed: 8575351]
- Prendergast MA, Little HJ (2007) Adolescence, glucocorticoids and alcohol. *Pharmacol Biochem Behav* 86:234–245 [PubMed: 16930684]
- Prinz M, Hanisch UK (1999) Murine microglial cells produce and respond to interleukin-18. *J Neurochem* 72:2215–2218 [PubMed: 10217305]
- Ranki A, Nyberg M, Ovod V, Haltia M, Elovaara I, Raininko R, Haapasalo H, Krohn K (1995) Abundant expression of HIV Nef and Rev proteins in brain astrocytes in vivo is associated with dementia. *AIDS* 9:1001–1008 [PubMed: 8527071]
- Reid W, Sadowska M, Denaro F, Rao S, Foulke J, Jr., Hayes N, Jones O, Doodnauth D, Davis H, Sill A, O'Driscoll P, Huso D, Fouts T, Lewis G, Hill M, Kamin-Lewis R, Wei C, Ray P, Gallo RC, Reitz M, Bryant J (2001). An HIV-1 transgenic rat that develops HIV-related pathology and immunologic dysfunction. *Proc Natl Acad Sci U S A* 98: 9271–9276
- Rostasy K, Monti L, Yiannoutsos C, Wu J, Bell J, Hediye J, Navia BA (2000) NFkappaB activation, TNF-alpha expression, and apoptosis in the AIDS-dementia-complex. *J Neuro-Oncol* 6:537–543
- Sanhueza C, Garcia-Moreno LM, Exposito J (2011) Weekend alcoholism in youth and neurocognitive aging. *Psicothema* 23:209–214 [PubMed: 21504671]
- Shiu C, Barbier E, Di Cello F, Choi HJ, Stins M (2007). HIV-1 gp120 as well as alcohol affect blood-brain barrier permeability and stress fiber formation: involvement of reactive oxygen species. *Alcohol Clin Exp Res* 31: 130–137 [PubMed: 17207111]

- Shu J, Zhang F, Zhang L, Wei W (2016). G protein coupled receptors signaling pathways implicate in inflammatory and immune response of rheumatoid arthritis. *Inflamm Res*
- Siegel M, DeJong W, Naimi TS, Fortunato EK, Albers AB, Heeren T, Rosenbloom DL, Ross C, Ostroff J, Rodkin S, King C, Borzekowski DL, Rimal RN, Padon AA, Eck RH, Jemigan DH (2013) Brand-specific consumption of alcohol among underage youth in the United States. *Alcohol Clin Exp Res* 37:1195–1203 [PubMed: 23398328]
- Siegel M, DeJong W, Naimi TS, Heeren T, Rosenbloom DL, Ross C, Ostroff J, Jemigan DH (2011) Alcohol brand preferences of underage youth: results from a pilot survey among a national sample. *Subst Abus* 32:191–201 [PubMed: 22014249]
- Silveri MM (2012) Adolescent brain development and underage drinking in the United States: identifying risks of alcohol use in college populations. *Harv Rev Psychiatry* 20:189–200 [PubMed: 22894728]
- Simopoulos AP (1991) Omega-3 fatty acids in health and disease and in growth and development. *Am J Clin Nutr* 54:438–463 [PubMed: 1908631]
- Vigorito M, Connaghan KP, Chang SL (2015) The HIV-1 transgenic rat model of neuroHIV. *Brain Behav Immun* 48:336–349 [PubMed: 25733103]
- von Giesen HJ, Jander S, Koller H, Arendt G (2004) Serum and cerebrospinal fluid levels of interleukin-18 in human immunodeficiency virus type 1-associated central nervous system disease. *J Neuro-Oncol* 10:383–386
- Wang H, Yu M, Ochani M, Amelia CA, Tanovic M, Susarla S, Li JH, Yang H, Ulloa L, Al-Abed Y, Czura CJ, Tracey KJ (2003) Nicotinic acetylcholine receptor alpha7 subunit is an essential regulator of inflammation. *Nature* 421:384–388 [PubMed: 12508119]
- Wang P, Liu Z, Chen H, Ye N, Cheng X, Zhou J (2017). Exchange proteins directly activated by cAMP (EPACs): emerging therapeutic targets. *Bioorg Med Chem Lett*
- Wrona D (2006) Neural-immune interactions: an integrative view of the bidirectional relationship between the brain and immune systems. *J Neuroimmunol* 172:38–58 [PubMed: 16375977]
- Yang J-Y, Xue X, Tian H, Wang X-X, Dong Y-X, Wang F, Zhao Y-N, Yao X-C, Cui W, C-F W (2014) Role of microglia in ethanol-induced neurodegenerative disease: pathological and behavioral dysfunction at different developmental stages. *Pharmacol Ther* 144:321–337 [PubMed: 25017304]

a Weekly Intra gastric Treatment

b LMA Measurements on Days 5, 6, and 7

Fig. 1.

a Schematic representation of treatment for the Saline, Ethanol (EtOH), Docosahexaenoic acid (DHA), and DHA + EtOH groups during each week. The Saline group was treated with 4 mL/kg saline daily. The EtOH group was treated with 4 mL/kg/d saline on day 1 to day 4 of every week; during the first week, they were treated with 1 g/kg 52% w/v ethanol (EtOH) on day 5, 2 g/kg 52% w/v EtOH on day 6, and 4.8 g/kg on day 7, and from weeks 2 to 5 were treated with 4.8 g/kg/day 52% w/v EtOH on day 5 to day 7. The DHA group was treated with 20 mg/kg docosahexaenoic acid (DHA) daily. The DHA + EtOH group was treated with 20 mg/kg/day DHA on day 1 to day 7, and on day 5 to day 7, the rats also received EtOH 1 h after DHA administration using the same EtOH dosages as in the EtOH group, **b** Schematic representation of LMA measurements on days 5, 6, and 7. Measures were made 2 min before treatment and for 2 min at 45, 90, 135, and 180 min after treatment

Fig. 2.

Mean travel distance prior to administration of EtOH in the Saline group, DHA group, ETOH group, and DHA + EtOH group in HIV-1Tg rats (**a**) and F344 rats (**b**). The data were measured 2 min prior to EtOH administration on EtOH treatment days 5, 6, and 7. Travel distances were analyzed as a Genotype (2) $\times$ EtOH (2) $\times$ DHA (2) $\times$ blocks (3) mixed ANOVA with blocks as the repeated measure and the three other factors as between-groups factors. The HIV-1Tg and F344 groups treated with EtOH showed a decrease in distance traveled over blocks whereas the groups not receiving EtOH did not show this decline (blocks $\times$ EtOH interaction: $F(2, 70) = 17.05$, $p < 0.001$). LMA decreased consistently over the three blocks in all EtOH-treated groups of both genotypes except the HIV-1Tg DHA + EtOH group

Fig. 3.

Mean travel distance after EtOH administration of EtOH in the Saline group, DHA group, EtOH group, and DHA + EtOH group in HIV-1Tg rats (**a**) and F344 rats (**b**). Travel distance within 2 min was measured at 45, 90, 135, and 180 min after treatment. Travel distances were analyzed as a Genotype (2) $\times$ EtOH (2) $\times$ DHA (2) $\times$ blocks (3) mixed ANOVA with blocks as the repeated measure and the three other factors as between-groups factors. The only significant interaction was EtOH $\times$ blocks, $F(2,70) = 6.69$, $p = 0.002$

Fig. 4.

The data from the multifactorial ANOVA are replotted to show the mean total distance traveled of the rats treated with or without EtOH treatment, revealing an interaction of EtOH $\times$ block

Fig. 5. Plasma endotoxin concentrations after 5 weeks of saline, docosahexaenoic acid (DHA), ethanol (EtOH), or DHA + EtOH treatment in HIV-1Tg and F344 rats. The data are expressed as mean $\pm$ SD. ** $p < 0.01$

Fig. 6.

Inflammation-related gene expression in the striatum after 5 weeks of saline, docosahexaenoic acid (DHA), ethanol (EtOH), or DHA + EtOH treatment in HIV-1Tg and F344 rats. mRNA expression of IL-6 (a), IL-18 (b), and NF-κB (c). The data are expressed as mean ± SD. * $p < 0.05$, ** $p < 0.01$

Table 1

RT-qPCR gene-specific primers

Gene	Forward primer	Reverse primer
<i>IL-6</i>	GCCCTTGCTGGTGATGTT	GAGAGGGAGTGTGCTTGGA
<i>IL-18</i>	AAACCGCCTGTGTTCA	TCAGTCTGGTCTGGGATTTCGT
<i>NF-κB</i>	GCTTACGGTGGGATTGCATT	GTTTATGGTGGCCATGGGTGAT
<i>ACTB</i>	AAGTCCCTCACCCCTCCCAAAG	AAGCAAATGCTGTACACCTTCCC
<i>SFRS4</i>	TGGCAAAGACCTCTGTGGTG	CACTGGGTCCAGAACCCGTAA

ACTB β-actin; *IL* interleukin; *NF-κB* nuclear factor κB; *SFRS4* splicing factor, arginine/serine-rich 4

Table 2

Rats dropped from the analysis

Group	Genotype	
	HIV-1Tg	F344
Saline	1	0
DHA	0	1
EtOH	1	1
EtOH + DHA	1	0